REVIEW

Carotenoids and apocarotenoids in cellular signaling related to cancer: A review

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The basis for the vivid color of carotenoids and their antioxidant activity is the multiple conjugated double bonds, which are characteristic for these phytonutrients. Moreover, the cleavage of these oxidation-prone double bonds leads to the formation of apocarotenoids. A large number of carbonyl-containing oxidation products are expected to be produced as a result of carotenoid oxidation and these can be further metabolized into the corresponding acids and alcohols. As discussed in this review, many, but not all, of these potential products have been detected and identified in plants as well as in human and animal plasma and tissues. Some of these compounds were found to be biologically active as anticancer agents. In addition to the inhibition of cancer cell proliferation, several carotenoid metabolites were shown to modulate the activity of various transcription systems. These include ligand-activated nuclear receptors, such as the retinoic acid receptor, retinoid X receptor, peroxisome proliferator-activated receptor and estrogen receptor, as well as other transcription systems that have an important role in cancer, such as the electrophile/antioxidant response element pathway and nuclear factor-κB. Therefore, apocarotenoids can be considered as natural compounds with multifunctional, rather than monofunctional, activity and, thus, can be useful in the prevention of cancer and other degenerative diseases.

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1 Introduction

Carotenoids are lipophilic plant pigments typically containing a series of conjugated double bonds, which makes them susceptible to oxidative cleavage. For example, lycopene contains 11 conjugated double bonds, which are accessible to oxidative cleavage. Indeed, arrays of apolycopenals, the products of such oxidation, were found in tomatoes, tomato products and in mammalian tissues. This suggests that apolycopenals and other carotenoid oxidation products have biological roles and therefore studying their biological effects is of great importance and is a major subject of this review. In addition, we will address the formation of carotenoid cleavage products by chemical and enzymatic oxidation and their presence in eatable plants and in animal plasma and tissues.

The most studied and known carotenoid derivatives are those related to vitamin A. Retinal or related compounds, such as 3-hydroxyretinal, serve as the chromophores of various visual pigments (rhodopsins) throughout the animal kingdom. Retinal oxidation by aldehyde dehydrogenase leads to the formation of retinoic acid, a nuclear receptor ligand that is a major signal controlling a wide range of transcriptional processes. In humans, four carotenoids

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Abbreviations: **BCDO2,** β-carotene 9′-10′-dioxygenase; **BCMO1,** β,β-carotene-15,15′ monooxygenase; **EpRE/ARE,** electrophile/antioxidant response element; **IκB,** inhibitor of NF-κB; **LXR,** liver X receptor; **NFκB,** nuclear factor-κB; **Nrf2,** nuclear factor E2-related factor 2; **PPAR,** peroxisome proliferator-activated receptor; **RAR,** retinoic acid receptor; **RXR,** retinoid X receptor

(β-carotene, α-carotene, γ-carotene and β-cryptoxanthin) are pro-vitamin A compounds (meaning they can be converted to retinol). As these derivatives were the focus of many excellent reviews (for example, see [1, 2]) they will not be discussed here any further.

2 Formation of carotenoid oxidation products

Cleavage and oxidation of carotenoids can occur by chemical reactions in plants or in foods containing carotenoids. Alternatively, intact carotenoids, which are absorbed into the body, can be converted enzymatically into these derivatives. The various routes for carotenoid oxidation and the resulting derivatives are discussed below.

Two different nomenclatures can be used for the oxidized carotenoid derivatives. One is the generic chemical nomenclature that describes the carbon chain and its substitutions. The second is based on the numbering of the 30 carbons in the backbone of the major dietary carotenoids, as exemplified below for the structural formula of lycopene. In this nomenclature, the number of the oxidized carbon is given together with the nature of the oxidized group, namely, aldehyde (-al), ketone (-one) or acid. For example, the derivative resulted from oxidation of lycopene to a dialdehyde at the 5–6 and 13′–14′ double bonds is termed diapocarotene-6–14′-dial (Fig. 1). In the same way, a monoaldehyde formed at carbon 10′ is termed apo-10′-lycopenal, and a similar derivative from β-carotene is termed β-apo-10′-carotenal. This nomenclature will be used throughout the review.

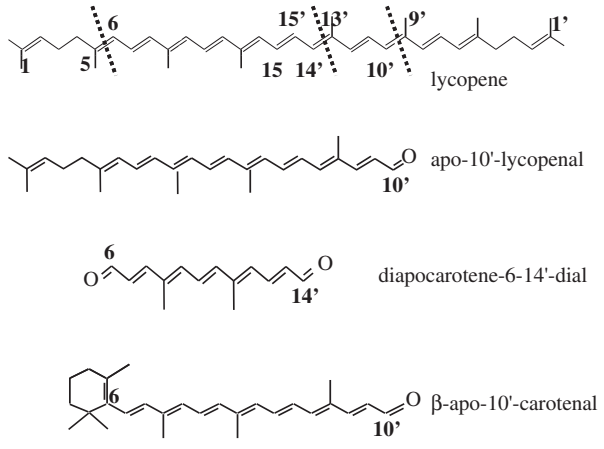

Figure 1. Structural formulae of lycopene and representative apocarotenals. The formulae depict part of the carbon numbers to highlight the positions of the double bonds that are cleaved (dashed lines) in the formation of the indicated lycopenals. The numbering is from 1 to 15 up to the center of the molecule and then from 15′ to 1′ toward its opposite end.

