with non-substituted β-ionone cycles and provitamin A properties exhibits several biological activities, including the scavenging of free radicals, enhancement of gap junctions, immunomodulation and regulation of the enzyme activity involved in carcinogenesis [97,98]. The most common sources of β-cryptoxanthin are citrus fruits and red sweet peppers. β-Cryptoxanthin is reported to inhibit mouse skin tumorigenesis [58] and rat colon carcinogenesis [99]. Narisawa *et al.* also reported that 25 ppm of β-cryptoxanthin administered for 30 weeks in the diet significantly suppressed *N*-methylnitrosourea-induced colon carcinogenesis in rats [99]. This suggested that dietary β-cryptoxanthin may affect colon carcinogenesis after accumulation in the colonic mucosa, perhaps due to absorption from the colon as well as the small intestine. β-Cryptoxanthin-rich juice (Satsuma mandarin juice [MJ]) has also been found to inhibit colon [59] and lung [100] carcinogenesis. Hesperidin, present in several vegetables and fruits, has antioxidant properties, and anti-inflammatory and inhibitory effects on prostaglandin biosynthesis. This flavonoid has been shown to inhibit chemically induced carcinogenesis in several organs [87–90,101–105]. β-Cryptoxanthin and hesperidin are thus considered to be potential cancer chemopreventive compounds. However, edible plants contain only small amounts of these chemicals. Therefore, to obtain higher contents of these compounds in foods we prepared a pulp (CHRP) containing high amounts of β-cryptoxanthin and hesperidin during the process of making MJ. CHRP (100 g) contained 0.67 g of β-cryptoxanthin and 3.58 g of hesperidin; the contents of β-cryptoxanthin and hesperidin were 583 times and 38 times greater than those in the edible parts of Satsuma mandarin, respectively. In addition, we prepared Satsuma mandarin juices, which we called MJ2 (1.7 mg of β-cryptoxanthin and 84 mg of hesperidin/100 g) and MJ5 (84 mg of β-cryptoxanthin and 100 mg of hesperidin/100 g), by adding CHRP to the standard Satsuma mandarin juice (MJ: 0.8 mg of β-cryptoxanthin and 79 mg of hesperidin/100 g). We have demonstrated the chemopreventive effects of CHRP and MJs on chemically induced oncogenesis in rat colon and tongue and mouse lung [59,100,106,107].

Citrus compounds act on multiple key elements in signal transduction pathways related to cellular proliferation, differentiation, apoptosis, inflammation and obesity. We have found that *Citrus unshiu* segment membrane (CUSM) containing β-cryptoxanthin and fiber suppresses colitis- and obesity-related colon tumorigenesis in animal models [108,109]. Feeding involving a diet with CUSM treatment also decreased the serum level of triglycerides.

6.3. Lycopene

There are relatively few reports on the cancer chemopreventive effects of lycopene or other tomato carotenoids in animal models. The majority, but not all, of these studies have indicated a protective effect. Inhibitory effects were seen in two studies using aberrant crypt foci (putative precursors of colon cancer) [96] and colon cancer [110] as biomarkers, and in two mammary tumor studies, one using the dimethylbenz(*a*)anthracene model [111] and the other the spontaneous mouse model [112]. Inhibitory effects were also reported in mouse lung [113] and rat hepatocarcinoma [114] and bladder cancer [115] models. However, a study by Cohen *et al.* [116] found no effect in the *N*-nitrosomethylurea-induced mammary tumor model when crystalline lycopene or a lycopene-rich tomato carotenoid oleoresin was administered in the diet. Unfortunately, differences in routes of administration (gavage, intraperitoneal injection, intra-rectal instillation, drinking water and diet

supplementation), species and strain differences, form of lycopene (pure crystalline, beadlet and mixed carotenoid suspension), varying diets (grain-based and casein based) and dose ranges (0.5–500 ppm) resulted in no prevention effect on development of chemically induced mammary cancer. It is clear that the majority of ingested lycopene is excreted in the feces and that 1,000-fold more lycopene is absorbed and stored in the liver than in other target organs. Nonetheless, physiologically significant (nanogram) levels of lycopene are assimilated by key organs such as breast, prostate, lung and colon, and there is a rough dose-response relationship between lycopene intake and blood levels. Pure lycopene was absorbed less efficiently than the lycopene-rich tomato carotenoid oleoresin, and blood levels of lycopene in rats fed a grain based diet were consistently lower than those in rats fed lycopene in a casein-based diet. The latter suggests that the matrix in which lycopene is incorporated is an important determinant of lycopene uptake.

High intake of lycopene has been associated with a lower risk of a variety of cancers including lung cancer. Lycopene can be converted to apo-10'-lycopenoids [117] in mammalian tissues and can be cleaved by carotene 9',10'-oxygenase at its 9',10' double bond to form apo-10'-lycopenoids, including apo-10'-lycopenal, apo-10'-lycopenol, and apo-10'-lycopenoic acid. Among apo-10'-lycopenoids, apo-10'-lycopenoic acid has been recently shown to inhibit lung carcinogenesis both *in vivo* and *in vitro* [118]. Since enzymatic metabolites of lycopene induce NF-E2-related factor 2 (Nrf2)-mediated expression of phase II detoxifying/antioxidant enzymes including heme oxygenase-1 (HO-1), NQO1, GSTs, and glutamate-cysteine ligases in human bronchial epithelial cells, BEAS-2B [119] and human liver cell cancer cells, HepG2 [120], the anti-carcinogenic and antioxidant functions of lycopene are mediated by apo-10'-lycopenoids, especially apo-10'-lycopenoic acid, via activating Nrf2 and inducing phase II detoxifying/antioxidant enzymes [119].

