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REVIEW ARTICLE

Antioxidant and anti-proliferative properties of lycopene

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Abstract

The recent search for new anti-cancer drugs focuses more on natural compounds from the regular human diet because these compounds rarely exhibit severe side-effects yet efficiently act on a wide range of molecular targets involved in carcinogenesis. One promising compound, which is now being tested in clinical studies, is the tomato-derived carotenoid lycopene. This review summarizes the current knowledge about the cellular action of lycopene and presents the molecular targets responsible for its remarkable chemopreventive and anti-proliferative activity. Its antioxidant effects include a considerable reactive oxygen species (ROS) scavenging activity, which allows lycopene to prevent lipid peroxidation and DNA damage. Simultaneously, lycopene induces enzymes of the cellular antioxidant defense systems by activating the antioxidant response element transcription system. As another chemopreventive strategy, lycopene increases gap junctional communication, which is suppressed during carcinogenesis. This review focuses also on the synergistic effects of lycopene with other natural antioxidants that might be important for its future application in anti-cancer treatment. Lastly, this review provides evidence for the biological activity of some oxidized lycopene metabolites, which seem to be partially responsible for the strong and manifold anti-cancer potential of lycopene.

Keywords: *Lycopene, antioxidant, chemoprevention, synergistic effects, bioactive metabolites*

Introduction

Lycopene is a fat-soluble red pigment produced by plants and some microorganisms [1]. It represents the major carotenoid in tomatoes and is found to a lesser extent in guava, pink grapefruit, watermelon and papaya [2,3]. In contrast to other carotenoids, this lipophilic acyclic isomer of β-carotene lacks vitamin A activity [4] and, although it represents the most predominant carotenoid in human plasma that is enriched in (very-) low-density lipoprotein fractions [5,6], no physiological function in humans has been described thus far. Lycopene is especially interesting because of its considerable antioxidant activity that highly exceeds that of β-carotene and α-tocopherol [7,8]. In this respect, skin lycopene has been reported to be more sensitive to UV light stress than β-carotene. As carotenoids are consumed during radical quenching, the observed lycopene destruction might be a hint for the protective role of lycopene [9]. Similar to resveratrol and curcumin, it possesses an impressive spectrum of health beneficial properties, ranging from hypocholesterolemic and cardioprotective effects [10] to anti-inflammatory and anti-mutagenic activity and a remarkable anti-cancer potential [11–14]. Beside this, a high lycopene serum level might be linked to a lower risk for age-related macular degeneration [15]. Data from epidemiological and clinical studies have revealed a correlation between a high consumption of tomato products and a reduced cancer risk [16–18]. Accordingly, Stahl and Sies [19] cited in their review several studies that clearly revealed an inverse correlation between lycopene serum levels and cancer risk, namely bladder and pancreatic cancer as well as cervical intraepithelial neoplasia [20–22]. Lycopene particularly exhibits strong anti-cancer activity against prostate cancer, even against the advanced and aggressive forms [17]. This might be explained by the fact that the highest tissue level of lycopene could be found in the testes, followed by adrenals and liver [19]. Among carotenoids, lycopene is of special interest, as it is the

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only compound of this class for which no association exists between its long-term use as a dietary supplement and an elevated risk of lung cancer [23].

The antioxidant effects of lycopene

The chemopreventive potential of lycopene can partially be explained by its strong singlet oxygen quenching activity (equation 1 [24]) , which is the most effective of 600 naturally occurring carotenoids [7] and is based on its chemical structure; lycopene consists of a tetraterpene hydrocarbon polyene chain with 11 conjugated and two unconjugated double bonds that can easily be attacked by electrophilic reagents, resulting in an extreme reactivity toward oxygen and free radicals [24]. For example, Mortensen et al. [25] provided evidence for the ability of lycopene to scavenge nitrogen dioxide and thiyl and sulphonyl radicals. Carotenoids in general might react with free radicals in three major ways, including electron transfer, hydrogen abstraction and radical addition (equations 2–4, respectively [26]). Galano et al. [27] recently reported that lycopene and torulene are more reactive than β-carotene toward peroxyl radicals. These authors further identified the C5 position as the main $-$OOH addition site. Lycopene has also been reported to trap peroxynitrite, an important biological oxidant, both *in vitro* and *in cellulo* [28–30], a reaction probably leading to the generation of oxidized, biologically active lycopene products [31]. Furthermore, Bast et al. [32] suggested that lycopene might enhance the cellular antioxidant defense system by regenerating the non-enzymatic antioxidants vitamins E and C from their radicals and, indeed, the ability of lycopene to reduce the δ-tocopheryl radical has been demonstrated (equation 5). The resulting lycopene radical cations then react with each other to form stable products in the absence of tocopherols (equation 6) [25].

$$^1O_2 + LYC \rightarrow {}^3O_2 + {}^3LYC$$
$$^3LYC \rightarrow LYC + \text{heat} \quad (1)$$
$$ROO^{\bullet} + LYC \rightarrow ROO^- + LYC^{\bullet +} \quad (2)$$
$$ROO^{\bullet} + LYC \rightarrow ROOH + LYC^{\bullet} \quad (3)$$
$$ROO^{\bullet} + LYC \rightarrow (ROO\text{-}LYC)^{\bullet} \quad (4)$$
$$LYC + TO^{\bullet} + H+ \rightarrow LYC^{\bullet +} + TOH \quad (5)$$
$$2\ LYC^{\bullet +} \rightarrow \text{products} \quad (6)$$