2.1 Chemical cleavage of carotenoids

To study the biological activities of carotenoid oxidation products, they were produced by various chemical reactions. Two different methods of lycopene oxidation were used by Caris-Veyrat and colleagues and yielded a myriad of degradation products [3]. One method used potassium permanganate and the other atmospheric oxygen in the presence of metalloporphyrin, which served as a catalyst. Oxidation of lycopene with potassium permanganate produced 11 apolycopenals/ones and 6 diapocarotene-dials [3]. The latter products are of special interest as these diapocarotene-dials were found to have biological activities (see Section 4). The diapocarotene-dials are formed from the cleavage of two carbon–carbon double bonds. It is interesting that only the double bonds 5–6 (or 5′–6′), 7–8 (or 7′–8′), 9–10 (or 9′–10′) and 11–12 (or 11′–12′) were cleaved. Diapocarotene-dials that may result from the cleavage of the double bonds located either at the center of the molecule (15–15′ double bond) or closer to its end (i.e. the 1–2 double bond) were not detected. Surprisingly, the expected diapocarotene-10–10′-dial was not detected as well. Oxidation of lycopene by atmospheric oxygen catalyzed by a metalloporphyrin (a model system of the active center of cytochrome P450 enzymes) resulted in isomerization to *cis*-isomers and then in the addition of oxygen to double bonds and their cleavage to a series of apolycopenals. No diapocarotene-dial products, which can result from a double oxidative cleavage, were detected over the 96-h period, although almost all the apolycopenals/ones that were obtained by oxidation of lycopene with potassium permanganate were detected. Similarly, production of apolycopenal (apo-6-lycopenal) without diapocarotene-dials was obtained also by another oxidation method of lycopene using full spectrum light irradiation (incandescent tungsten lamp) in an atmosphere of oxygen and in the presence of a sensitizer (methylene blue [4]). In contrast, a dialdehyde derivative was obtained by complete oxidation of lycopene with hydrogen peroxide/osmium tetroxide [5]. This dialdehyde was identified as 2,7,11 trimethyltetradecahexaene-1,14-dial (diapocarotene-6-12′-dial) and was shown to have a biological activity (see Section 4.1). It could be formed by a fragmentation at the 5,6 and 12′,11′ positions. These positions are preferred targets of oxidants within the lycopene molecule since apo-12′-lycopenal, apo-6′-lycopenal and the 5,6-epoxides have been identified as decomposition products of lycopene [6].

Nara et al. [7] have reported that the tomato acyclic carotenoids lycopene, phytoene, phytofluene and ζ-carotene are unstable when incubated in regular tissue culture medium. These carotenoids were completely lost within 5 h of incubation. On the other hand, β-carotene was stable when incubated in similar conditions. Oxidation mixtures of phytoene, phytofluene, ζ-carotene and lycopene were prepared by incubation at 37°C in toluene for 24 h [7] and were found to inhibit cell growth stronger than each of the intact carotenoids alone. The potentially active compounds

from the oxidation mixtures of the acyclic carotenoids were not isolated. However, Kim et al. [6] identified acyclo-retinal, apolycopenals and acyclo-retinoic acid as products of autoxidation of lycopene in toluene. The identified apolycopenals correspond to the products formed by cleavage of the respective 11 conjugated double bonds of lycopene and include apo-14′-lycopenal, apo-12′-lycopenal, apo-10′-lycopenal, apo-8′-lycopenal and apo-6′-lycopenal, which are similar to those obtained from lycopene by the other oxidation methods discussed above. However, it was not mentioned whether diapolycopene-dials were generated by oxidation in toluene. Autoxidation of β-carotene [8] resulted in apocarotenals that were similar to the apolycopenals identified by Kim et al. [6], except for a compound that cannot be obtained by the cleavage between carbons 5′ and 6′ because these carbons are part of the β-ionone ring of β-carotene. In another study, the same group reported that autoxidation of lycopene at positions 5,6 and 13,14 produced a diapolycopene-dial – (E,E,E)-4-methyl-8-oxo-2,4,6-nonatrienal (diapocarotene-6-al,13-one) [9]. To induce this autoxidation, purified lycopene was treated with toluene or ozone. Interestingly, only few carotenoids other than lycopene, such as neurosporene and γ-carotene, have the potential structure to produce this oxidation product, which was found to cause apoptosis of leukemic cells (see Section 4.1).