Of the various carotenoids lycopene has been found to be very protective, particularly for prostate cancer. The major dietary source of lycopene is tomatoes, with the lycopene in cooked tomatoes being more bioavailable than that in raw tomatoes. Several prospective cohort studies have found associations between high intake of lycopene and reduced incidence of prostate cancer, although not all studies have produced consistent results [121,122]. Some studies suffer from a lack of good correlation between lycopene intake assessed by questionnaire and actual serum levels, and other studies measured intakes among a population that consumed very few tomato products. In the Health Professionals Follow-up Study there was a 21% decrease in prostate cancer risk, when comparing the highest quintile of lycopene intake with the lowest quintile. Combined intake of tomatoes, tomato sauce, tomato juice and pizza (which accounted for 82% of the lycopene intake) was associated with a 35% lower risk of prostate cancer. Furthermore, lycopene was even more protective for advanced stages of prostate cancer, with a 53% decrease in risk [123]. A more recent follow-up report on this same cohort of men confirmed these original findings that lycopene or frequent tomato intake is associated with about a 30–40% decrease in the risk of developing prostate cancer, especially advanced prostate cancer [124]. In addition to the two reports detailed above, a nested case control study from the Health Professional Follow-up Study involving 450 cases and controls found an inverse relationship between plasma lycopene and prostate cancer risk (OR 0.48) among older subjects (>65 years of age) without a family history of prostate cancer [125].

In addition to these observational studies, two clinical trials have been conducted to supplement lycopene for a short period before radical prostatectomy. In one study 30 mg/day of lycopene were

given to 15 men in the intervention group, while 11 men in the control group were instructed to follow the National Cancer Institute's recommendations to consume at least five servings of fruits and vegetables daily. Results showed that lycopene slowed the growth of prostate cancer. Prostate tissue lycopene concentration was 47% higher in the intervention group. Subjects that took lycopene for 3 weeks had smaller tumors, less involvement of the surgical margins and less diffuse involvement of the prostate by pre-cancerous high-grade prostatic intraepithelial neoplasia [126]. In another study carried out before radical prostatectomy surgery, 32 men were given a tomato sauce-based pasta dish every day, which supplied 30 mg of lycopene per day. After 3 weeks serum and prostate lycopene levels had increased 2-fold and 2.9-fold, respectively. Prostate-specific antigen (PSA) levels had decreased by 17%, as also reported by Kucuk *et al.* [126]. Oxidative DNA damage was 21% lower in the patients' leukocytes and 28% lower in prostate tissue, as compared with the non-study controls. The apoptotic index was 3-fold higher in the resected prostate tissue, relative to biopsy tissue [127].

A number of issues remain to be resolved before any definitive conclusions can be drawn concerning the anticancer effects of lycopene. These include the following: the optimal dose and form of lycopene; interactions among lycopene and other carotenoids and fat soluble vitamins such as vitamin E and D; the role of dietary fat in regulating lycopene uptake and disposition; organ and tissue specificity; and the problem of extrapolation from rodent models to human populations [128].

6.4. Lutein and Zeaxanthin

In addition to playing pivotal roles in ocular health, lutein and zeaxanthin are important nutrients for the prevention of CVD, stroke and lung cancer. They may also be protective in skin conditions attributed to excessive ultraviolet (UV) light exposure. In a 10-year study following 120,000 U.S. men and women, a significant reduction in lung cancer was observed in patients with the highest intake of total carotenoids including lutein and zeaxanthin [129]. A second 14-year study assessed the same relationship in 27,000 Finnish male smokers via a food-item questionnaire. Consumption of carotenoid containing fruits and vegetables was associated with a decreased risk of lung cancer. A decreased risk of lung cancer was also observed in individuals in the highest quintiles of lutein/zeaxanthin intake *versus* the lowest quintiles. A population-based survey of 20 South Pacific Island populations examined the association between lutein consumption and lung cancer rates. Researchers found an inverse association between lutein and lung cancer and a markedly lower incidence rate for lung cancer among Fijians, as compared with other South Pacific populations. Fijians consume an average of 200 g of dark green vegetables (25 mg lutein) daily; whereas inhabitants of other South Pacific countries consume diets in which colorful fruits and vegetables are less plentiful [130].