Due to its highly lipophilic nature, lycopene exerts its maximal antioxidant activity at the level of cellular membranes and interacts with lipid components [4]. Through protecting membranes from lipid peroxidation, it counteracts tumour initiation. Several studies have indicated that lycopene prevents nitrogen dioxide-induced oxidation of lipid membranes and subsequent cell death more efficiently than β-carotene [33,34]. Rao et al. [35] studied the impact of a tomato-rich diet on serum lycopene levels and the cellular oxidative state. Their findings indicated that serum lycopene levels increased following dietary supplementation of tomato products, suggesting that this carotenoid was easily absorbed. As a consequence of the enhanced plasma lycopene levels, lipid peroxidation, as measured by the formation of thiobarbituric acid reactive substances (TBARSs), was significantly lowered, as was both protein and DNA oxidation [35]. In a more recent study, the authors confirmed that a consumption of 30 mg lycopene per day, administered through a diet of processed tomato products, such as juice, ketchup and spaghetti sauce, led to a significant increase in lycopene serum levels and total antioxidant capacity, while oxidative stress, as measured by the damage on lipids and proteins, was diminished [36]. These observations correspond with the data from Agarwal et al. [37], which revealed a strong decrease of serum lipid and LDL oxidation in response to tomato consumption. Further analysis of processed tomato products indicated that the processing operations affected neither the content nor the stability of lycopene in tomatoes and that heat processing induced a conformational change in the *cis* isomeric form that improved its bioavailability [37–39]. Another clinical trial recently provided evidence that a tomato-rich diet reduces oxidative stress and subsequent damage of the plasma lipoproteins, serum proteins and lymphocyte DNA in prostate cancer patients. In addition, lycopene has been shown to inhibit cancer development and reduce the aggressiveness of prostate tumours in patients by decreasing prostate-specific antigen (PSA) and inducing connexin expression [40].

Antioxidants can prevent cancer development by protecting DNA from oxidative damage. Scolastici et al. [41] used micronucleus and comet assays to show that the rescuing effect of lycopene on DNA damage was induced either directly by hydrogen peroxide (H_2O_2) or indirectly by *n*-nitrosodiethylamine (DEN) in HepG2 cells, which are used as a model for the identification of anti-mutagens [42]. Because DEN is a pro-carcinogen that needs to be transformed to react with DNA, lycopene only prevented DEN-induced primary DNA damage when added before DEN exposure. This protective effect of lycopene has been confirmed by further studies showing that lycopene prevents DEN-induced neoplasia [43]. Moreover, the antioxidant activity of lycopene has been corroborated by the observation that lycopene significantly inhibits the mutagenic and genotoxic effects of H_2O_2 exposure [41,44–46]. The anti-mutagenic activity of lycopene has also been shown with the Ames test on two *Salmonella typhimurium* strains treated with different mutagenic agents. Significant anti-mutagenic effects could be observed with two indirect mutagens (2-amino-3-methylimidazol[4,5-f] quinoline and aflatoxin B1 [AFB1]), whereas lycopene was less effective against the direct mutagenesis

induced by N-nitroso-N-methylurea [47]. Moreover, lycopene and tomato purée protected mice from carcinogenesis when administered for 3 days before the mutagen treatment [47]. Conversely, lycopene was unable to prevent DNA damage and the generation of pre-neoplastic foci in rat liver upon AFB1 treatment [48]. Muzandu et al. [30] used SIN-1, a peroxynitrite generator, and authentic peroxynitrite to investigate the effect of lycopene on peroxynitrite-induced cellular damage. Using the alkaline comet assay, the authors observed a strong protection against peroxynitrite-induced DNA strand breaks due to lycopene treatment in Chinese hamster lung fibroblast cells, without detecting any toxicity. Although the exact mechanism of how lycopene prevents this DNA damage remains unclear, the proof for a direct quenching of peroxynitrite was provided with the indicator fluoro-probe dihydrorhodamine 123 [30]. Lycopene was similarly able to inhibit oxidative DNA damage *in vivo*. Matos et al. [49], for example, have reported that the administration of lycopene 5 days prior to ferric nitrilotriacetate (Fe-NTA) treatment inhibited the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) levels in male Wistar rats by nearly 70%. Data from a randomized, placebo-controlled trial substantiated the DNA-protecting activity of lycopene, as the authors detected decreased levels of urinary 8-OHdG [50]. Importantly, lycopene was detected in the nucleus of prostate cancer cells, explaining its effect at the DNA level [51]. Consequently, it is not surprising that lycopene also modulates transcriptional regulation. In fact, nuclear lycopene [51] counteracts oxidative stress also indirectly by up-regulating the phase II detoxifying enzymes to protect cells from ROS and electrophilic molecules [52]. This mechanism is known to contribute to the chemopreventive action of various natural compounds [53,54]. The *cis*-regulatory sequences in the promoter region of these enzymes, the so-called electrophile response element/antioxidant response element (EpRE/ARE) transcription system, allow their coordinated induction [55]. Carotenoids are known to activate this EpRE/ARE transcription system through the disruption of the cytosolic interactions between the major ARE-activating transcription factor, NF-E2-related factor 2 (Nrf2) and its inhibitor, Kelch-like ECH-associated protein 1 (Keap1) [56]. When Ben-Dor et al. [57] investigated the effect of lycopene and other tomato-derived carotenoids on the induction of phase II enzymes, their findings revealed that lycopene transactivated the ARE sequences of the phase II enzymes NAD(P)H:quinone oxidoreductase (NQO1) and glutamate cysteine ligase (GCL), which led to increased mRNA and protein levels. These results are in agreement with previous reports demonstrating that lycopene induced the expression of NQO1 in rat and GCL in hamster buccal pouch tumours [13,58]. Due to the induction of GCL, the rate-limiting enzyme in GSH synthesis, GSH levels were also up-regulated [13,59]. Recently, it has been reported that the activation of the Nrf2 signalling pathway through lycopene counteracts the cisplatin-induced reduction of heme oxygenase expression. Subsequently, lycopene was able to improve the nephrotoxic phenotype in rats that occurred as a side effect of the chemotherapeutic treatments for solid tumours [60]. It is noteworthy that, because of its lack of any electrophilic group, it is doubtful that lycopene directly targets Keap1. Instead, it has been proposed that the hydrophilic, oxidized products of lycopene, but not its intact form, mediate EpRE/ARE transactivation and thus indirectly amplify the cellular antioxidant defense [57,61]. Based on the analysis of 11 synthetic lycopene derivatives, Linnewiel et al. [61] provided detailed structure-activity rules necessary for the activation of this transcription system. After its release from Keap1, Nrf2 is known to translocate to the nucleus and induce the expression of phase II enzymes [62]. Indeed, after lycopene treatment of HepG2 cells, Nrf2 was found in the nucleus [57], where it co-localized with the promyelocytic leukaemia (PML) protein, which is known to regulate the transcriptional activity of various transcription factors [63]. Even though lycopene was the most potent ARE activator among the tested carotenoids, these compounds did not differ significantly in their ROS scavenging properties. This observation suggests that ARE activation is independent of the radical scavenging activity of carotenoids [57].

