

Carotenoids and lung cancer prevention

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1. ABSTRACT

Understanding the molecular actions of carotenoids is critical for human studies involving carotenoids for prevention of lung cancer and cancers at other tissue sites. While the original hypothesis prompting the beta carotene intervention trials was that beta carotene exerts beneficial effects through antioxidant activity, the harmful effects of beta carotene led to further animal and cell culture studies showing that the free radical rich but antioxidant poor environment of smoker's lungs could decrease the stability of the beta carotene molecule and increase beta carotene oxidative metabolites or decomposition products. In addition, the beneficial vs. detrimental effects of carotenoids are related to the carotenoid dose administered *in vivo*, and the tissue accumulation of carotenoids and their metabolites. This review will discuss the recent understanding that the biological functions of carotenoids are mediated *via* their oxidative metabolites through their effects on several important cellular signaling pathways and molecular targets, as well as smoke-related lung cancer.

2. INTRODUCTION

Lung cancer is the most common cause of cancer death in the world today. Despite great efforts to improve the treatment of patients with lung cancer, the survival rate for people diagnosed with this disease has not significantly improved over the past 30 years. Cigarette smoking is the dominant cause of lung cancer and tobacco control remains a key focus in lung cancer prevention. While progress has been made in smoking cessation efforts, former smokers remain at an increased risk of developing lung cancer for decades after they quit smoking. Further, the addictive power of nicotine cannot be dismissed, and there are a number of smokers who are unwilling or unable to quit. Finally, 13-15% of lung cancer patients are never-smokers, emphasizing that the risk is not restricted to smokers. Thus, dietary intervention is one of the main strategies for preventing lung cancer in individuals with a history of exposure to environmental carcinogens and/or genetic factors that put them at risk.

Clinical intervention trials conducted to determine if beta carotene functioned as a chemopreventive

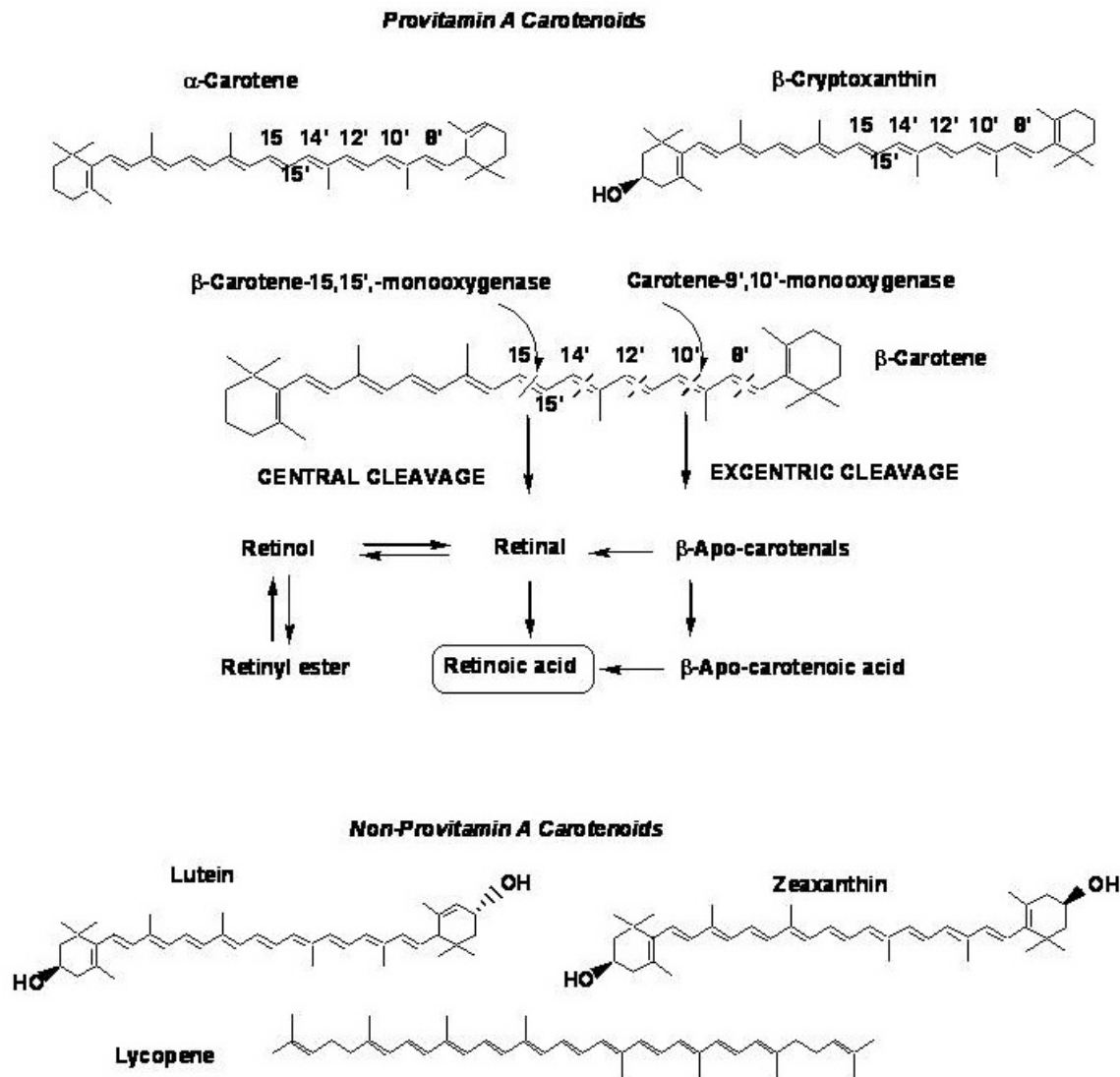


Figure 1. Metabolic pathway of beta carotene and chemical structures of provitamin A carotenoids (alpha carotene and beta cryptoxanthin) and non-provitamin A carotenoids (lutein, zeaxanthin and lycopene).