Carotenoids singly or in combination could lower cancer risk due to their antimutagenic properties and ability to scavenge free radicals, to protect against tumor development and to improve immune response [131,132]. Lutein and β-carotene quench peroxy radicals and demonstrate antioxidant properties against oxidative damage *in vitro* [133,134]. Plasma lutein analyzed from 37 women correlated inversely with measured oxidative indices [135]. It has been shown *in vitro* using multilamellar liposomes, that carotenoids in combination elicit a greater antioxidant defense than singly. The strongest synergistic effect was obtained in the presence of lutein or lycopene [136]. Lutein may be anticarcinogenic as well. This is suggested by its ability to interact with the mutagens 1-nitropyrene

and aflatoxin B_1 (AFB_1) [137,138]. Lutein may also exert an anticarcinogenic effect by stimulating certain genes involved in T-cell transformations activated by mitogens, cytokines and antigens [139].

Investigation of lutein's protective effects in relation to site-specific cancers is beginning to evolve in epidemiologic studies and animal models. No associations have been detected between plasma lutein and zeaxanthin concentrations and gastric cancer [140]. Slattery *et al.* [141] detected an inverse association between dietary lutein intake and colon cancer in men and women. The reduction in risk was significant only in patients who were diagnosed with colon cancer at a younger age [141]. Carotenoid esters are found in human skin [142]. A combination of carotenoids may protect against the development of erythema in human skin [143] and are correlated with the presence or absence of skin cancer and precancerous lesions [141]. The specific effects of lutein on skin cancer are yet to be determined. Previous research has shown modest relationships between the consumption of nutrients found in carotenoid rich foods such as β-carotene and vitamin A, and a reduced risk of breast cancer [144–146]. Focus on the potential protective effects of lutein in relation to developing breast cancer has evolved only recently. Recent research in mice showed that low levels of dietary lutein at 0.002 and 0.02% of the diet inhibited mammary tumor incidence, growth and latency [19]. Lutein has been shown to induce apoptosis in transformed but not in normal human mammary cells, and to protect normal cells from apoptosis induced in cell culture [147]. Freudenheim *et al.* [148] have shown that the intake of carotenoid-rich foods, specifically vegetables, as well as lutein and zeaxanthin, is significantly associated with a lower risk of developing premenopausal breast cancer. In a case-control study, increasing serum levels of lutein and zeaxanthin were associated with a reduced breast cancer risk, but the trend was only marginally significant [149]. A decreased risk of cancer was associated with increasing levels of breast adipose tissue lutein and zeaxanthin concentrations in women with breast cancer as compared with women with benign breast biopsies, but the association was not significant [150]. The Nurse's Health Study [12] showed a weak, but significant, inverse association between lutein and zeaxanthin intake and the risk of developing breast cancer among premenopausal women. The protective effect of lutein and zeaxanthin in relation to breast cancer was strongest among women with a family history of breast cancer. A nested case-control study from the prospective New York University Women's Health Study [151] indicated an inverse association between plasma lutein, but not zeaxanthin, and risk of breast cancer. However, plasma α- and β-carotene levels were also significantly related to a decrease in risk. Other case-control studies have shown no differences in breast adipose tissue concentrations of lutein and zeaxanthin between women with benign breast tumors and those with breast cancer [152].

6.5. Astaxanthin

Because astaxanthin has not typically been identified as a major carotenoid in human serum, information on its epidemiology in human health is lacking. Salmon, the principal dietary source of astaxanthin, is an important component of the traditional diets of Eskimos and certain coastal tribes in North America; these groups have shown an unusually low prevalence of cancer [153,154]. This low cancer incidence has been attributed to the high levels of certain fatty acids in salmon, notably eicosapentaenoic acid [154], yet it is possible that astaxanthin has played a role in cancer chemoprevention among these peoples as well. Regardless, the existing data on the potential for

astaxanthin to directly prevent cancer is limited to *in vitro* cell culture studies and *in vivo* studies with rodent models.

We previously investigated the possible preventive effects of astaxanthin and canthaxanthin on *N*-butyl-*N*(4-hydroxybutyl)nitrosamine (OH-BBN)-induced mouse urinary bladder carcinogenesis [155], 4-NQO-induced rat oral carcinogenesis [156] and azoxymethane (AOM)-induced rat colon carcinogenesis [157]. Both of these xanthophylls exhibited inhibitory activity in relation to cancer development in urinary bladder [155], tongue [156] and colorectum [157] through the suppression of cell proliferation. In urinary bladder carcinogenesis, the inhibitory effect of astaxanthin was greater than that of canthaxanthin through the suppression of cell proliferation [155]. A recent study of ours demonstrated the anti-inflammatory ability and anti-carcinogenesis effects of astaxanthin in inflamed colon due to modulation of the expression of several inflammatory cytokines that are involved in inflammation-associated carcinogenesis [158]. Indeed, astaxanthin may aid cyclooxygenase (COX)-2 down-regulation [159]. A recent study using a 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis model also showed that daily administration of astaxanthin (15 mg/kg body weight) significantly inhibited colon carcinogenesis by modulating nuclear factor kappaB (NF-kB), COX-2, matrix etalloproteinases (MMP) 2/9, extracellular signal-regulated kinase (ERK)-2 and protein kinase B (Akt) [160]. Astaxanthin, canthaxanthin and β-carotene, but not lycopene, are reported to be able to suppressed the development of preneoplastic liver cell lesions induced by AFB_1 in rats through the deviation of AFB_1 metabolism towards detoxification pathways [161]. In addition, tetrasodium diphosphate astaxanthin has been reported to completely inhibit methylcholanthrene-induced neoplastic transformation of C3H/10T1/2 cells by upregulation of connexin 43 and gap junctional intercellular communication (GJIC) [162].