Although many studies provided evidence for the antioxidant properties of lycopene *in vitro* and supporting *in vivo* data have been published, recently some doubt has emerged as to whether the health benefits of lycopene really arise from its antioxidant activity. Most of the *in vivo* studies have been performed with lycopene extracts and the few human studies with pure lycopene (reviewed in [64]) did not clearly point to a lycopene-induced decrease of LDL oxidation or plasma lipid oxidation. Additionally, only 0.9 molecules of lycopene per LDL molecule have been found compared to 11.6 molecules of α-tocopherol. This low number is unlikely to allow a significant impact on LDL oxidation [64].

Moreover, it has been suggested that carotenoids might exert pro-oxidant, potentially harmful, effects depending on their concentration and the partial O_2 pressure (pO_2) [26,65,66]. However, most of these data are related to β-carotene while no conclusive evidence exists for lycopene. In fact, Eichler et al. [66] reported that lycopene protects skin fibroblasts from UV-induced formation of TBARS only at concentrations up to 0.15 nmol/mg protein, whereas at higher concentrations a pro-oxidant effect could be observed. Likewise, only low doses of β-carotene and lycopene were able to prevent cellular DNA damage [67]. However, according to Young and Lowe [26], clear

evidence for a pro-oxidant activity of carotenoids at physiological relevant pO_2 is still missing. In addition, Collins et al. [68] found neither significant protective nor harmful effects of carotenoid supplementation on the level of DNA damage in human lymphocytes. Young and Lowe [26] suggested that rather than exerting pro-oxidant effects, the antioxidant effectiveness of carotenoids might be decreased *in vivo*.

In the following part we focus on further biological activities of lycopene linked to its ability to counteract tumour promotion and progression.

Lycopene affects the proliferation and apoptosis of cancer cells

Whereas the chemopreventive effects of lycopene are primarily based on its antioxidant activity, lycopene prevents the promotion of carcinogenesis by interfering with various cellular processes, including cell cycle progression and the modulation of signal transduction pathways [12,69] (see Figure 2). In this respect, lycopene has been reported to affect the inflammatory cascade. A recent review summarized the anti-inflammatory effects of lycopene, which include the modulation of cyclooxygenase (COX) and lipoxygenase (LOX) expression, interference with nuclear factor (NF)-κB, activator protein (AP)-1 and mitogen activated protein kinase (MAPK) signalling and subsequent regulation of inducible nitric oxide synthase (iNOS) [70]. The suppression of the latter enzyme has been demonstrated by transcriptional analysis of the prostate tumour tissue of lycopene-treated Dunning rats (Siler 2004) [81]. In LNCaP prostate carcinoma cells, lower concentrations of lycopene only affected lipid oxidation, while higher concentrations induced DNA damage and arrested LNCaP cells in the G2/M phase [72]. This effect might be due to a pro-oxidant activity of elevated lycopene concentrations, as has been reported for curcumin [73–75]. Because cancer cells are deficient in normal cell cycle control and repair mechanisms, DNA damage can eventually cause cell cycle arrest. When Salman et al. [76] tested the effect of lycopene on different cell lines, they found a dose-dependent growth inhibition of human colon carcinoma (HuCC), erythroleukaemia (K562) and Burkitt's lymphoma (Raji) cells, whereas an inhibitory effect on chronic lymphoblastic leukaemia (CLL) (EHEB) cells was only detectable with the highest lycopene concentration (i.e. 4 μM) [76]. The anti-proliferative and pro-apoptotic activity of lycopene has also been observed in malignant T-lymphoblast cells (Jurkat E6) [77].

Lycopene-induced inhibition of DNA synthesis was observed in HL-60 promyelocytic leukaemia cells using an (^{3}H) thymidine incorporation assay and resulted in cell cycle arrest in the G0/G1 phase [78]. This effect was further enhanced by co-treatment with 1,25-dihydroxyvitamin D3, which displayed only weak anti-proliferative activity when administered alone [78]. In breast and prostate cancer cell lines, lycopene treatment inhibited cell cycle progression mainly in the G0/G1 phase via down-regulation of IGF-1R expression and the subsequent reduction of cell cycle regulatory proteins, including cyclin D1, cyclin E and cyclin-dependent kinases (CDK) 2 and 4 [79–81]. As a result, the phosphorylation of retinoblastoma (Rb) at serine 780 was strongly decreased in lycopene-treated prostate cancer cells [79]. The authors observed a concomitant decrease in the constitutive phosphorylation level of Akt, another downstream target of IGF-1 signalling, indicating that the PI3K signalling pathway was suppressed [79]. In addition to PI3K/Akt signalling, the MAPK pathways, including extracellular signal-regulated kinase (ERK) 1, p38Hog1 and c-Jun N-terminal kinase (JNK), were down-regulated in response to lycopene treatment. Although lycopene-induced apoptosis was observed only in androgen-sensitive LNCaP (but not in PC-3) cells, androgen-responsive luciferase reporter assays showed that lycopene did not affect androgen receptor signalling [79]. Conversely, in the Dunning prostate cancer model, lycopene was shown to interfere with the androgen pathway. Accordingly, Siler et al. [71] presented convincing microarray data of MatLyLu prostate tumour tissues of male Copenhagen rats fed with lycopene that showed a repression of steroid 5-α-reductase 1. Consequently, the mRNA levels of the androgen target genes were down-regulated. In combination with vitamin E, used as a stabilizer in lycopene formulations, an additive inhibitory effect on androgen metabolism was observed. Additionally, the local expression of IGF-1 and IL-6 in this tissue was found to be suppressed [71]. This is in accordance with the results of Karas et al. [82], who confirmed the inhibition of the IGF-1 pathway as the major mechanism of the anti-proliferative activity of lycopene on MCF7 mammary cancer cells [82]. However, in contrast to other data [79,71,83], the authors reported that neither the number of insulin-like growth factor-1 receptors (IGF-1R) nor their affinity was changed. Instead, they measured a higher amount of IGF-binding proteins, known to be negative regulators of IGF-1R activation [82].