2.2 Enzymatic cleavage of carotenoids

In mammals, including humans, two homologous genes exist that encode enzymes responsible for carotenoid oxidative cleavage: β,β-carotene-15,15′ monooxygenase (BCMO1) catalyzes retinal production from pro-vitamin A carotenoids, while a second carotenoid-oxygenase, β-carotene 9′-10′-dioxygenase (BCDO2), catalyzes asymmetric cleavage of carotenoids to apocarotenoids.

Enzymatic central cleavage of carotenoid molecules is a major pathway leading to vitamin A formation. The enzyme BCMO1 was cloned and was shown to cleave β-carotene at the 15,15′-double bond [10, 11]. The enzyme localizes to the cytoplasm and converts limited types of carotenoids into retinoids [12]. Studies in knockout mice showed that BCMO1 is a major enzyme for vitamin A production as its deficiency abolishes vitamin A generation from β-carotene and alters lipid metabolism in mice [2, 13, 14]. Recombinant human BCMO1 catalyzes the cleavage of pro-vitamin A carotenoids with at least one nonsubstituted β-ionone ring, such as β-carotene, α-carotene and β-cryptoxanthin, but fails to promote cleavage of nonprovitamin A carotenoids such as lycopene or zeaxanthin [12].

Besides the symmetric cleavage, an asymmetric cleavage of β-carotene is carried by the other carotenoid-oxygenase, BCDO2. This enzyme has been cloned from human, mouse, zebrafish and ferret sources, and recombinant ferret and mouse BCDO2 have been biochemically characterized [15, 16]. Recombinant ferret BCDO2 catalyzed the formation of β-apo-10′-carotenal and β-ionone [15], suggesting that BCDO2 is a carotenoid 9′-10′ oxygenase. Besides β-carotene, the acyclic carotenoid lycopene is also a substrate for this enzyme. The ferret BCDO2 cleaved 5-*cis*- and 13-*cis*-isomers of lycopene but not the all-*trans* stereoisomer [15]. The physiological function of BCDO2 is less well understood. Such eccentric cleavage has been suggested as an alternative route for vitamin A production. However, BCMO1 knockout mice become vitamin A deficient despite the expression of BCDO2, suggesting a different physiological function of the latter enzyme [14]. Indeed, BCDO2 was shown recently to be localized in the mitochondria and to have broad substrate specificity, including lycopene and xanthophylls such as lutein [17]. In BCDO2-deficient mice, carotenoid metabolism was abrogated leading to deranged carotenoid homeostasis and their accumulation in several tissues with remarkable consequences [17]. For example, in hepatic mitochondria, the accumulated carotenoids reduced rates of ADP-dependent respiration. This was associated with induction of various markers for oxidative stress. Mammalian cells thus express a mitochondrial carotenoid-oxygenase that degrades carotenoids to protect these vital organelles. In addition to these mitochondrial functions, several publications have shown that various apocarotenals, which can be produced by BCDO2, modulate major cellular processes (see Section 4).

The role of lycopene *cis*-isomers in apolycopenal production is of special interest. All-*trans* lycopene supplementation in ferrets and humans [18] resulted in a significant accumulation of *cis*-isomers of lycopene. Hu et al. [15] demonstrated that the recombinant ferret BCDO2 catalyzes the eccentric cleavage of both all-*trans* β-carotene and the 5-*cis* and 13-*cis* isomers of lycopene at the 9′,10′ double bond but not all-*trans* lycopene. The cleavage activity of ferret BCDO2 was higher toward lycopene *cis*-isomers as the substrate than toward β-carotene. In addition, in vitro incubation of apo-10′-lycopenal with the post-nuclear fraction of hepatic homogenates of ferrets resulted in the production of both apo-10′-lycopenoic acid and apo-10′-lycopenol. These authors suggested that the bioconversion of *cis*-isomers of lycopene into apo-10′-lycopenoids by BCDO2 is substantial because these are the predominant isomeric form of lycopene in mammalian tissues and apolycopenoids may have specific biological activities related to human health.

3 The presence of carotenoids oxidation products in foods, and in mammalian plasma and tissues

Carotenoid oxidation products, apo-6′- and apo-8′-lycopenal, were reported to be present in raw tomatoes already in the early 1970s [19]. Recently, several other apolycopenals were found in both raw and processed foods as well as in human plasma, including apo-10′-, apo-12′-, apo-14′- and apo-15′-

lycopenal [20]. Some of these metabolites were found in individual's plasma who had consumed tomato juice for 8 wk. These include apo-6′-, apo-8′-, apo-10′-, apo-12′- and apo-14′-lycopenals, which were detected and quantified in all tested food products as well as in plasma. The concentration of these apolycopenals in foods is very low and amount to roughly 1000 times lower than that of lycopene. Similar relative concentrations were found in human plasma. Interestingly, apo-15′-lycopenal, which is equivalent to retinal, was not detected in the plasma of any of the seven subjects included in that study [20]. This is in agreement with the facts that this central cleavage product is not found in foods and that lycopene is not a good substrate for BCMO1, the central cleavage enzyme, and thus there is no valid source for apo-15′-lycopenal in the human body. As discussed above (Section 2.1), lycopene oxidation leads to the formation of diapocarotene-dials in addition to the mono-apolycopenals; however, such dialdehydes were not detected in animal and human tissues. The reason may stem from the high reactivity of the diapocarotene-dials in biological systems. For example, it was suggested that they are highly reactive toward SH groups present in various regulatory proteins [21] (see Section 4.2.2.1).