6.6. Canthaxanthin

Epidemiological data on canthaxanthin in disease prevention is lacking. However, this carotenoid has exhibited potential anticancer properties *in vitro* and in animal models. In earlier studies, canthaxanthin exerted cancer chemopreventive activities in UV-B-induced mouse skin tumorigenesis [163] and chemically-induced gastric [164] and breast carcinogenesis [164,165]. Canthaxanthin can also suppress the proliferation of human colon cancer cells [166], and protect mouse embryo fibroblasts from transformation [167] and mice from mammary and skin tumor development [16,168]. Canthaxanthin has also proved effective in inhibiting both oral and colon carcinogenesis in rats [156,157]. Canthaxanthin and astaxanthin have been found to lower the incidence of urinary bladder cancers induced by OH-BBN, but the inhibitory effects of canthaxanthin were weak when compared to astaxanthin [155]. As was the case with astaxanthin and β-carotene, canthaxanthin suppressed AFB1-induced preneoplastic hepatocellular lesions in rats [161]. Although it is a potent antioxidant, the chemopreventive effects of canthaxanthin may also be related to its ability to up-regulate gene expression, resulting in enhanced gap junctional cell-cell communication [74,169]. The chemopreventive effects of canthaxanthin may also be related to its ability to induce xenobiotic metabolizing enzymes, as has been demonstrated in the liver, lung and kidney of rats [170,171]. The apoptosis-inducing effects of canthaxanthin may also contribute to its cancer chemopreventive effects [172]. Unfortunately, canthaxanthin overuse as a sunless tanning product has led to the appearance of crystalline deposits in

the human retina [173]. Although these retinal inclusions are reversible [174] and appear to have no adverse effects [173], their existence has prompted caution regarding the intake of this xanthophyll.

6.7. Fucoxanthin

There are several *in vitro* studies that have demonstrated the inhibitory effects of fucoxanthin on human cancer cell lines developed in liver (HepG2) [175], colon (Caco-2, HT-29 and DLD-1) [176] and urinary bladder [177]. The induction of apoptosis [176,177] and the suppression of cyclin D levels [175] have been considered to be the biochemical mechanisms by which fucoxanthin exerts its inhibitory effects on the growth of cancer cells. Since mice actively convert fucoxanthin into keto-carotenoids by oxidizing the secondary hydroxyl groups and accumulating them in tissues [178], it may be possible that keto-carotenoids are active chemicals responsible for the effects of fucoxanthin.

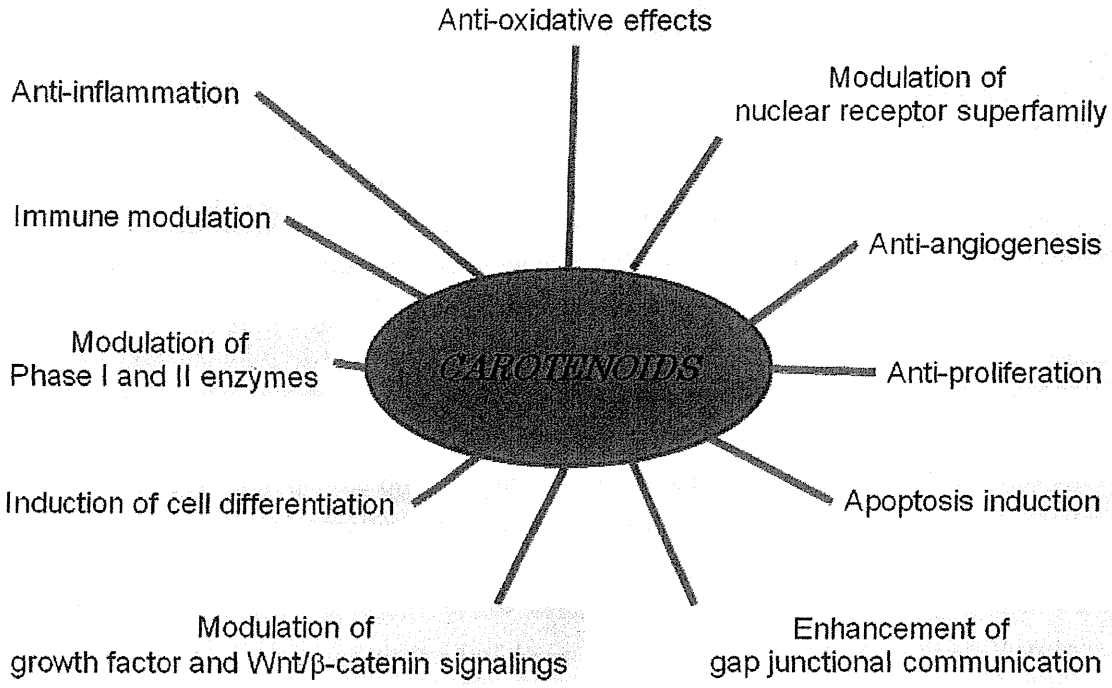
In a preclinical study, fucoxanthin was found to significantly inhibit DMH-induced mouse colon carcinogenesis [179]. Fucoxanthin has been proven to suppress spontaneous liver tumorigenesis in C3H/He male mice and showed antitumor-promoting activity in a two-stage carcinogenesis experiment involving the skin of ICR mice, initiated with 7,12-dimethylbenz[*a*]anthracene and promoted with 12-*O*-teradecanoylphorbol-13-acetate and mezerein [180]. In addition, fucoxanthin has been reported to inhibit duodenal carcinogenesis induced by *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine in mice [181].