Recent data from Palozza et al. [84] clearly demonstrated that lycopene affects Ras signalling in prostate and colon carcinoma cells by inhibiting the expression of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and thereby modulating the mevalonate pathway. The authors found that lycopene treatment prevented the membrane localization of Ras, likely through a lack of farnesylation. Consequently, this GTPase remained inactive and incapable of activating the MAPK cascade, which ultimately suppressed the Ras-dependent activation of NF-κB. The inhibition of NF-κB activation then resulted in cell cycle arrest in G1/S phase, with altered expression

levels of cyclin D1, p21, p27 and p53, and was followed by apoptosis [84]. Moreover, after co-treatment with mevalonate, lycopene lost its anti-proliferative activity. Together with the fact that lycopene showed stronger activity in cells expressing a mutated, cytosolic form of Ras, this supports the finding that lycopene acts via an alteration of the mevalonate pathway [84]. It is also noteworthy that modulating Ras signalling could explain the ability of lycopene to block ROS production, as a role for Ras proteins in redox regulation has been proposed [85–87]. By preventing the binding of the transcription factors NF-κB, stimulatory protein (Sp1) and, to a lesser extent, AP-1 to the regulatory sequences of the metalloproteinase (MMP-9) gene, lycopene further affected the transcription of MMP-9 [83]. As lycopene interferes with IGF-1 signalling, which is known to activate NF-κB and Sp1 [88,89], this suppression might, in part, explain the decreased binding ability of these transcription factors [83]. The mRNA and protein levels of MMP-9 were similarly affected by lycopene treatment, whereas gelatin zymography did not provide any evidence for a direct inhibition of MMP-9 enzymatic activity. As MMP-9 has been implicated in both tumour invasion and angiogenesis, its suppression explains the inhibitory effect of lycopene on SK-Hep-1 invasion [83]. Previous work has demonstrated that lycopene exhibits an anti-metastatic activity in a highly invasive hepatocarcinoma cell line by increasing the expression of the metastasis suppressor gene, *nm23-H1* [90]. Whereas an inverse correlation exists between the up-regulation of *nm23-H1* and MMP-2 expression [91], no connection has been found for MMP-9 thus far.

In addition, lycopene has been shown to exhibit a chemopreventive activity at the level of gap junctional communication (GJC). Gap junctions connect the cytosol of neighbouring cells to allow the exchange of signalling molecules and nutrients through channels formed by the assembly of connexin proteins [92]. Communication via these channels is important in the control of cell growth and the loss of GJC has been implicated in carcinogenesis [93,94]. Growing evidence indicates that lycopene inhibits the cell growth of chemically transformed cells by inducing GJC [95]. Stahl et al. [96] demonstrated by a dye transfer method that in human foetal skin fibroblasts, lycopene significantly enhanced GJC after 1 and 3 days of treatment. In the same study, acyclo-retinoic acid exhibited a similar effect only at much higher concentrations, indicating that this metabolite may not account for the GJC-inducible activity of lycopene *in vivo*. In contrast to retinoic acid, a cleavage product from β-carotene, acyclo-retinoic acid, mediated the stabilization of the connexin 43 (Cx43) mRNA only at high concentrations, whereas lycopene itself had no significant effect [96]. In contrast, Cx43 expression was up-regulated in prostate cancer patients upon a high lycopene intake [97]. This is in agreement with the findings from Livny et al. [98], which showed a significant increase in the transcription and expression of Cx43 in KB-1 oral epidermoid cancer cells following lycopene treatment.

A genome-wide microchip array study substantiated the modulation of these different pathways by lycopene treatment [99]. The study was performed on oestrogen-positive (MCF-7) and oestrogen-negative (MDA-MB-231) breast cancer cells, as well as on a fibrocystic breast cell line (MCF-10A) after 48 h of treatment with 10 μM lycopene. Depending on the oestrogen receptor state, 391 genes were differentially expressed, whereas 726 genes showed modified expression between the breast cancer cells and the fibrocystic cell line. These genes were assigned to 34 pathways. In summary, the apoptosis-related genes of the PI3K/Akt pathway, as well as MAPK-related genes, were up-regulated, similar to genes involved in cell cycle control, especially those involved in the G1/S transition. Other pathways that were affected include xenobiotic metabolism, fatty acid biosynthesis and GJC [99]. This result is in agreement with a previous study in which the transcriptional profiles of 202 *BRCA1/2* interacting genes were analysed. Most of the 56 genes affected possess a role in apoptosis, cell cycle or MAPK signalling [100]. Figure 1 summarizes schematically the different modes of action of lycopene and mentions its main cellular targets. A more detailed overview of the signalling pathways affected by lycopene is presented in Figure 2.

Co-treatments/synergistic effects

Many studies have focused on a possible chemopreventive effect of tomatoes. There is strong epidemiological evidence for an inverse correlation between a high consumption of tomatoes or processed tomato products and prostate cancer risk [101,102] and lycopene, which represents the most abundant carotenoid in tomatoes [103], may be primarily responsible for the observed positive effects. However, even if lycopene alone exerts anti-tumour activity against different types of cancer [80,82,98,104–107], it might act more efficiently in combination with other bioactive phytochemicals present in the whole tomato extract—or in the diet in general—that might target different steps during carcinogenesis. In this respect, Ettorre et al. [108] found that a lycopene phytocomplex, but not pure lycopene, induced apoptosis in HL-60 cells. Similarly, Boileau et al. [109] demonstrated a chemopreventive activity of tomato powder on prostate carcinogenesis in male rats, whereas no significant effect was observed for lycopene beadlets. However, this finding was not confirmed by a more recent pre-clinical study on TRAMP mice, in which a lycopene-beadlet diet significantly prevented prostate carcinogenesis and no difference was observed with a tomato-paste