Although it has been shown that BCDO2 cleaves both β-carotene and lycopene at the single 9′–10′ double bond [15], an array of different derivatives was found in human and animal plasma and tissues [15, 20, 22] This suggests further metabolism of BCDO2 cleavage products. Feeding of lycopene to ferrets resulted in the detection of apo-10′-lycopenol in the lung tissue, which probably is the reduction product of the aldehyde apo-10′-lycopenal [15]. In addition, the liver of rats consuming a lycopene-containing diet for 30 days produced apo-8′-lycopenal and putative apo-12′-lycopenal [22]. It was suggested that the presence of apolycopenals in the plasma and tissues, either from the diet or as metabolic products of enzymatic cleavage, supports a hypothesis that these compounds may have bioactivity and potentially mediate some of the health-promoting beneficial effects proposed for tomato products [20, 22–24].

4 Biological activities of carotenoid oxidation products

Anticancer activity is probably the best characterized biological activity of carotenoid and their derivatives. This includes inhibition of cancer cell proliferation and induction of cell death by activation of apoptosis program. Another frequent effect is the modulation of the activity of various transcription factors, e.g. nuclear factor E2-related factor 2 (Nrf2), which activates the electrophile/antioxidant response element (EpRE/ARE), nuclear factor-κB (NF-κB), as well as nuclear receptors, such as the retinoic acid receptor (RAR), retinoid X receptor (RXR), peroxisome proliferator-activated receptor (PPAR) and estrogen receptor. These transcription factors regulate the expression of genes that are closely related to carcinogenesis and its prevention.

4.1 Inhibition of cancer cell proliferation

Nara et al. [7] have reported that oxidized derivatives produced by incubation of tomato carotenoids (lycopene, phytoene, phytofluene and ζ-carotene) with toluene inhibit the growth of HL-60 leukemia cells to a greater extent than each of the intact carotenoids. For example, lycopene at 10 μM showed a slight inhibitory effect, whereas a remarkable growth inhibition was caused by the oxidation mixture of lycopene containing various apolycopenals, as described above (Section 2.1). The authors suggested that the inhibition of cell growth by acyclic carotenoids is mediated by their oxidation products through the induction of apoptosis [7]. This was further studied by Zhang et al. [9] who found that a specific diapocarotenoid (diapocarotene-6-al,13-one) produced by autoxidation of lycopene with toluene or ozone (see Section 2.1) induced apoptosis in HL-60 leukemia cells. In addition to the identified diapocarotenoid, other, not identified, derivatives were also produced during lycopene autoxidation, and it is remarkable that the mixture of lycopene oxidation products was more active than the isolated derivative alone, indicating the presence of other unidentified active oxidation products in that mixture.

We have determined the effects of synthetic diapocarotene-dials on the growth of estrogen-dependent breast (MCF-7, T47D) and androgen-dependent prostate (LNCaP) cancer cells [21]. These compounds, diapocarotene-10–10′-dial, diapocarotene-8–12′-dial, diapocarotene-8–8′-dial and diapocarotene-12–12′-dial, can potentially be derived from double oxidative cleavage of lycopene. We observed similar relative potencies of these four derivatives to inhibit cancer cell growth and to induce the EpRE/ARE transcription system (see more details in Section 4.2.2.1). It is not clear whether there is a direct connection between the EpRE/ARE system and regulation of cell growth. However, these results suggest that in addition to activating EpRE/ARE (as discussed below) the carotenoid derivatives containing conjugated carbonyls can affect other cellular processes. Interestingly, acyclo-retinoic acid, a central cleavage product of lycopene, which is an open chain analog of retinoic acid, was also found to inhibit cancer cell proliferation [25, 26]. This putative lycopene metabolite was not detected yet in biological tissues and since its potency in inhibition of cancer cell growth was similar to that of lycopene [26], it is probably not an active metabolite of this carotenoid.