Although the antitumor effects of fucoxanthin are known, the precise mechanism of action has yet to be elucidated [182]. The anticancer activity of fucoxanthin has been shown to be partly based on its regulative effect on biomolecules related to the cell cycle and apoptosis [183,184] and those associated with antioxidant activity through its pro-oxidant action [185]. In addition, fucoxanthin has been found to be able to selectively inhibit mammalian DNA polymerase activities, especially replicative DNA polymerases (*i.e.*, pol α, δ and ε), and thus has anti-neoplastic activity [186]. Further investigations using animal models are needed to assess the details of the molecular mechanisms involved in fucoxanthin's activity against different types of cancer cells.

7. Mechanisms of Cancer Chemoprevention by Carotenoids

The mechanisms underlying the anticancer and/or cancer chemopreventive activities of carotenoids may involve changes in pathways leading to cell growth or cell death. These include immune modulation, hormone and growth factor signaling, regulatory mechanisms of cell cycle progression, cell differentiation and apoptosis. Examples of carotenoid effects on some of these pathways are listed below, with the emphasis being placed on the changes in protein expression associated with these effects. The main question is, by what mechanism do carotenoids affect so many and diverse cellular pathways as described above? The changes in the levels of many proteins suggest that the initial effect involves modulation of transcription. As described below, such modulation can occur at the level of ligand-activated nuclear receptors or other transcription factors. As illustrated in Figure 2, carotenoids have multiple targets that contribute to their efficacy as chemoprevention agents.

Figure 2. Proposed mechanisms by which certain carotenoids suppress carcinogenesis.



7.1. Gap Junctional Intercellular Communication

One of the earliest discoveries related to carotenoids and modulation of protein level was made by Bertram's group. They found that carotenoids increase gap junctional intercellular communication (GJIC) and induce the synthesis of connexin43, a component of the gap junction structure [74,187]. This effect was independent of provitamin-A and the antioxidant properties of the carotenoids. Loss of GJIC may be important for malignant transformation, and its restoration may reverse the malignant process.

7.2. Growth Factor Signaling

Growth factors, either in the blood or as part of autocrine or paracrine loops, are important for cancer cell growth. Recently, insulin growth factor (IGF)-1 has been implicated as a major cancer risk factor [188,189] and a target of potential for dietary intervention strategies for cancer prevention [189]. It has been reported that high blood levels of IGF-1, existing years before the detection of malignancy, can predict an increase in risk for prostate [190], breast [191], colorectal [192] and lung [193] cancers. A recent study of ours indicated that *db/db-Apc*$^{Min/+}$ with increased expression of IGF-1, IGF-1R and IGF-2 in the intestine was associated with an increased incidence of spontaneous intestinal neoplasms [194]. Accordingly, two possible strategies might be used to reduce IGF-related cancer risk, namely a reduction in IGF-1 blood levels and interference with IGF-1 activity in the cancer cell. Preliminary results of our studies on the former strategy suggest that tomato phytonutrients lower IGF-1 blood levels. In addition, lycopene inhibits the mitogenic action of IGF-1 in human cancer cells. In mammary cancer cells, lycopene treatment markedly reduced IGF-1 stimulation of both tyrosine phosphorylation of insulin receptor substrate-1 and the DNA binding capacity of the activator 1 (AP-1)

transcription factor [195]. These effects were not associated with changes in the number or affinity of IGF-1 receptors, but rather with an increase in membrane-associated IGF binding proteins (IGFBPs). This finding can explain the suppression of IGF-1-signaling by lycopene based on the finding that membrane-associated IGFBP-3 inhibits IGF-1 receptor signaling in an IGF-dependent manner [196].

7.3. Cell Cycle Progression

Growth factors have a major effect in promoting cell cycle progression, primarily during the G1 phase. Lycopene treatment of MCF-7 mammary cancer cells has been shown to slow down IGF-1-stimulated cell cycle progression [195], which was not accompanied by either apoptotic or necrotic cell death. Lycopene-induced delay in progression through the G1 and S phases has also been observed in other human cancer cell lines (leukemia and cancers of endometrium, lung and prostate) [197]. Similar effects of another carotenoid, α-carotene, were reported in human neuroblastoma cells (GOTO) [198]. Likewise, β-carotene was found to induce a cell-cycle delay in the G1 phase in normal human fibroblasts [199]. Fucoxanthin is reported to alter cell cycle progression [182,184,200]. In addition, metabolites of lycopene, apo-10'-lycopenoic acid [118] and apo-12'-lycopenal [201] can induce cell cycle arrest in cancer cells. Cancer cells arrested by serum deprivation in the presence of lycopene are incapable of returning to the cell cycle after serum re-addition [202]. This inhibition correlated with a reduction in cyclin D1 protein levels that resulted in inhibition of both Cdk4 and Cdk2 kinase activity and in hypophosphorylation of pRb.