agent against lung cancer in smokers found either no protective effect or a harmful effect. However, supporting evidence for a protective role of carotenoid rich fruits and vegetables in the prevention of certain cancers and other chronic disease (e.g., atherosclerosis, age-related macular degeneration, UV damage in skin) continues to be reported in human epidemiological studies, small intervention trials and in mechanistic studies using cell culture and animal models (1, 2). There are three provitamin A carotenoids (beta carotene, alpha carotene, and beta cryptoxanthin) and three non-provitamin A carotenoid (lycopene, lutein and zeaxanthin) that can be found routinely in human plasma and tissues (Figure 1), and that have been studied for their potential beneficial roles in various cancers (e.g., lung, prostate, breast, colorectal and stomach) (1, 2). These carotenoids are lipophilic plant pigments with polyisoprenoid structures, typically containing a series of

conjugated double bonds in the central chain of the molecule, making them susceptible to oxidative cleavage (3), isomerization from the *trans* to the *cis* forms (4), and to the formation of potentially bioactive metabolites (5). Within the last few years, we have gained greater knowledge of the biological effects of carotenoids, particularly the impact of oxidation on these carotenoids and the potential for beneficial effects with small quantities or harmful effects with large quantities of the resulting metabolic products (Figure 2). While the initial impetus for studying the benefits of carotenoids in cancer prevention was their antioxidant capacity, significant advances have been made in understanding the actions of carotenoids with regard to other mechanisms such as, modulation of intracellular redox status involving cell proliferation and induction of apoptosis (6, 7), interaction with growth factors and hormones *via* transcription systems (8) and

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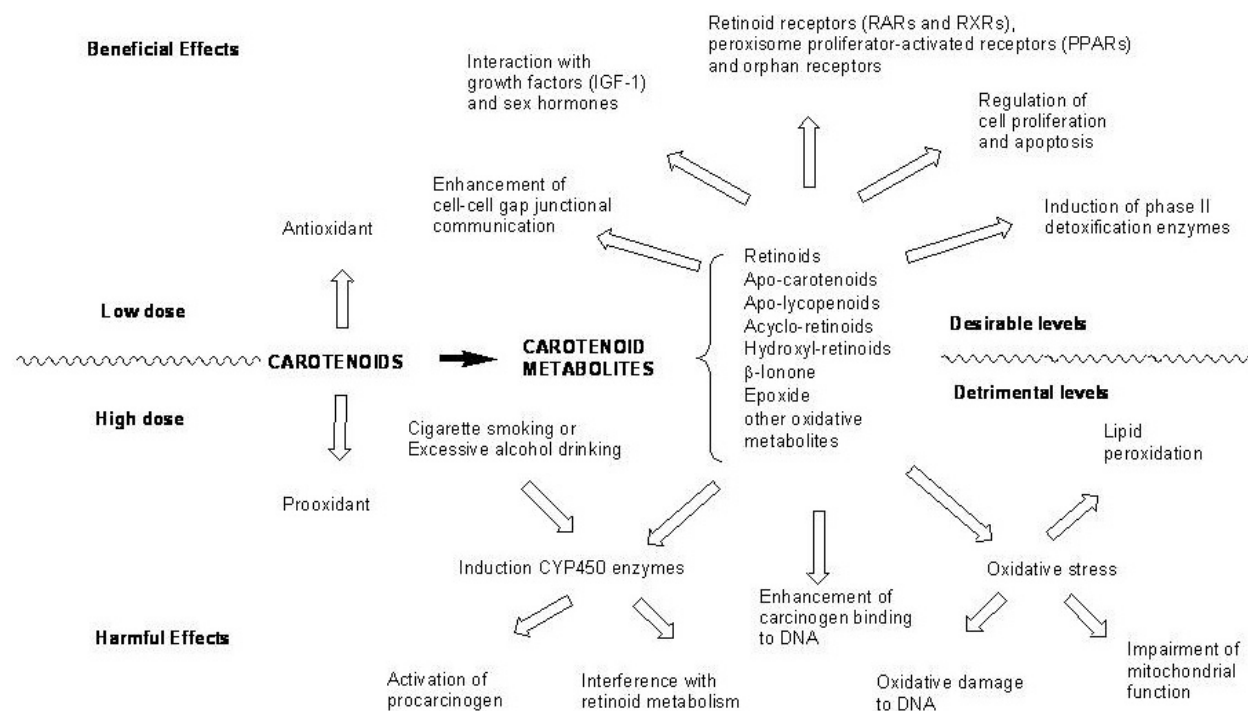


Figure 2. Schematic illustration of possible mechanism(s) of carotenoid dose and oxidative metabolites on beneficial and detrimental effects to human health (see text for details).

enhancement of cellular gap junctional communication without retinoid activity (9). This review will discuss the recent findings of the biological functions of carotenoids and their significance in lung cancer prevention.

3. CAROTENIDS AND LUNG CANCER RISK

The association between carotenoids and lung cancer risk has been investigated in several studies including epidemiological and interventional studies. Epidemiological data, both cohorts and case control studies and interventional studies conducted in humans and in animal models have provided important information regarding the effects of the various carotenoids on lung cancer. Evidence examining the association between carotenoids and lung cancer is reviewed below.

3.1. Evidence from epidemiological studies

Several epidemiological studies have reported the protective effect of increased intake of carotenoid rich yellow/orange and red fruits and vegetables on the risk of developing lung cancer in several populations (10-17). In these studies, subjects in the highest quintile of fruit and vegetable consumption had significantly lower risk of lung cancer vs. those in the lowest quintile. Other studies have reported non-significant inverse associations between high consumption of carotenoids (18) and vitamin A (19) and lung cancer risk. In addition to studying the effect of fruits and vegetables, several studies have also examined the association between dietary intake/serum levels of specific carotenoids and lung cancer risk. The protective effects of carotenoid rich vegetables on lung cancer risk were

previously attributed to beta carotene, possibly making it the most commonly researched carotenoid for its association with lung cancer risk.