In a recent study, it was reported that treatment of androgen-independent DU145 prostate cancer cells by supra-physiological levels of lycopene and apo-12′-lycopenal reduced cell proliferation. However, it is not clear if the lycopenal's effect is physiologically important. The inhibition of cell growth was achieved through inhibition of the normal cell cycle progression but not by the induction of the

gap junction protein, connexin 43 [27]. In contrast, another study has shown that a lycopene oxidation product can induce gap junctions. This compound, diapocarotene-6–12′-dial, obtained by complete oxidation of lycopene with hydrogen peroxide/osmium tetroxide (see Section 2.1) was found to enhance cell-to-cell communication via induction of gap junctions [5]. Similarly, Bertram et al. [28] found that another type of oxidation product of lycopene, 2,6 cyclolycopene-1-5-diol, which was found in both tomato products and human serum, had somewhat greater activity than lycopene to induce connexin 43 in mouse and human cells. This activity was previously suggested to partially explain the anticancer activity of carotenoids [29]. It is interesting to note that although the up-regulation of connexin 43 by retinoids involves RAR transactivation, its induction by the carotenoids astaxanthin or lycopene is not RAR-dependent [30]. In contrast, connexin 43 induction by astaxanthin, but not by a specific retinoid, was inhibited by an antagonist of the nuclear receptor PPAR-γ, indicating separate molecular mechanisms of gap-junctions control by pro-vitamin A and non-pro-vitamin A carotenoids.

It may be concluded from the above studies that carotenoid oxidation products inhibit cancer cells growth to a greater extent than the intact carotenoids. The physiological significance of these findings and the mechanism of action of these carotenoid derivatives are only partially understood and should await further research.

4.2 Modulation of transcription

4.2.1 Nuclear receptors

Nuclear receptors which reside inside the cells are ligand-activated transcription factors. The hydrophobic nature of carotenoids and their oxidation derivatives makes them good candidates for interaction with nuclear receptors, which bind ligands that easily transverse the cell membrane. Indeed, carotenoid derivatives other than the known retinoids were shown to affect the activity of several nuclear receptor families such as RAR, RXR, PPAR and estrogen receptor. However, it is not yet clear if the observed changes in the transcriptional activity of these nuclear receptors are related to direct interaction of carotenoid derivatives with the relevant receptors as ligands (as suggested for RXR and PPARs; see Sections 4.2.1.1 and 4.2.1.2) or to their indirect effects, which may result from modulation of other cellular pathways (as suggested from our findings regarding estrogen receptor; see Section 4.2.1.3).

4.2.1.1 Retinoid receptors

Retinoid receptors are composed of two families of nuclear receptors, RAR and RXR. When a ligand binds to a member of the RAR family, the receptor hetero-dimerizes with a member of the RXR family and attaches to the retinoic acid response element in the promoter region of RAR target genes. RARβ, one of these target genes [31], has been suggested to be a tumor suppressor gene, playing a critical role in mediating the growth-inhibitory effects of retinoids in various cancer cells [32].

Acyclo-retinoic acid, the open chain analog of retinoic acid, was shown to activate RAR transcriptional activity and to inhibit cancer cell proliferation [25, 26]. These effects were compared with those of retinoic acid in human mammary cancer cell line (MCF-7) [26]. Acyclo-retinoic acid transactivated a reporter gene containing the retinoic acid response element with a 100-fold lower potency than retinoic acid suggesting that activation of RAR is not an important function of this derivative. In contrast to the results of the transactivation studies, acyclo-retinoic acid, retinoic acid and lycopene inhibited cell growth with a similar potency. These results show that RAR does not mediate the growth inhibitory effect of acyclo-retinoic acid.

A similar experimental approach was followed by Lian et al. [33]. Apo-10′-lycopenoic acid (an eccentric cleavage product of lycopene) was found to transactivate the promoter of the RARβ gene and to induce the expression of this receptor. Previously, it was demonstrated by the same group that lycopene can be converted to apo-10′-lycopenoids (including apo-10′-lycopenoic acid) in mammalian tissues both in vitro and in vivo [15]. Apo-10′-lycopenoic acid was also shown to inhibit the growth of several normal and malignant lung cell lines; however, some of these results were achieved at high concentrations of this molecule [33]. The transactivation of RAR by apo-10′-lycopenoic acid, although with a much lower potency as compared to all-*trans* retinoic acid, and the induction of expression of RARβ may provide at least partial explanation for the described growth inhibition of lung cancer cells [33]. These observations demonstrate that apo-10′-lycopenoic acid is a biologically active metabolite of lycopene and suggest that it is a potential chemopreventive agent against lung tumorigenesis.

Marsh et al. [34] have recently tested the hypothesis that apocarotenoids formed by asymmetrical cleavage of β-carotene are able to stimulate transcription by activating RARs. The effects of long- and short-chain apocarotenals and apocarotenoic acids such as β-apo-8′-carotenoic acid, β-apo-14′-carotenoic acid and β-apo-13-carotenone were examined on the transactivation of RARα and RARβ. None of these apocarotenoids showed significant effect when compared with all-*trans* retinoic acid. The results suggest that biological effects of these apocarotenoids are through mechanisms other than activation of RARα and RARβ. In a more recent study, the same group determined the effects of these eccentric cleavage products of β-carotene on RXRα signaling [35]. None of the products tested activated RXRα. However, β-apo-13-carotenone was found to antagonize the activation of RXRα by 9-*cis*-retinoic acid and was effective at concentrations as low as 1 nM. Molecular modeling studies revealed that β-apo-13-carotenone should be capable of acting as an RXRα antagonist. This prediction, together with

the low effective concentration of this specific apocarotenoid, suggests that it has a physiological role in RXR regulation.