7.4. Differentiation-Related Proteins

Induction of malignant clonogenic cells to differentiate into mature cells with distinct functions similar to those of nonmalignant cells has been proposed as an alternative to cytotoxic chemotherapy, and may be useful for chronic chemoprevention. Differentiation therapy has been quite effective in treating acute promyelocytic leukemia and is currently being investigated for the treatment of solid tumors. Differentiation inducers that are presently under laboratory and clinical investigation include vitamin D and its analogs, retinoids, polyamine inhibitors and others. We have shown that lycopene alone induces differentiation of HL-60 promyelocytic leukemia cells [197]. A similar effect has also been described for other carotenoids such as β-carotene, lutein and the saffron carotenoids [197,203,204]. The differentiation effect of lycopene was associated with elevated expression of several differentiation-related proteins such as cell surface antigen (CD14) and oxygen burst oxidase (as measured by phorbol ester-stimulated reduction of nitroblue tetrazolium) [197]. The mechanism of the differentiating activity of lycopene and its ability to synergize with $1,25(OH)_2D_3$ in this effect [197] is largely unclear. However, the differentiation-enhancing effect of another phytonutrient, carnosic acid from rosemary, is associated with the induction of multiple differentiation-related proteins such as Cdk inhibitor, p21Cip1, early growth response gene (EGF)-1 and Cdk5 and its activator protein, p35Nck5a [205,206]. Most importantly, carnosic acid and its combinations with $1,25(OH)_2D_3$ and retinoic acid transcriptionally activated the expression of nuclear hormone receptors such as vitamin D_3 receptor (VDR), retinoic acid receptor (RAR)α, and retinoid X receptor (RXR)α [205,206]. This may represent a molecular basis for synergy between phytonutrients and differentiation inducers. The

possibility that lycopene, as well as other carotenoids and/or their derivatives, may affect nuclear signaling pathways is an attractive suggestion, but requires experimental proof.

7.5. RAR

The structural similarity between lycopene and β-carotene suggests that lycopene or some of its oxidized derivatives may activate retinoid-like receptors. Acyclo-retinoic acid, a hypothetical oxidation product of lycopene, is the open chain analog of retinoic acid [207] and was found to be able to transactivate RARα, but the growth-inhibitory effect of lycopene was not mediated directly via this classical retinoid receptor [208]. In addition, acyclo-retinoic acid has been reported not to have a role in gap junctional communication [207]. Muto *et al.* [209] synthesized acyclo-retinoic acid and tested its biological activity as part of a series of acyclic retinoids, but did not observe transactivation by this compound in the RAR or RXR reporter gene systems [210]. However, they did find that other acyclic retinoids, lacking one or two double bonds (geranyl geranoic acid and 4,5-didehydrogeranylgeranoic acid), caused transactivation of the reporter gene comparable to that achieved by retinoic acid. It is interesting to note that these acyclic retinoids may be potential derivatives of phytoene and phytofluene carotenoids present in tomatoes. These studies suggest that carotenoids, their oxidized derivatives, and other phytonutrients interact with a network of transcription factors that are activated by different ligands at low affinity and specificity. The activation of several transcription factor systems by different compounds may lead to the synergistic inhibition of cell growth. In addition to the retinoid receptors, other candidate transcription systems that may participate in this network are the peroxisome proliferator-activated receptors (PPARs) [211–214], ARE [215,216], AP-1 [217], the xenobiotic receptors [218] and yet unidentified orphan receptors.

Recent elucidation of the pathways that are activated by retinoids will help to exploit the beneficial aspects of this class of compounds for cancer therapy and prevention [219,220]. Retinoids and carotenoids are important dietary factors which regulate cellular differentiation and growth, so that they are thought to be particularly effective at preventing the development of certain tumors. They play this role as ligands of the nuclear retinoic acid receptors, RAR and RXR [220]. These ligand-activated nuclear receptors induce the transcription of target genes by binding to retinoic acid-responsive elements in the promoter regions. Among these target genes, the RARβ gene is of great interest, being able to encode a potential tumor suppressor. It should be emphasized that most breast carcinomas and breast cancer cell lines show loss or down-regulation of RARβ receptor expression, whereas RARα and γ, as well as RXRs, appear to be variably expressed in both normal and tumor cells [220]. Expression of RARβ could be modulated by chemopreventive intervention [221,222] and may therefore serve as an intermediate biomarker in chemoprevention trials for some cancers [223]. Provitamin A carotenoids, such as β-carotene and its excentric cleavage metabolites, can serve as direct precursors for (all-*trans*)-retinoic acid and (9-*cis*)-retinoic acid which are ligands for RAR and RXR, respectively. β-Carotene and its oxidative metabolite, apo-14'-carotenoic acid, are reported to reverse the down-regulation of RARβ by smoke-borne carcinogens in normal bronchial epithelial cells [224]. In addition, the transactivation of the RARβ promoter by β-apo-14'-carotenoic acid appears to occur via its metabolism to all-*trans*-retinoic acid [224]. Therefore, the molecular mode of the action of β-carotene might be mediated by retinoic acid through transcriptional activation of a series of genes

with distinct anti-proliferative or pro-apoptotic activity, which allows for the elimination of neoplastic and preneoplastic cells with irreparable alterations.