Many early studies measuring plasma/dietary beta carotene found inverse associations between high beta carotene status and cancer risk (16, 20-24) though some studies found no significant association between the two (25-28). A case control study conducted in New York state found a 30% reduction in the probability of getting lung cancer in individuals with high dietary intakes of beta carotene (16). Data from the Missouri Women's Health Study showed that women with high beta carotene intakes had a 42% reduction in their chances for developing lung cancer (29). A review of several earlier studies examining the association between beta carotene and lung cancer risk has been published earlier (30). Based on epidemiological evidence supporting a protective effect of beta carotene against lung cancer risk, clinical intervention trials were designed to test the efficacy of high doses of beta carotene in cancer prevention in various populations. These studies yielded unexpected results when it was found that high dose beta carotene either potentially increased risk of lung cancer in smokers and asbestos workers (31, 32) or had no effect on risk in a relatively healthy, predominantly non-smoking population of physicians (33). These data suggested that high dose beta carotene could increase the risk of lung cancer in individuals who were already at a higher risk for developing lung cancer (smokers, asbestos workers). Data from an earlier study conducted in a Chinese population found that supplementing beta carotene with vitamin E and selenium reduced total mortality and

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Table 1. Relative Risk and Odds Ratio of lung cancer risk with various carotenoids

| Exposure | Relative Risk/ Odds Ratio (95% CI) | | | | | Ref |
|----------------------------------|------------------------------------|--------------------|------------------|------------------|-------------------------------|-----|
| | Alpha CAR | Beta CAR | Beta CRYP | LYC | LUT/ ZEA | |
| Dietary carotenoids ¹ | NA | 0.92 (0.55-1.54) | NA | 1.21(0.69-2.10) | 1.09 (0.70-1.70) ⁴ | 38 |
| Dietary carotenoids | 0.61 (0.39-0.95) | 0.79 (0.50-1.24) | 0.72 (0.46-1.11) | 1.00 (0.67-1.50) | 0.87 (0.57-1.31) | 14 |
| Serum carotenoids ¹ | 0.60 (0.30-1.30) | 0.50 (0.20-1.30) | 0.80(0.30-2.00) | NA | 0.40 (0.20-1.10) | 45 |
| Dietary carotenoids | 0.85 (0.60-1.16) | 0.81 (0.59-1.11) | 0.71 (0.52-0.96) | 0.91 (0.67-1.25) | 0.72 (0.53-0.99) | 41 |
| Dietary carotenoids ² | 0.75 (0.59-0.96) | 0.90 (0.67-1.21) | 0.82 (0.65-1.02) | 0.80 (0.64-0.99) | 0.81 (0.64-1.01) ⁴ | 35 |
| Serum carotenoids ¹ | 1.15 (0.62-2.15) | 0.74(0.42-1.30) | 0.45 (0.22-0.92) | 0.59 (0.31-1.14) | 0.97 (0.55-1.17) | 42 |
| Dietary carotenoids | 0.94 (0.81-1.09) | 0.92 (0.79-1.07) | 0.85 (0.72-0.99) | 0.72 (0.61-0.84) | 0.83 (0.71-0.99) | 12 |
| Dietary carotenoids ³ | 0.90 (0.51-1.58) | 1.40 (0.76 - 2.59) | 0.66(0.33-1.32) | 1.04 (0.61-1.76) | 1.26(0.70-2.24) ⁴ | 36 |
| Dietary carotenoids | 1.06 (0.79-1.42) | 1.00 (0.74-1.35) | 0.73 (0.54-0.98) | 0.89 (0.65-1.21) | 1.12 (0.84-1.50) ⁴ | 43 |
| Dietary carotenoids | 0.93 (0.82-1.06) | 0.98 (0.87-1.11) | 0.76 (0.67-0.86) | 0.91 (0.78-1.07) | 0.91 (0.81-1.03) | 34 |
| Dietary carotenoids | NA | 0.70 (0.50-0.99) | NA | NA | NA | 16 |
| Dietary carotenoids ¹ | 0.58 (0.21-1.64) | 0.92 (0.20-4.16) | NA | 0.56(0.26-1.24) | 0.76 (0.29-2.02) ⁴ | 28 |
| Dietary carotenoids ¹ | 0.82 (0.55-1.20) | 0.58 (0.39-0.86) | 0.64(0.43-0.96) | 0.73 (0.48-1.1) | 0.52(0.35-0.78) | 44 |
| Serum carotenoids ¹ | 0.77 (0.45-1.32) | 1.07(0.63-1.83) | 0.76 (0.44-1.28) | 0.86 (0.52-1.43) | 0.55 (0.33-0.93) ⁴ | 46 |
| | | | | | 0.56(0.33-0.95) ⁵ | |

Abbreviations: alpha CAR: alpha carotene, beta CAR: beta carotene, beta CRYP: beta cryptoxanthin, LYC: lycopene, LUT: lutein, ZEA: zeaxanthin, NA: not analyzed. Relative risk and Odds Ratio are for highest vs. lowest quintile/ quartile/ tertile of nutrient intake/ status. ¹Numbers are odds ratio, ²Data from pooled multivariate analyses, ³Incidence rate ratios, ⁴Lutein only, ⁵Zeaxanthin only

mortality from cancer (esophagus and stomach) (34) suggesting that a combination of antioxidants may be a more safe and effective method to reduce cancer deaths instead of high doses of a single nutrient. In addition to beta carotene, there is evidence to suggest that other carotenoids such as alpha carotene, lycopene, beta cryptoxanthin, lutein and zeaxanthin are inversely associated with lung cancer risk.