4.2.1.2 PPARs and LXR (liver X receptor)

PPARs are a subfamily of nuclear receptors activated by various lipids and other biologically active molecules [36, 37]. Long-chain fatty acids have been proposed as natural PPAR ligands, and indeed, specific endogenous pathways of lipid metabolism can activate distinct PPAR responses. All three PPAR isomers, PPARα, PPARβ/δ and PPARγ, are involved in gene expression in multiple aspects of fatty acid [38] and glucose metabolism [39] as well as in inflammation [40].

The possibility that carotenoid oxidation products may regulate PPAR activity stems from their structural similarities to the natural ligands of this family of receptors. β-Carotene can undergo asymmetric cleavage, producing a series of apocarotenals with varying chain length [41]. Ziouzenkova et al. [42–44] suggested that apocarotenals might be involved in transcription. One specific apocarotenal, β-apo14′-carotenal, but not other structurally similar apocarotenals, could inhibit PPARγ, PPARα, and RXR activation [42]. Inhibition of PPARγ and RXR activity by this derivative in adipocytes resulted in the suppression of both adipogenesis and the expression of the PPARγ target genes Fabp4 and adiponectin. In endothelial cells, β-apo14′-carotenal inhibited PPARα activation and augmented expression of vascular cell adhesion molecule-1 (VCAM-1), which was previously shown to be reduced by PPARα [42]. In addition to β-apo14′-carotenal, retinaldehyde, the product of the central cleavage of β-carotene found in fat, was shown to inhibit RXR and PPARγ activation in vitro and PPARγ-induced adipogenesis both in vitro and in vivo [43]. These data indicate that specific apocarotenals might act as biologically active mediators in transcription regulation. This suggestion is supported by our findings [21] showing that the EpRE/ARE transcription system could be activated by a series of apocarotenals (see below).

The above studies by Ziouzenkova et al. established the role of β-carotene derivatives in inhibition of PPARs activity. A recent study stressed the role of PPARγ and LXRα activation in the lycopene inhibition of prostate (LNCaP) cancer cell proliferation [45], although the role of lycopene derivatives was not examined in this study. Lycopene significantly increased the protein and mRNA expression of PPARγ and LXRα. The reduced prostate cancer cell proliferation observed in the presence of lycopene was restored by specific antagonists of PPARγ and LXRα. In addition, LXRα knockdown by siRNA significantly enhanced the proliferation of LNCaP cells, whereas si-LXRα knockdown followed by incubation with lycopene reduced cell proliferation.

4.2.1.3 Estrogen receptors

It is well known that endogenous or exogenous estrogens are crucial risk factors for the development and progression of breast cancer [46]. Therefore, inhibition of estrogenic activity by antiestrogens is an important treatment for breast cancer and can also be used for prevention of the disease in women who are at high risk for this malignancy [47, 48]. The probability that carotenoids can attenuate estrogenic activity was suggested by a study that found that an inverse association of breast cancer risk with dietary intake of carotenoids (α-carotene, β-carotene and lycopene) exists for postmenopausal women with hormone-dependent mammary tumors but not for other breast cancer patient groups [49]. We tested the ability of carotenoids to inhibit estrogenic signaling in estrogen-dependent cancer cells [50], which could explain the reduction in cancer risk by dietary intake of carotenoids. Indeed, the tomato carotenoids lycopene, phytoene, phytofluene and β-carotene inhibited estrogen-induced breast and endometrial cancer cell proliferation. Moreover, these carotenoids inhibited transactivation of the estrogen response element by both estrogen receptor alpha and beta [50]. In a more recent study, we found that synthetic diapocarotene-dials also inhibited estrogen receptor transactivation in breast cancer cells [51]. Although the action of estrogens in breast and endometrial cancer is harmful, it is beneficial for bone formation. Thus, we examined the effects of lycopene derivatives on estrogenic activity in osteoblasts which are bone forming cells. We found that the diapocarotene-dials, which inhibited estrogenic activity in cancer cells, did not inhibit and even stimulated estrogen receptor transactivation and the expression of estrogen-responsive genes in the bone cells [51].

4.2.2 Non-ligand activated transcription factors

In this review, we will discuss primarily two major transcription systems that are important in malignant processes – the EpRE/ARE system and its major transcription factor, Nrf2, and the NF-κB system. The EpRE/ARE mediates induction of detoxifying and antioxidant enzymes, which are responsible for reducing the mutagenic effects of carcinogens and reactive oxygen species [52]. In contrast, activation of the NF-κB transcription system contributes to cancer progression and also exerts harmful effects on bone health [53]. Interestingly, under un-stimulated conditions, both Nrf2 [52] and the NF-κB transcription factors [54] are retained in the cytoplasm by their respective inhibitory proteins, Keap1 and IκB (inhibitor of NF-κB), which harbor cysteine thiols. The interaction of various electrophiles, including dietary derived compounds, with these cysteines results in activation of Nrf2 [52] and inhibition of NF-κB activity [54]. The opposite modulation of these two transcription systems by dietary compounds including carotenoids and their derivatives can partially explain the prevention of cancer and the improvement of bone health by various carotenoids.