7.6. PPAR

These nuclear receptors have a key role in the differentiation of adipocytes, but recently their role in cancer cell growth inhibition and differentiation has also been demonstrated. PPARγ is expressed at significant levels in a variety of human primary and metastatic carcinomas [214,225–227]. Human colorectal cancer was found to be associated with loss-of-function mutations in PPARγ [228]. Ligand activation of PPARγ was reported in cultured breast cancer cells [213]. Human prostate cancer cells have been shown to express PPARγ at prominent levels, while its expression in normal prostate tissues was very low [212,213]. Activation of this receptor with specific ligands such as troglitazone exerts an inhibitory effect on the growth of prostate cancer cells, and favorable changes in PSA dynamics in prostate cancer patients [213]. The presence of PPARγ receptors in various cancer cells, their activation by fatty acids, prostaglandins and related hydrophobic agents in the μM range makes this liganded transcription factor an interesting target for carotenoid derivatives. We have previous demonstrated that fucoxanthin can induce apoptosis and enhance the antiproliferative effects of the PPARγ ligand, troglitazone, and inhibit the growth of human colon cancer cells [176].

Recently, Simone *et al.* [229] reported new molecular mechanisms by which lycopene regulates cigarette smoke-driven inflammation in human macrophages, THP-1. They have shown that lycopene inhibits the production of the pro-inflammatory cytokine interleukin (IL)-8 induced by cigarette smoke. More recently, Yang *et al.* [230] demonstrated that the anti-proliferative effect of lycopene on human prostate cancer cells (LNCaP) involves the activation of the PPARγ-LXRα-ATP-binding cassette transporter 1 (ABCA1) pathway.

7.7. Xenobiotic and other Orphan Nuclear Receptors

Orphan receptors include gene products that are structurally related to nuclear hormone receptors, but lack known physiological ligands. Thus, like all the recognized nuclear receptors they should have multiple regulatory roles, some of which may be related to diet-derived compounds. Mammals encounter numerous xenobiotics which are metabolized and eliminated mainly by cytochrome P450 (CYP) enzymes [218]. CYP enzymes are induced by various xenobiotic substrates, including phytonutrients, through the response element of several orphan nuclear receptors such as the steroid and xenobiotic receptor/pregnane X receptor (SXR/PXR), and the constitutive androstane receptor (CAR) [218,231]. St. John's wort, the herbal remedy used widely for the treatment of depression, illustrates the possible role of phytonutrients in this system. It has recently been found that its active compound, hyperforin, is a potent ligand for PXR that promotes the expression of CYP 3A4 [232].

7.8. Antioxidant Response Element

Induction of phase 2 enzymes that neutralize reactive electrophiles and act as indirect antioxidants appears to be an effective means for achieving protection against a variety of carcinogens in animals and man. Transcriptional control of the expression of these enzymes is mediated, at least in part,

through the antioxidant response element (ARE) found in the regulatory regions of their genes. The transcription factor Nrf2, which binds to the ARE, appears to be essential for the induction of prototypical phase 2 enzymes such as glutathione *S*-transferases (GSTs), NAD(P)H:quinone oxidoreductase (NQO1) [233] and thioredoxin [234]. The constitutive hepatic and gastric activities of GST and NQO1 were reduced by 50–80% in Nrf2-deficient mice as compared with wild-type mice [65]. Under basal conditions, Nrf1 and Nrf2 are located in the cytoplasm and are bound to the inhibitory protein, Keap1. Upon challenge with inducing agents, they are released from Keap1 and translocate to the nucleus [235,236]. Within the nucleus, these basic region leucine zipper transcription factors are recruited to the ARE as heterodimers with either small Maf proteins, FosB, c-Jun or JunD. Several studies have shown that dietary antioxidants such as terpenoids [237], phenolic flavonoids including green tea polyphenols and epigallocatechin-3-gallate [238,239] and isothiocyanates, may work as anticancer agents by activating this transcription system. By way of illustration, an isothiocyanate compound from Japanese horseradish extract has been demonstrated to induce both nuclear localization of Nrf2, which binds to the ARE, and expression of phase 2 enzyme genes. These effects were completely abrogated in Nrf2-deficient mice [240].