Several studies have reported a reduced risk of lung cancer with high tomato/lycopene intake (10, 12, 18, 35-37). A Finnish cohort reported a 28% reduction in risk in individuals in the highest quintile of lycopene consumption vs. those in the lowest quintile (38). Pooled analyses from two cohorts showed a 20% reduction in lung cancer risk with high lycopene intake (37). Some studies have reported no significant association between the two (14, 20, 39-42). Two of these studies reported a non-significant positive association between cancer risk and high tomato/lycopene intake (39, 41). Most case control studies that reported protective associations, assessed tomato intake as apposed to lycopene intake itself (10, 11, 16, 18, 43), therefore it is possible that the beneficial effects seen are attributable to lycopene and a combination of other nutrients present in tomatoes. The chemoprotective effects of lycopene are currently being tested in a prostate cancer clinical intervention trial, however to date; there are no similar trials to investigate the effects of lycopene on lung cancer.

Epidemiological data also supports a protective association between dietary and serum levels of beta cryptoxanthin and lung cancer risk in populations in Finland, the USA and China (12, 29, 36, 44-46). In some cases, among the various carotenoids analyzed, beta cryptoxanthin was the only carotenoid significantly associated with a lower risk of lung cancer (36, 46). In the pooled analysis of data from seven large cohorts in North America and Europe with 3,155 incident cases of lung cancer, beta cryptoxanthin intake was associated with a significant 24% reduction in lung cancer risk, comparing

the highest to the lowest quintile (36). Other cohort (14, 37, 39, 47) and case control studies (48) have reported inverse, but non-significant associations between dietary/serum beta cryptoxanthin levels and lung cancer risk.

There is limited data examining the associations between lutein, zeaxanthin, alpha carotene and lung cancer. Previous studies have shown an inverse association between lutein and zeaxanthin together (12, 29, 44), or separately (48) as seen in the CARET study with lung cancer risk. Holick and colleagues reported a 17 % reduction in lung cancer risk with increased intake of lutein and zeaxanthin in male smokers in Finland (38). Michaud and colleagues and Knekt and colleagues reported an inverse association between dietary alpha carotene and lung cancer risk (14, 37). In the case of the former, alpha carotene was the only carotenoid that was significantly associated with a reduced risk (39%) for cancer. Michaud *et al.*, reported that increased alpha carotene intake was related to a 63% lower risk of lung cancer in non-smokers (37) and a recent cohort study in Japan showed that alpha carotene had the strongest inverse association with cancer mortality for lung cancer (49) and colorectal cancer (50). However, there are many studies that showed no significant associations between alpha carotene (12, 29, 36, 39, 44-48), lutein and zeaxanthin (14, 36, 37, 39, 41, 45-47) and lung cancer risk. Some of these studies found non-significant positive associations between alpha carotene (45, 46), lutein and zeaxanthin (39, 46) and lung cancer risk. Table 1 reviews some studies investigating the association between carotenoids and lung cancer risk. In addition to affecting lung cancer risk, the protective effects of carotenoids are also associated with smoking status (29, 36, 37, 44, 45), alcohol consumption (51), type and location of cancer (29, 36, 44, 52), cancer prognosis (43) and mortality (50).

Several studies have shown a protective effect of high amounts of both total carotenoids (29, 45) and individual carotenoids (29, 36, 37, 44, 45) with lung cancer

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risk among current smokers. While Wright and colleagues reported inverse associations between beta carotene and lutein and zeaxanthin and lung cancer risk in current smokers in the highest quartile of nutrient consumption vs. those in the lowest quartile (29), several others reported a similar effect of beta cryptoxanthin (36, 44, 45). A similar association was not seen in never/former smokers. Michaud and colleagues also observed a greater protective effect of lycopene against lung cancer risk in current smokers (37) and more recently, Mannisto *et al.*, reported a marginal association between high lycopene intake and reduced risk of lung cancer in current smokers in pooled data from seven US cohorts (36). While these data suggest that foods rich in carotenoids may be especially beneficial for smokers, the results of the beta carotene intervention trials caution against the use of high doses of single nutrients in this population. Interestingly, in a Finnish population, carotenoids were associated with a non-significantly protective association against lung cancer in non-smokers but not in smokers (53). Finally, data from a population of tin miners in China showed that alcohol drinkers with high serum beta cryptoxanthin, lutein and zeaxanthin had an increased risk for lung cancer while high levels of the same carotenoids were inversely, but non-significantly associated with cancer risk among non-drinkers (47).

There is also data to show an inverse association between dietary intakes of beta carotene, lutein and zeaxanthin (29), beta cryptoxanthin, lutein and zeaxanthin (36, 44) and risk of adenocarcinomas and small cell/squamous cell carcinoma in various populations. In a Norwegian cohort, consumption of tomatoes 6-13 times/month was associated with a 13% reduction in the risk for developing lung tumors and a 46% reduction in the risk for developing squamous and small cell carcinoma of the lung (35). Lee and colleagues reported that low intake of yellow-orange vegetables was a significant predictor of tumors in the upper lobe of the lung (52). Goodman and colleagues reported that increased consumption of foods like tomatoes and oranges appeared to improve survival in men with lung cancer in a population in Hawaii (54). Finally, Ito and colleagues reported significantly lower risk of lung cancer death in Japanese men with high serum levels of beta cryptoxanthin and lycopene vs. those with lower serum levels in that population (55). To conclude, considerable evidence from epidemiological studies supports a protective role of carotenoids against lung cancer.

3.2. Evidence from animal studies

3.2.1. Beta carotene

The protective effects of beta carotene have been well documented mostly for UV induced skin cancers. Moon and colleagues showed that supplementing different doses of beta carotene to hamsters injected with a carcinogen had no effect on lung tumor formation. However, when the animals were supplemented with a combination of beta carotene and retinol, they reported an inhibition of tumor formation (56). Later studies showed that a beta carotene rich diet had no effect on respiratory tract cancers induced by a carcinogen in hamsters (57). A detailed description of the effect of beta carotene on lung

cancer can be found in an earlier review by DeLuca (58). The authors found that beta carotene had no protective effects against lung cancer in the models that they reviewed. However, it must be noted that there was limited information regarding the absorption and metabolism of beta carotene in these models. If beta carotene was inefficiently absorbed and if circulating levels failed to reach biologically significant amounts, it could explain the results of these studies, showing no effect of beta carotene.