diet [110]. Instead, the authors found a higher ratio of *cis* to *trans*-lycopene in the serum of mice fed with lycopene beadlets. To clarify the possible positive or negative effects of combinational treatments, studies aiming at the potential synergistic effects of lycopene with other natural anti-cancer compounds are of growing importance. In this respect, data from Canene-Adams [111] indicate that tomato powder is more effective in reducing the net weight of prostate tumours in the Dunning R3327-H prostate adenocarcinoma model of Copenhagen rats than lycopene alone, which only led to an insignificant decrease of tumour weight. An even higher decline was achieved with a diet containing both tomato and broccoli powders (52% loss of tumour weight compared to a 34% or 18% decrease in rats fed with tomato powder or lycopene, respectively) [111]. Amir et al. [78] demonstrated that the anti-proliferative and differentiating activities of lycopene on HL-60 pro-myelocytic leukaemia cells were amplified in a synergistic manner when 1,25-dihyroxyvitamin D3 was simultaneously administered at low concentrations. Stahl et al. [112] compared the antioxidant effects of different carotenoid mixtures on the lipid peroxidation of multilamellar liposomes induced by 2,2'-Azo-bis(2,4-dimethylvaleronitrile) (AMVN). Their data indicated that, among the tested carotenoids, lycopene was the most effective in preventing TBARS formation. The synergistic effects of most carotenoid mixtures were observed, with the combination of lycopene and lutein showing the greatest synergy. From this finding it was concluded that combinations of different carotenoids inhibit oxidative damage more effectively than the pure compounds and the authors hypothesized that their differential membrane localization and/or physicochemical properties might account for their improved antioxidant activity [112]. Moreover, other studies have demonstrated that the combined administration of tomato and garlic exerts anti-carcinogenic and pro-apoptotic effects in hamster buccal pouch carcinogenesis [11,113]. However, these studies did not differentiate between the combined and single treatments, nor did they test the isolated compounds. However, another study on Wistar rats revealed that both S-allylcysteine (SAC) from garlic and lycopene were efficient in preventing the development of N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) and saturated sodium chloride (S-NaCl)-induced squamous cell carcinomas of the stomach [114,115]. Although the treatment with each natural compound alone led to a significant suppression of gastric carcinogenesis by modulating the cellular redox state and inducing apoptosis, the efficacy was notably increased when both of the compounds were administered in combination [114,115]. Vaishampayan et al. [116] compared the influence of lycopene on serum prostate-specific antigen (PSA) in patients suffering from prostate cancer with the impact of a combined administration of lycopene with soy isoflavones. In this case, the authors found that the lycopene administration alone stabilized the serum PSA level in 95% of the evaluable patients, whereas no additive effect was observed after the combined administration of lycopene plus soy isoflavones. Only 67% of patients in this group achieved a stabilization of their disease.

In addition to these studies comparing the anti-carcinogenic properties of tomatoes and isolated lycopene with other phytonutrients, there are additional reports that have analysed the antioxidant or chemopreventive effects of lycopene in combination with cellular antioxidants. The hormone melatonin, produced by the human pineal gland and some special cell types such as bone marrow cells [117,118], is known to exhibit an antioxidant activity. It protects proteins and lipids from oxidation and prevents damage of the nuclear and mitochondrial DNA by acting as a direct scavenger of harmful free radicals, such as peroxynitrite anion [119–121]. Moreover, melatonin exerts its antioxidant activity indirectly by inducing enzymes of the cellular antioxidant defense system and by inhibiting iNOS and LOX [122–125]. Moselhy and Al mslmani [126] investigated the chemopreventive potential of lycopene and melatonin on 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary tumours in female rats. They reported that melatonin enhanced the ability of lycopene to decrease the levels of both malondialdehyde (MDA), a marker of lipid peroxidation, and nitric oxide in the serum and breast tissues of DMBA-treated Sprague Dawely rats. Similarly, the co-administration of melatonin significantly increased the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in this tissue. Compared to the lycopene supplementation, which protected 66.5% of the DMBA-injected rats from carcinogenesis, the co-administration with melatonin protected 80% of the animals [126]. In another study, Wang et al. [127] tested coenzyme Q10 (CQ) on rats in combination with different micronutrients, including the two carotenoids lycopene and lutein. CQ is a component of the electron transport chain, primarily functions in ATP generation and represents an important antioxidant in its reduced form. A diet supplement of 10 mg/(kgxd) CQ resulted in increased levels of the enzymes involved in the cellular antioxidant defense system, such as SOD and GPx, in the plasma and liver of rats. After the combined treatment with CQ and lycopene or lutein, these levels were increased further. In parallel, MDA levels and DNA damage were diminished.

Additionally, food processing leads to multiple chemical transformations of the food components. During heating and dehydration, the so-called Maillard reaction occurs and leads to the generation of ketosamines, which are carbohydrate derivatives

Table I. Lycopene derivatives: their occurrence and bioactivity *in vivo*.

Lycopene derivative	Synthetic generation	Detected *in vivo*	Bioactivity	References
apo-6'-lycopenal	Solubilization in toluene,	in human plasma		[143–145]
apo-8'-lycopenal	aqueous Tween 40 or liposomal suspension	in rat liver		[143–146]
		in human plasma		
apo-10'-lycopenal	Oxidation with $KMnO_4$	not detectable in rat liver	• Transactivation of EpRE/ARE	[59,61,143–146]
		in human plasma	• Induction of phase II detoxifying enzymes	
apo-12'-lycopenal		in rat liver		[143–147]
		in human plasma		
apo-14'-lycopenal		in human plasma		[143–145]
apo-15'-lycopenal = acycloretinal		not detectable in human plasma		
		in pig liver		
apo-7-lycopenal	Oxidation with $KMnO_4$			[145]
apo-11-lycopenal	Solubilization in toluene, aqueous Tween 40 or liposomal suspension; Oxidation with $KMnO_4$			[143,145]
apo-5-lycopenone	Oxidation with $KMnO_4$			[145]
apo-9-lycopenone				
apo-13-lycopenone	Solubilization in toluene, aqueous Tween 40 or liposomal suspension; Oxidation with $KMnO_4$			[143,145]
apo-10'-lycopenoic acid		in ferret lung tissue	• Transactivation of EpRE/ARE • Induction of phase II detoxifying enzymes • Growth inhibition of human lung cancer cells *in vitro* • Inhibition of cell cycle progress in G1 phase with reduction of cyclin E and increase of p21 and p27 • *In vivo* suppression of lung carcinogenesis in A/J mice	[59,61,142]
apo-10'-lycopenol		in ferret lung tissue	• Induction of phase II detoxifying enzymes	[59,136]
acyclo retinoic acid			• Induction of apoptosis in prostate cancer cells (PC-3 and DU145, but not LNCaP) • Inhibition of cell proliferation of MCF-7 cells • Inhibition of cell cycle progression from G1 to S with reduction of cyclin D1 and increase of p21 • Weak GJC stimulation	[96,139,140,143]
apo-6,6'-carotenedial	Oxidation with $KMnO_4$			[61,145]
apo-6,10'-carotenedial			• Transactivation of EpRE/ARE	
apo-8,8'-carotenedial			• Modest effect on EpRE/ARE	
apo-6,12'-carotenedial	Oxidation with $KMnO_4$, H_2O_2 and osmium tetroxide		• Stimulation of GJC	[61,141,145]

(Continued)

Table I. *(Continued)*.