4.2.2.1 The electrophile/antioxidant response element transcription system

Under resting conditions, Nrf2 is bound to its cysteine-rich partner, Keap1, which is known to repress Nrf2 activity. Various phytonutrients, such as polyphenols and isothiocyanates [52, 55], interact with Keap1 and activate Nrf2 and EpRE/ARE. Inducers of this system are diversified in their chemical structure but all are chemically reactive and nearly all are electrophiles, which react with Keap1 to disrupt its inhibitory activity on Nrf2. However, hydrophobic carotenoids such as lycopene lack any electrophilic group and, thus, are unlikely to interact directly with Keap1. Therefore, we hypothesized that oxidation products are the active mediators in the stimulation of the EpRE/ARE transcription system by carotenoids. To identify the putative activators, we collaborated with H. Ernst (BASF, Ludwigshafen, Germany) and C. Caris-Veyrat (INRA and Avignon University, Avignon, France) to design a series of pure synthetic and fully characterized apocarotenoids that can potentially be derived from in vivo metabolism of lycopene and other carotenoids or during their spontaneous oxidation [21]. The ability of these compounds to stimulate the EpRE/ARE system was determined in order to elucidate the structure-activity relationship of these derivatives and their mechanism of action at the molecular level. We have demonstrated that oxidized derivatives, extracted by ethanol from partially oxidized lycopene, transactivated EpRE/ARE in $HepG_2$ human hepatocellular carcinoma cells [56] with a potency similar to that of the unextracted lycopene mixture, whereas the intact carotenoid showed a small insignificant effect [21]. The modest activation of EpRE/ARE by the intact lycopene could result from oxidation of the carotenoid in the course of the experiment or from its enzymatic cleavage by cellular BCDO2. Indeed, transfection of T47D breast cancer cells with BCDO2 increased the activity of intact lycopene (Salman et al., unpublished results). Using the series of characterized mono- and diapocarotenoids described above, we demonstrated that aldehydes, and not acids, are the active molecules which stimulate EpRE/ARE and that this activity depends on the relative position of the methyl group to the terminal aldehyde, which determines the reactivity of the conjugated double bond.

As discussed previously in this review, various apolycopenals are found in tomatoes and in human serum and tissues [20, 22, 57]. These oxidized derivatives, for example, apo-8′- and apo-12′-lycopenals [22], are similar to some of the molecules that were shown in our study to activate EpRE/ARE. Furthermore, such oxidation products can be formed by spontaneous oxidation [7], or after chemical [3, 5] or enzymatic [58] oxidation, as described above (Section 2). For example, Caris-Veyrat et al. [3] have reported that lycopene is readily oxidized with potassium permanganate giving rise to a range of apocarotenals and diapocarotenedials, such as apo-10′-lycopenal and diapocarotene-10, 10′-dial [16]. It is important to emphasize that this diapocarotene-dial can be formed not only from lycopene but also from several other carotenoids by enzymatic or chemical cleavage at the 10 and 10′ carbons of the molecule.

4.2.2.2 NF-κB

The NF-κB transcription system is important for the normal functioning of the immune system but is also crucial for the deleterious inflammatory response. NF-κB consists of five homologous subunits (RelA/p65, c-Rel, RelB, p50 and p52), which dimerize and are retained in the cytoplasm by specific proteins, the IκBs. The IκB bound to an NF-κB dimer is phosphorylated by the IκB kinase complex, which in turn is activated by several receptor-activated pathways. The phosphorylated IκBs are targeted to ubiquitination and degradation by the 26S proteasome, and the liberated NF-κBs translocate to the nucleus and regulate the expression of NF-κB-dependent genes [54].

The attenuation of NF-κB activity by carotenoids, as part of their intracellular activity, was suggested in several studies. For example, lycopene decreased the invasive ability of hepatoma cells by suppressing the activity of NF-κB [59]. This inhibitory effect of lycopene may be related to down-regulation of the Insulin-like growth factor-I receptor. Another study suggested that lycopene, via the inhibition of NF-κB, might be useful in the treatment of benign prostate hyperplasia, a non-malignant condition associated with inflammation [60]. Inhibition by lycopene of this transcription system in several cancer cell lines including prostate (LNCaP and PC-3), colon (HCT-116 and HT-29) and lung (BEN) was reported by Palozza et al. [61]. Interestingly, in contrast to the inhibitory effect of lycopene on NF-κB activity, the same group reported that β-carotene stimulated NF-κB in human leukemic (HL-60) and colon adenocarcinoma (LS-174 and WiDr) cells [62].