Previous research has demonstrated that ferrets (*Mustela putorius furo*), are a good model to study carotenoid absorption, metabolism and tissue distribution because they closely mimic that of humans (59-61). Furthermore, the lung structure and the types of tumors that ferrets develop in response to exposure to cigarette smoke and a carcinogen are very similar to that of humans (62). Therefore, to study the association between carotenoids and lung cancer specifically, ferrets may be superior to other existing animal models because of the above mentioned biological and pathological similarities to humans. We have previously demonstrated that low dose (equivalent to 6 mg/day in human) beta carotene supplementation in smoke-exposed ferrets partially prevent the decrease in lung retinoic acid levels and inhibit smoke-induced lung precancerous lesions, when compared with smoke-exposed ferrets without supplementation (63). Conversely, ferrets that were supplemented with high dose beta carotene and exposed to cigarette smoke had increased squamous metaplasia and destruction of alveolar walls vs. ferrets that were treated with cigarette smoke alone (64). However, beta carotene supplemented in combination with vitamins C and E reduced the incidence of preneoplastic and neoplastic lesions in the lungs of ferrets injected with a carcinogen and exposed to cigarette smoke vs. animals that were not supplemented (65).

3.2.2. Lycopene

Relatively fewer studies have examined the effects of lycopene supplementation on lung cancer. *In vitro* work using lung cancer cell lines demonstrated that lycopene prevented cell proliferation and growth, DNA damage and suppressed IGF-1 stimulated growth (66-68). Levy and colleagues found that lycopene was more effective in inhibiting lung cancer cell proliferation compared to alpha carotene and beta carotene (66). The data from *in vivo* studies are more complex, suggesting that the effects of lycopene on lung cancer are dependent on the dose of lycopene used. Lycopene supplementation at different doses in rats and mice initiated with a carcinogen had either no effect on tumor incidence (69), decreased tumor incidence and multiplicity in male mice but not in female mice (70) or increased lung mutagenesis in mice (71). However, it is unknown whether the concentrations of lycopene reached biologically significant levels because none of these studies reported tissue/plasma levels of lycopene. Previously we have shown that male ferrets exposed to cigarette smoke and supplemented with lycopene had reduced lung squamous metaplasia, reduced proliferating cell nuclear antigen staining (PCNA) and reduced phosphorylated BAD expression. Lycopene

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treatment also restored caspase 3 expression levels and increased IGFBP-3 levels in the ferrets (72).

3.2.3. Beta cryptoxanthin

Animals studies investigating the effects of beta cryptoxanthin on lung cancer are relatively recent. Kohno and colleagues demonstrated that in mice initiated with a carcinogen, supplementation with mandarin juice rich in beta cryptoxanthin and hesperidin significantly reduced lung tumor incidence (73). We have shown that beta cryptoxanthin can also inhibit growth of both lung cancer cells and immortalized human bronchial epithelial cells and can upregulate the expression of retinoic acid receptor beta (74).

3.2.4. Alpha carotene

There are very few studies that have examined the effects of alpha carotene on lung cancer. In carcinogen initiated mice, alpha carotene was found to be more effective in decreasing mean number of lung tumors/mouse compared to beta carotene (75). Furthermore, alpha carotene supplementation reduced the area of lesions (hyperplasia and adenomas) in the lungs of mice injected with multiple carcinogens (76). To date, there are no studies that have investigated the effect of lutein and zeaxanthin on induced/ spontaneous lung tumors. To conclude, while there is evidence both *in vitro* and *in vivo*, to support a protective effect of carotenoids against lung cancer in experimental studies, the mechanisms underlying these effects need to be further investigated.

4. MECHANISMS OF ACTION OF CAROTENOIDS AGAINST LUNG CANCER

4.1. Carotenoid metabolites and the retinoid signaling pathway

Retinoids, the important biologically active oxidative products from provitamin A carotenoids (Figure 1), play an important role in several critical life processes. Considerable evidence demonstrates that retinoids may be effective in the prevention and treatment of a variety of human chronic diseases, including cancer (77, 78). The mechanism by which retinoids are able to elicit these effects resides in their ability to regulate gene expression at specific target sites within the body. Both retinoic acid receptors (RAR alpha, RAR beta and RAR gamma) and retinoid X receptors (RXR alpha, RXR beta and RXR gamma) function as transcription factors and regulate gene expression by binding as dimeric complexes to the retinoic acid response element (RARE) and the retinoid X response element (RXRE), which are located in the 5' promoter region of susceptible genes (78). All-*trans* retinoic acid binds and transactivates only RAR, whereas 9-*cis*-retinoic acid binds and transactivates both RAR and RXR. However, recent data showing that 9-*cis*-retinoic acid is not a RXR ligand in mouse epidermis keratinocytes (79) suggests the need for more research to understand its role in signaling. Recent results have shown that decreased expression of all RAR and RXR receptor subtypes is a frequent event in non-small cell lung cancer (80). Particularly, both *in vivo* and *in vitro* studies indicate that RAR beta expression, which can be induced by retinoic

acid, is frequently reduced in various cancer cells and tissues (81). Recent evidence suggests that the RAR beta subtypes, RAR beta 2 and RAR beta 4, have contrasting biological effects (tumor suppressor and tumor promoter, respectively) in human carcinogenesis (82). The down-regulation of all retinoid subclasses suggests a fundamental dysregulation of the retinoid pathway in lung cancer (80). Conversely, restoration of RAR beta 2 in a RAR beta-negative lung cancer cell line has been reported to inhibit tumorigenicity in nude mice (83) and retinoic acid can reverse benzy(a)pyrene diol epoxide suppressed RAR beta protein by increasing transcription of RAR beta in immortalized esophageal epithelial cells (84) and lung cancer cells (85). In a small human trial, daily treatment with 9-*cis*-retinoic acid for three months restored RAR beta expression in the bronchial epithelium of former smokers (86). Recently, we also reported that supplementing carcinogen initiated AJ mice with 9-*cis*-retinoic acid decreased lung tumor multiplicity and increased lung RAR beta mRNA levels (87).