Lycopene derivative	Synthetic generation	Detected *in vivo*	Bioactivity	References
apo-8,12'-carotenedial	Oxidation with $KMnO_4$		• Transactivation of EpRE/ARE • Growth inhibition of MCF-7, T47D and LNCaP cells	[61,145]
apo-10,10'-carotenedial			• Transactivation of EpRE/ARE • Induction of NQO1 expression • Growth inhibition of MCF-7, T47D LNCaP cells	[61]
apo-8,15-carotenedial			• Transactivation of EpRE/ARE	
(E,E,E)-4methyl-8-oxo-2,4,6-nontrienal (MON)			• Induction of apoptosis in HL-60 cells	[138]
2,6-cyclolycopene-1,5-epoxide	Epoxidation by MCPBA (=m-chloroperbenzoic acid)			[147]
lycopene-1,2-epoxide				
lycopene-5,6,5',6'-diepoxide	Incubation of [D]-lycopene with the postmitochondrial fraction of rat intestinal mucosa and soybean lipoxygenase			[148]
2-apo-5,8-lycopenal-furanoxide				
3-keto-apo-13-lycopenone				
3,4-dehydro-5,6-dihydro-15,15'-apo-lycopenal				
lycopene-5,8-furanoxide isomer				
lycopene-5,8-epoxide isomer				
3-keto-lycopene-5',8'-furanoxide				
2,6-cyclolycopene-1,5-diols	Oxidation with $KMnO_4$	in human breast milk + serum		[147,149]
5,6-dihydroxy-5,6-dihydrolycopene		in human serum		[150,151]
5,6-dihydrolycopene		in preruminant calf serum		[152]
undefined tetrahydrolycopene				

containing an amino group [128]. Mossine et al. [128] hypothesized that such compounds, present in dehydrated tomato products, might interact synergistically with the properties of lycopene to suppress rat prostate tumorigenesis. They observed the highest survival of Wistar-Unilever rats with *N*-nitroso-*N*-methylurea- and testosterone-induced prostate carcinogenesis after feeding them a tomato paste/FruHis diet. Compared to rats fed with tomato paste or tomato powder alone, the combination of tomato paste with the ketosamine FruHis was even more effective in preventing the death of the rats carrying macroscopic prostate tumours. The authors further demonstrated that FruHis might act via an antioxidant activity, as this compound avoided oxidative DNA degradation *in vitro*, whereas ascorbate and phenolic antioxidants from tomato failed [128].

Lycopene derivatives, metabolites and oxidation products

Due to its highly unsaturated structure, lycopene can easily be oxidized. Thus, bioactive lycopene metabolites might explain the beneficial activity of tomato extracts compared to purified lycopene. In fact, it has been hypothesized that the oxidation products of lycopene might, at least in part, be responsible for its ability to induce the expression of phase II enzymes [61]. Many studies have focused on the ability of lycopene to prevent prostate carcinogenesis. The finding that prostate tissue primarily contains lycopene *cis*-isomers, as well as polar lycopene derivatives, suggests that lycopene is metabolized/oxidized in this tissue [129,130]. Carotene-15,15'-oxygenase (CMO I), the main carotenoid cleavage enzyme in mammals, was excluded as a candidate enzyme for lycopene cleavage due to the results of different studies indicating that lycopene is a poor substrate for this enzyme [131,132]. Moreover, the theoretical cleavage products resulting from CMO I activity (i.e. acycloretinal and acycloretinoic acid) show much less bioactivity than lycopene and are therefore unlikely to account for the chemopreventive effects of lycopene [133,134]. In contrast, carotene-9',10'-oxygenase (CMO II) was identified as a key enzyme mediating lycopene cleavage *in vivo* [135]. Furthermore, Hu [136] demonstrated that, indeed, the *cis*-isomers are the favoured substrates of this enzyme and that transfection with CMO II was able to amplify the *in vivo* activity of lycopene [61].

Lindshield et al. [134] presented a comprehensive overview of the metabolic products of lycopene, including apo-lycopenals, apo-carotenedials, apo-lycopenones and epoxides. Bioactive properties have been reported for some of these metabolic products. For example, allowing cells to metabolize lycopene *in vitro* during a period of 3 days without changing the lycopene-supplemented culture medium significantly enhanced its anti-proliferative activity against connexin 43 wild-type mouse embryonic fibroblasts [134]. Moreover, a study by Nara et al. [137] revealed that a mixture of lycopene oxidation products, obtained by incubation with toluene, displayed an enhanced ability to inhibit the growth of HL-60 leukaemia cells via apoptosis induction compared to native lycopene. Similarly, Zhang et al. [138] reported that