7.9. AP-1 Transcriptional Complex

The activation of the AP-1 transcriptional complex is a middle-term event (1–2 h) in the mitogenic signaling pathway of IGF-1 and other growth factors [241]. The AP-1 complex consists of protein from the Jun (c-Jun, JunB and JunD) and Fos (c-Fos, FosB, Fra-1 and Fra-2) families, which associate as homo- (Jun/Jun) or heterodimers (Jun/Fos). These proteins are often induced by mitogenic stimuli and tumor-promoting agents. They bind to the AP-1 site, known also as the TPA response element (TRE), on the promoter of many genes that are related to cell proliferation such as cyclin-D [242]. Interestingly, some of these proteins participate in the ARE transcription complex as well. This transcriptional system is modulated by carotenoids. It is possible that lycopene and retinoic acid reduce growth factor-induced stimulation of AP-1 transcriptional activity by altering the composition of AP-1 complexes that bind to DNA [39,243]. Wang *et al.* [217] reported that the expression of c-Jun and c-Fos genes in the lungs of ferrets, supplemented with high-dose β-carotene and exposed to tobacco smoke, was elevated 3- to 4-fold. In addition, they observed a strong proliferative response in lung tissue and squamous metaplasia, as well as an increase in the level of a cell proliferation marker, proliferating cell nuclear antigen. In β-carotene-supplemented animals, this increase was enhanced further by tobacco smoke. Their report offers a possible explanation for the enhancing effect of β-carotene supplementation on lung carcinogenesis in smokers, as has been reported in large intervention studies [13,15].

7.10. Wnt/β-Catenin Pathway

The Wnt/β-catenin pathway has been demonstrated to modulate cell proliferation, migration, apoptosis, differentiation and stem cell self-renewal [244]. It has been shown that Wnt/β-catenin signaling is implicated in the maintenance of stem cells in a variety of cancers, including colorectal cancer [245]. The link between Wnt/β-catenin and the PI3K/Akt pathway has been established by several studies. Activated Akt was shown to be able to phosphorylate Ser9 on glycogen synthase

kinase 3β (GSK3β), which may decrease the activity of GSK3β, thereby stabilizing β-catenin [246]. Furthermore, the PI3K/Akt pathway is important in regulating the mammary stem/progenitor cells by promoting β-catenin downstream events through the phosphorylation of GSK3β [247]. In colon cancer cells, lycopene suppressed Akt activation and nonphosphorylated β-catenin protein levels, and augmented the phosphorylated form of β-catenin, which were associated with reduced protein expression of cyclin D1 [248]. Hence, lycopene may inhibit Wnt/β-catenin signaling via the connection along the Akt/GSK3β/β-catenin [249]. Further studies on cancer stem cells in response to lycopene would perhaps provide promising new data.

7.11. Inflammatory Cytokines

Cancer frequently develops in inflamed tissues, suggesting that the inflammatory condition is closely related to carcinogenesis [250,251]. Examples of this relationship are: chronic hepatitis (HBV and HCV infection) and liver cancer; Barrett dysplasia and esophageal cancer; chronic gastritis (*H. pylori* infection) and gastric cancer; and inflammatory bowel disease and colorectal cancer [250]. The common denominator of all these conditions is that chronic inflammation leads to an increased incidence of cancer [250]. Thus, suppression of inflammatory cytokine expression leads to inhibition of carcinogenesis. These inflammatory cytokines include IL-1β, IL-6 and tumor necrosis factor (TNF)-α. Cytokine expression is mainly regulated by NF-κB. A recent study of ours demonstrated that astaxanthin suppressed the expression of these inflammatory cytokines and NF-κB, and inhibited inflammation-associated colon carcinogenesis in mice [158]. In addition, lycopene is reported to inhibit pancreatitis [252]. Chronic pancreatitis and hereditary pancreatitis are believed to increase the risk of pancreatic cancer [253,254].

8. Conclusions

Beneficial effects of carotenoid-rich vegetables and fruits in relation to cancer risk have been found in many epidemiological studies. However, the metabolism and molecular biological properties of carotenoids remain to be determined through further research. Provitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) combined with other antioxidants (ascorbic acid, α-tocopherol and lycopene) limit the oxidative cleavage products of carotenoids, formed in large quantities in the highly oxidative conditions of the smoke-exposed lung and enhance retinoid signaling, by blocking the activation of MAPK. In considering the efficacy and complex biological functions of carotenoids in the prevention of human lung cancer [255], it seems that these provitamin A carotenoids and antioxidants used in combination could be employed as a chemopreventive strategy against certain human cancers. However, there appear to be detrimental interactions between β-carotene, cigarette smoke and alcohol. In addition, the molecular mechanisms that underlie these interactions need to be understood before β-carotene can be further pursued for the prevention of carcinogenesis in man. As we await a better scientific understanding of carotenoid metabolism and the mechanisms of action, a prudent strategy to reduce the risk of cancer incidence and mortality would include increased consumption of vegetables and fruits as a part of a healthy, balanced diet. This would include eating between five to nine servings of fruits and vegetables every day. There is currently no evidence of any dangers associated with high levels of dietary β-carotene from natural food sources, aside from the occasional appearance of

carotenodermia, an accumulation of β-carotene in the skin that gives it a yellow or orange tint. At present, supplemental doses of β-carotene taken to meet vitamin A needs beyond the recommended dietary intake dose are not advisable for the general population. Smokers and alcohol drinkers are especially encouraged to avoid high doses of supplemental β-carotene.

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Conflict of Interest

The authors declare no conflict of interest.

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