Provitamin A carotenoids, such as beta carotene and its excentric cleavage metabolites (Figure 1), can serve as direct precursors for all-*trans*- and 9-*cis*-retinoic acid (88-90). It has been shown that beta carotene supplementation prevents both skin carcinoma formation by upregulating RAR beta (91) and lung carcinogenesis in AJ mice (92). Recently we have observed that the down-regulation of RAR beta by smoke-borne carcinogens was completely reversed by treatment with either beta carotene or its oxidative metabolite, apo-14'-carotenoic acid, in normal bronchial epithelium cells (93). We further demonstrated that the transactivation of the RAR beta 2 promoter by beta apo-14'-carotenoic acid appears to occur, mainly as a result of its metabolism to all-*trans*-retinoic acid (93). Therefore, the molecular mode of action of provitamin A carotenoids can be mediated by retinoic acid, by transcriptionally activating a series of genes with distinct antiproliferative or proapoptotic activity, thereby eliminating cells with irreparable alterations in the genome or killing neoplastic cells. Interestingly however, relatively more luciferase activity was observed from beta apo-14'-carotenoic acid than can be accounted for by the appearance of retinoic acid, when compared to the effect of retinoic acid alone (93). Since RXRs may function not only as heterodimeric partners of other nuclear receptors (e.g., the peroxisome proliferators-activated receptor (PPAR), the vitamin D receptor (VDR) and possibly other orphan receptors), but also as active transducers of tumor suppressive signals (77), it will be interesting to investigate whether the biological activity of carotenoids or their metabolites are mediated through interaction with RARs, RXRs, PPAR, VDR or other orphan receptors. Recently we showed that supplementation with 9-*cis*-retinoic acid and/or 1-alpha, 25-dihydroxyvitamin D3 decreased lung tumor multiplicity in AJ mice initiated with a carcinogen. Furthermore, 9-*cis*-retinoic acid supplemented in combination with 1-alpha, 25-dihydroxyvitamin D3 reduced vitamin D induced toxicity symptoms compared to mice that were supplemented with vitamin D alone suggesting an interaction between the two compounds (94). It has been shown that both the PPAR gamma ligand

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ciglitazone and an RXR ligand cooperatively promoted transcriptional activity of the RARE beta and induced RAR beta expression in human lung cancer cells (95). In addition, it has been reported that carotenoids may be beneficial for bone formation by up-regulating vitamin D receptor levels (96). However, further research on the wide range of biological functions of carotenoids and their metabolites, as well as their precise mechanism of action, is required.

Protective effects of other provitamin A carotenoids, such as alpha carotene and beta cryptoxanthin have been reported recently. Effects of alpha carotene and beta cryptoxanthin (Figure 1) and their metabolites on the retinoid signaling pathway are yet to be elucidated although they may have similar interactions with cleavage enzymes based on dose and the oxidative environment of the lungs. In our recent cell culture study, we observed that beta cryptoxanthin can inhibit lung cancer cell growth by increasing the expression of RAR beta and transactivating RARE (74). However, it is possible that various breakdown products of pro-vitamin A carotenoids play a role in regulating cell functions, apart from their ability to be metabolized to retinoic acid. Studies have shown that beta apo-12'-carotenoic acid can inhibit the growth of HL-60 cells (97) and beta apo-14'-carotenoic acid can stimulate the differentiation of U937 leukemic cells (98) and inhibit the growth of breast cancer cells, without detection of evidence of conversion to retinoids (99). This retinoid-independent activity of provitamin A carotenoid metabolites may be similar to the biological activity of non-provitamin A carotenoid metabolites. It has been shown that lycopene oxidation products enhance gap junctional communication (100) and a retinoic acid receptor antagonist did not suppress reporter activity induced by lycopene, indicating that retinoids have separate mechanisms of gene activation than non-provitamin A carotenoids (101). It has also been shown that *acyclo*-retinoic acid, a possible metabolite of lycopene (102) but not a ligand for RAR and RXR (103), inhibited the growth of HL-60 human promyelocytic leukemia cells (104), human mammary cancer cells (105), and human prostate cancer cells and this effect was significantly greater than both 9-*cis*-retinoic acid and *all-trans*-retinoic acid (106). Recently, the induction of phase II detoxification enzymes by lycopene and its hydrophilic derivatives *via* the antioxidant response element and the transcription factor Nrf2 has been reported (96).

On the other hand, the harmful effects of carotenoids could be also due to their metabolites or decomposition products (5, 107); therefore, the quantities of these metabolites should be considered (Figure 2). We have shown that one biological basis for the harmful effects of high dose beta carotene supplementation in smokers relates to the dosage used and the free radical-rich atmosphere in lungs of cigarette smokers (63, 64, 108). This environment alters beta carotene metabolism and produces undesirable oxidative metabolites (108), which can facilitate the binding of metabolites of benz(a)pyrene to DNA (109), down-regulate RAR beta (64), up-regulate activator protein 1 (AP-1, c-Jun and c-Fos) activity (63),