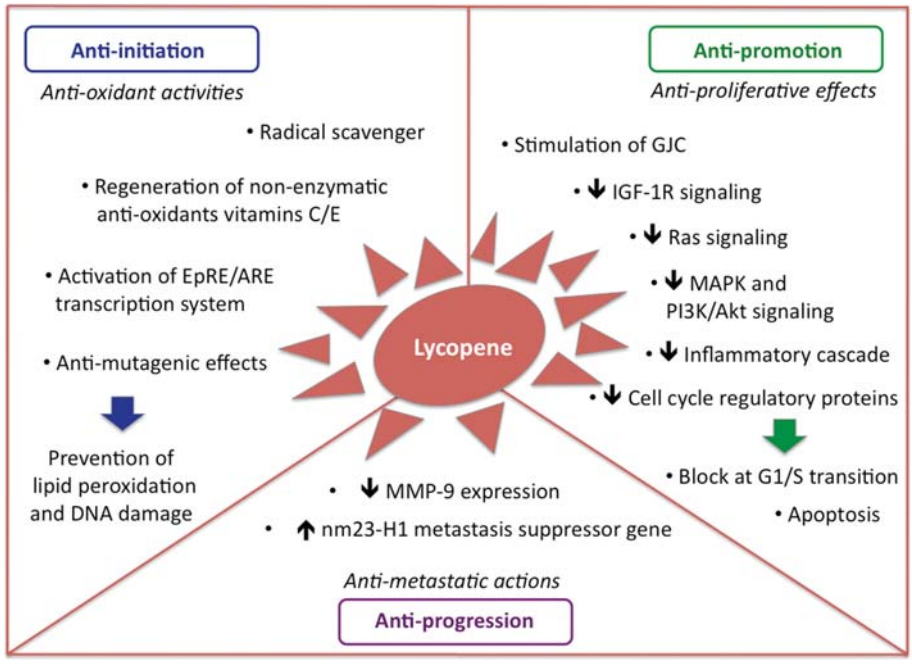

Figure 1. Schematic overview of the anti-oxidant, anti-proliferative and anti-metastatic activities of lycopene and its cellular targets as well as signalling pathways affected by this compound.

(E,E,E)-4-methyl-8-oxo-2,4,6-nonatrienal (MON), an auto-oxidation product of lycopene, triggers apoptosis in HL-60 leukaemic cells via caspase-dependent pathways. Acyclo-retinoic acid has similarly been reported to be a lycopene metabolite with potent anti-proliferative and pro-apoptotic activity against breast and prostate cancer cells [139,140]. It is known that one mechanism by which lycopene acts anti-proliferatively is by stimulating GJC [95]. Aust et al. [141] identified 2,7,11-trimethyltetradecahexaene-1,14-dial (apo-6,12'-carotenedial) as the bioactive oxidation product of lycopene that enhanced GJC in rat liver epithelial WB-F344 cells. In the literature, apo-10'-lycopenoic acid has been described to act anti-proliferatively *in vitro* and to suppress lung carcinogenesis *in vivo* [61,142]. Recently, a more detailed study by Lian and Wang [59] revealed that this metabolite, as well as apo-10'-lycopenal and a po-10'-lycopenol, led to the induction of phase II detoxifying enzymes via the nuclear accumulation and activation of the transcription factor Nrf2 in BEAS-2B human bronchial epithelial cells. As mentioned above, Linnewiel et al. [61] recently provided evidence that hydrophilic derivatives, including apo-10'-lycopenoic acid and apo-carotenedials, rather than lycopene itself, mediated the activation of the EpRE/ARE transcription system. Moreover, the authors presented some structure-activity rules of carotenoid derivatives for the induction of the EpRE/ARE system. In summary, their data showed that the aldehyde derivatives (apo-carotenals and, in particular, diapo-carotenedials) represented the active compounds and had a greater activity than their corresponding acids [61]. The activity was found to be further influenced by the position of the first methyl group relative to the terminal aldehyde group, as well as by the number of carbon atoms in the backbone chain. The most active derivatives, a group including apo-6,14'-carotenedial, carry the methyl group at a distant position, and their backbone optimally consists of 12 carbon atoms. From these observations, the authors suggested that such oxidized lycopene derivatives transactivate Nrf2 via the Michael addition of a double bond adjacent to the terminal aldehyde group to the SH-groups in the Nrf2-inhibitor, Keap1, and that the interaction