The role of NF-κB inhibition in the anti-inflammatory activity of another carotenoid, astaxanthin, was studied in vitro (lipopolysaccharide-treated RAW264.7 cells and primary macrophages) and in vivo (lipopolysaccharide-treated mice) [63]. The induction of various inflammatory markers and cytokines by lipopolysaccharide was attenuated by astaxanthin, and this was associated with inhibition of NF-κB activity. Moreover, astaxanthin blocked all the major signaling events in NF-κB activation, namely IκB kinase phosphorylating activity, IκBα degradation and the nuclear translocation of the NF-κB p65 subunit.

Oxidative stress plays an important role in the inflammatory process of celiac disease. Therefore, the effects of several natural antioxidants including lycopene were studied in a model system of the disease based on RAW 264.7 macrophages treated with a combination of interferon-γ plus gliadin [64]. This treatment enhanced the expression of the inflammatory markers, inducible nitric oxide synthase and cyclooxygenase-2, which was associated with increased binding of NF-κB to DNA. Lycopene inhibited all these effects, suggesting that the carotenoid can control the expression of pro-inflammatory genes involved in celiac disease.

The growth of tumors and the development of metastases are dependent on the formation of new blood vessels. For this reason, the antiangiogenic effect of β-carotene was evaluated using the in vivo model of B16F-10 melanoma in mice and several in vitro models [65]. β-Carotene treatment significantly reduced the number of tumor-directed capillaries accompanied by reduction of serum vascular endothelial growth factor and the proinflammatory cytokines Interleukin-1β, TNFα and Interleukin-6. Moreover, similar reduction of these cytokines was detected when the melanoma cells were treated with β-carotene in vitro. This reduction was associated with inhibition of nuclear translocation and DNA binding of p65, p50 and c-Rel subunits of NF-κB as well as of the activator protein-1 (AP-1) family transcription factors (see below), which, similar to NF-κB, are associated with inflammatory processes and cancer cell growth. We have previously shown that the AP-1 transcription system was inhibited by lycopene in MCF-7 mammary cancer cells [66]. This inhibition was associated with interference in Insulin-like growth factor-I receptor signaling and inhibition of cell cycle progression.

Consistent with the findings showing inhibition of NF-κB activity by various carotenoids, our preliminary studies indicate that apocarotenals and diapocarotene-dials inhibit the transcriptional activity of NF-κB as well as some of the related upstream signaling events in both cancer and bone cells (Linweill-Hermoni et al. unpublished results). The opposite modulation of the EpRE/ARE and NF-κB transcription systems by carotenoid derivatives can in part account for the prevention of cancer and the improvement of bone health by various carotenoids. It is interesting that a mixture of aldehydes derived from oxidation of β-carotene, lutein and zeaxanthin produced an opposite effect and enhanced nuclear DNA binding of NF-κB and AP-1 in human retinal pigment epithelial cells [67]. Some of the aldehydes produced by β-carotene oxidation were β-apo-14′-carotenal and β-apo-13-carotenone discussed above (Sections 4.2.1.1 and 4.2.1.2) but this mixture also included other unidentified carbonyl-containing derivatives and, thus, it is difficult to assess which oxidation products caused the observed enhancement of NF-κB activity.

It is obvious that the few transcription systems described above (EpRE/ARE, NF-κB and AP-1), which are important in cancer and inflammatory processes, are not the only transcription pathways modulated by carotenoid derivatives. Thus, we expect that further studies will shed more light on this subject. Furthermore, it is important to note that the expression of BCDO2, the enzyme which is responsible for the cellular production of apocarotenoids, is regulated by carotenoid derivatives. For instance, Reynaud et al. [68] have recently shown that apo-10′-lycopenoic acid and apo-14′-lycopenoic acid up-regulate this carotenoid asymmetric cleavage enzyme, BCDO2, while having no effect on BCMO1 expression. Although the transcription systems involved in the regulation of BCDO2 expression are not yet known, the latter results point to the importance of feedback mechanisms by which apocarotenoids can regulate the eccentric cleavage enzyme.

5 Concluding remarks

The basic idea that retinoic acid, a β-carotene metabolite, is a ligand for the retinoid receptor family and by that modulates the levels of multiple key cellular proteins was expanded in the last 10 years to a myriad of carotenoid oxidation products that modulate several other transcription systems. In this review, we focused on apocarotenoids, which affect multiple transcriptional pathways and, thus, can be considered as natural compounds with multifunctional, rather than monofunctional, activity. Such pleotropic action of these metabolites may be used in the prevention of cancer and other degenerative diseases. Although the studies reviewed here contribute significantly to our understanding of the mechanisms by which carotenoids and their oxidation metabolites may affect health, more work is required in order to establish their role in human physiology and in various pathologies such as cancer and inflammation.

Potential conflict of interest statement: Yoav Sharoni and Joseph Levy are consultants for Lycored Ltd., Beer Sheva, Israel.

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