induce carcinogen-activating enzymes (110), enhance the induction of BALB/c 3T3 cell transformation by benz(a)pyrene (111), inhibit gap junction communication in A549 lung cancer cells (112) and impair mitochondrial functions (113). The dosages of beta carotene used in the ATBC (Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study) and CARET (Beta-Carotene and Retinol Efficacy Trial) studies were 20 to 30 mg per day for 2-8 years, and these doses are 10- to 15- fold higher than the average intake of beta carotene in a the typical American diet (2 mg per day). Such a pharmacological dose of beta carotene in humans could result in the accumulation of relatively high levels of beta carotene and its oxidative metabolites in the lung tissue, especially after long periods of supplementation, which could also potentially lead to a decrease in lung retinoic acid concentration *via* induction of cytochrome p450 (CYP) enzymes (Figure 3) (114). It should be noted that excentric cleavage products, which may be formed in excess in cancerous lung tissue, have not been shown to competitively bind to RAR beta at physiologically relevant levels (99). However, it is possible that the excentric cleavage products of carotenoids interfere with retinoic acid binding to its receptors when retinoic acid level is low in tissues. This may be seen in the case of cigarette smoking and excessive alcohol drinking, resulting in higher CYP enzyme levels and breakdown of retinoic acid (114-116). The loss of or low levels of retinoic acid, including both *all-trans* and 9-*cis* isomers, or the "functional" down-regulation of retinoid receptors (because of the lack of retinoic acid) could interfere with retinoid signal transduction and result in enhanced cell proliferation and potentially malignant transformation. This is supported by our previous studies in ferrets where we showed that showed that high dose beta carotene supplementation (equivalent to an intake of 30 mg of beta carotene/day/70 kg human, considered a pharmacological dose) and/or cigarette smoke exposure decreased levels of retinoic acid and RAR beta protein, but increased levels of c-Jun and cyclin D1 proteins, and induced precancerous lesions in lung tissue (63, 64).

The anti- or procarcinogenic response to beta carotene supplementation reported in human intervention trials and in animal studies may be related to the stability of the beta carotene molecule and its metabolites in different organ environments (such as high oxidative stress in the lung due to smoking or low antioxidants levels). Recently, we observed that ascorbic acid (which facilitates both recycling and stability of beta carotene and alpha tocopherol, but not used in the ATBC study and expected to be low in this population of heavy smokers), beta carotene (equivalent to 12 mg/day in human) plus alpha tocopherol provides protection against lung cancer risk by maintaining normal levels of retinoic acid (65). This is in agreement with our previous *in vitro* study which showed that the addition of both ascorbic acid and alpha tocopherol to an incubation mixture of beta carotene with ferret lung tissue can inhibit the smoke-enhanced production of excentric cleavage metabolites of beta carotene, increase the formation of retinal and retinoic acid (117) and decrease the smoke-induced catabolism of retinoic acid (114). These studies and the known biochemical interactions of beta carotene, vitamin E and

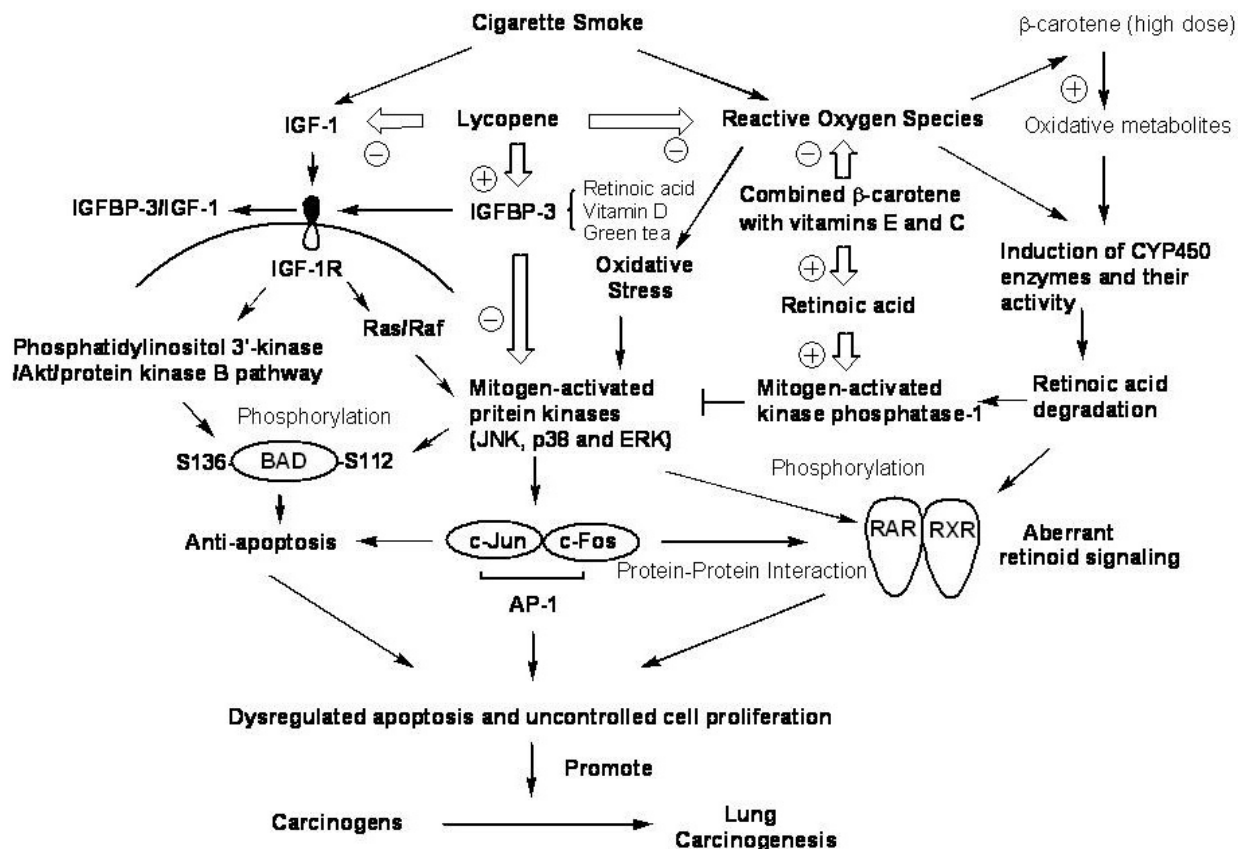


Figure 3. Simplified schematic illustration of possible molecular mechanism(s) of cigarette smoke and carotenoid supplementation in the presence of vitamins E and C on lung carcinogenesis. Beta Carotene, in the presence of vitamins E and C, may exert its protective effects against smoke-induced lung carcinogenesis by blocking smoke-induced free radicals, inhibiting the activation of MAP kinase pathway and the activity of cytochrome P450 enzymes, and by keeping retinoic acid signaling intact, inhibiting cell proliferation. Lycopene or lycopene metabolites may exert their protective effects against smoke-induced lung carcinogenesis by up-regulating IGFBP-3, interrupting the signal transduction pathway of IGF-1, down-regulating phosphorylation of BAD, promoting apoptosis and inhibiting cell proliferation. The combination of provitamin A carotenoids and non-provitamin A carotenoids, which target different signaling pathways and provides complementary or synergistic protective effects, would be a valuable strategy against lung cancer risk.