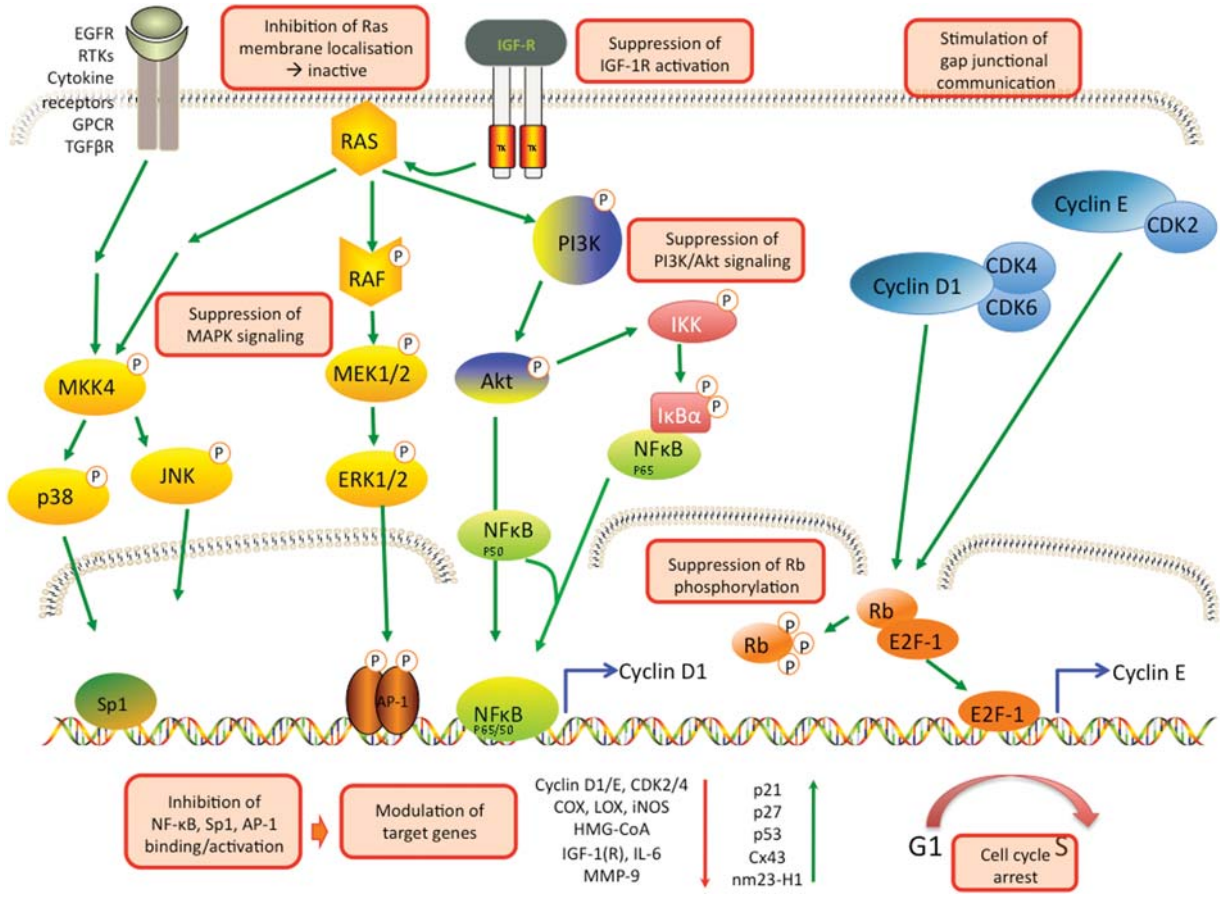

Figure 2. Detailed scheme of the signalling pathways targeted by lycopene in cancer cells. Lycopene strongly inhibits IGF-1R and Ras signalling but also affects downstream signalling via the MAPK (ERK, JNK, p38) and PI3K/Akt. Transcription factors like NF-κB, AP-1 and Sp1 consequently cannot be activated, thus modulating target gene expression. As most of the target genes are implicated in cell cycle regulation and proliferation, the cancer cells are finally blocked in cell cycle progression from G1 to S phase. Likewise the inflammatory cascade is repressed. Moreover, lycopene exhibits its anti-proliferative activity by stimulating gap junctional communication. Red frames indicate lycopene's points of action. This figure was created with the ScienceSlide software.

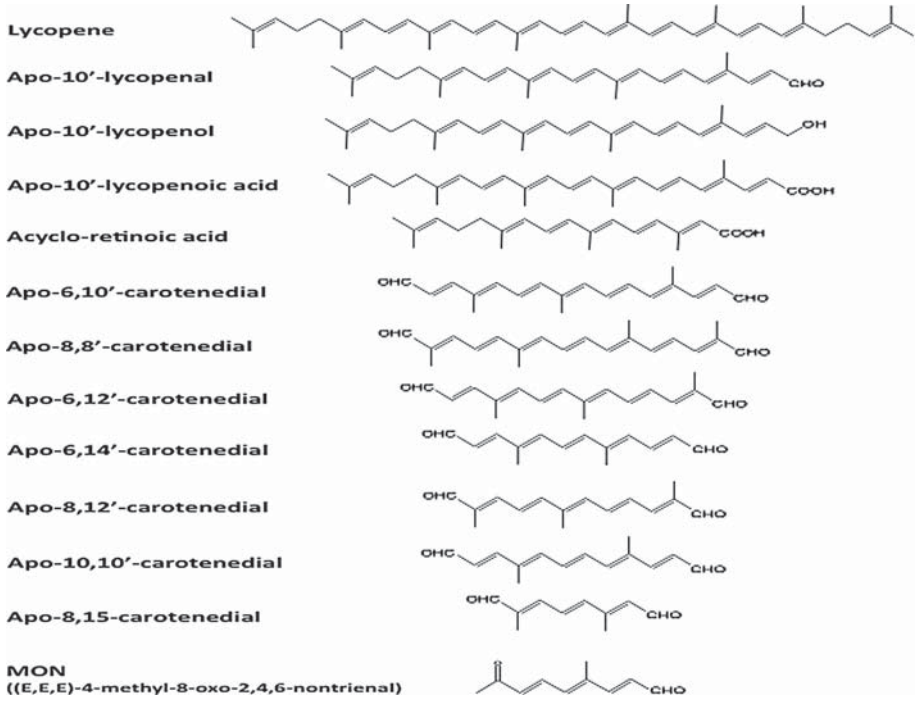

Figure 3. Chemical structures of lycopene and its bioactive derivatives/metabolites.

with the Keap1 dimer was most efficient with a 12-C-derivative [61]. Table I provides a list of the identified lycopene metabolites and summarizes the current knowledge about their occurrence *in vivo*, as well as their bioactivity, if known. Chemical structures of the described bioactive lycopene derivatives are presented in Figure 3.

Conclusion

Lycopene, a tomato-derived carotenoid lacking provitamin A activity, represents a natural compound that is ubiquitous in the diet of humans all over the world. Numerous epidemiological studies have proposed an inverse correlation between a high consumption of tomatoes and a risk of cancer. Therefore, during the last decade, many efforts have been made to uncover the role of lycopene in the anti-cancer effects of tomato products and to decipher the underlying molecular mechanisms responsible for the remarkable chemopreventive activity of this antioxidant. In this review, we summarized the current knowledge about the mode of action of lycopene as an antioxidant that prevents the cellular damage of lipid membranes and DNA, activates phase II detoxifying enzymes at the transcriptional level, modulates the cell cycle and induces apoptosis in cancer cells. By considering the relevant literature, we attempted to provide an overview of the implicated pathways and how their disturbance causes the inhibition of cancer cell growth (see Figure 1). Perhaps the most important target of lycopene is IGF-1 receptor signalling, as interfering with this pathway affects downstream Ras/MAPK and PI3K/Akt signalling and ultimately blocks cell cycle progression and leads to apoptosis. Moreover, lycopene counteracts the loss of GJC during carcinogenesis. We further illustrated how lycopene acts in an additive fashion and even synergistically in combination with some other natural antioxidants and suggest that future investigations in this field could help validate the benefit of such synergistic combinations in forthcoming chemotherapeutical applications of lycopene. Based on the data presented here, it is clear that at least a part of the anti-cancer activity of lycopene is mediated by its derivatives, which are generated *in vivo* through oxidation or cleavage. In this respect, the dialdehydes represent the most active metabolites, which function in the transcriptional activation of the EpRE/ARE system and GJC. As lycopene acts at different steps of carcinogenesis without exerting side-effects and has been shown to have anti-metastatic activity, this compound is a very promising candidate for chemoprevention and cancer treatment.

Declaration of interest

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