vitamin C suggest that the combination of nutrients, rather than individual agents, could be an effective chemopreventive strategy against lung cancer in smokers (Figure 3).

4.2. Carotenoid metabolites and the mitogen-activated protein kinase (MAPK) pathway

Cigarette smoke exposure is a major risk factor for lung cancer since it promotes genomic instability and the development of neoplasia by modulating molecular pathways involved in cell differentiation, cell proliferation and apoptosis. Jun N-terminal kinase (JNK), extracellular-signal-regulated protein kinase (ERK) and p38 mitogen-activated protein kinase belong to the mitogen-activated protein kinase (MAPK) family (Figure 3). They are activated by phosphorylation in response to extracellular stimuli and environmental stress and may play an important role in carcinogenesis (118, 119). It has been reported that components of smoke or smoke exposure itself can increase the phosphorylation of JNK and ERK in cell models (120,

121). JNK was shown to phosphorylate c-Jun on sites Ser-63 and Ser-73 and increase AP-1 transcription activity, mediating cell proliferation and apoptosis (118, 119). ERK induced c-Jun through phosphorylation and activation of the AP-1 component ATF1 at Ser-63 (122). On the other hand, MAPK phosphatases (MKPs), a family of dual-specificity protein phosphatases can dephosphorylate both phospho-threonine and phospho-tyrosine residues to inactivate JNK, ERK and p38 both *in vitro* and *in vivo* (123, 124). It has been shown that phosphorylated-JNK, phosphorylated-ERK, phosphorylated-p38 are preferred substrates for MKP-1 *in vivo* among isomers of MKPs (123, 124). Previously, we observed that activator protein 1 (AP-1, c-Jun and c-Fos) expression was up-regulated in the lungs of smoke-exposed ferrets supplemented with beta carotene (64), compared to the control animals. This overexpressions of AP-1 was positively associated with increased levels of cyclin D1 protein and squamous metaplasia in the lungs of animals with smoke-exposure (64). It is conceivable that the chronic excess beta carotene

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intake may modulate MAPK signaling and cause abnormal cell cycle regulation, and promote carcinogenesis. This hypothesis is supported by our recent observation that smoke exposure, high dose beta carotene, and their combination activated the phosphorylation of JNK and p38, but significantly reduced lung MKP-1 protein levels (125). In contrast, low dose beta carotene attenuated smoke-induced JNK phosphorylation by preventing down-regulation of MKP-1 due to smoke exposure (125). The mechanism behind the inhibitory effect of low dose beta carotene supplementation against the phosphorylation of JNK could be due to increased lung retinoic acid levels in smoke-exposed animals since it has been reported that retinoic acid can inhibit phosphorylation of MAPKs, such as JNK and ERK, by upregulation of MKP-1 (126-128). Interestingly, in our recent study, relatively high beta carotene supplementation (equivalent to 12 mg/day in human) in the presence of ascorbic acid and alpha tocopherol blocked smoke-induced phosphorylation of JNK and ERK completely by preventing smoke-induced reductions in retinoic acid levels in the lungs of ferrets (129). In addition, we observed that combined antioxidants inhibited smoke-induced oxidative stress assessed by Comet analysis (129). These data may help to explain the conflicting results of the negative human beta carotene intervention trials (which used high doses of beta carotene) vs. the positive observational epidemiological studies showing that diets high in fruits and vegetables containing beta carotene (but at much lower concentrations than in the intervention studies and with other antioxidants present) are associated with a decreased risk for lung cancer. Lycopene has also been shown to inhibit JNK, p38 and ERK, and the transcription factor, nuclear factor kappa B (130). Therefore, it is possible that the inhibition of JNK activation by combined antioxidants including both provitamin A- and non-provitamin A carotenoids may help to "rescue" the functions of RARs because it has been recently reported that activation of JNK contributes to RAR dysfunction by the phosphorylation of RAR alpha and by inducing its degradation through the ubiquitin-proteasomal pathway (131). It has been shown that RAR alpha does activate the RARE of RAR beta suggesting a possible accessory role for RAR alpha in RAR beta expression (132). Further examination of carotenoids including provitamin A- and non-provitamin A carotenoids on the stability and degradation of RARs through JNK-mediated pathways should be considered for future studies (Figure 3).

4.3. Carotenoid metabolites and the insulin-like growth factor-1 (IGF-1) pathway

The insulin-like growth factors (IGFs) are mitogens that play a pivotal role in regulating cell proliferation, differentiation, and apoptosis (133). The downstream pathway of the IGF-1R signaling involves the activation of both the phosphatidylinositol 3'-kinase (PI3K)/Akt/protein kinase B and the Ras/Raf/MAPK pathways (Figure 3). Disruptions of normal IGF-1 system components lead to hyperproliferation, survival signals and are implicated in the development of various tumor types. Several lines of evidence implicate IGF-1 and its receptor, IGF-1R in lung cancer and other malignancies (133, 134).

Epidemiological evidence indicates that increased levels of IGF-1, reduced levels of IGFBP-3, or an increased ratio of IGF-1 to IGFBP-3 in circulation are associated with an increased risk for the development of several common cancers, including those of the breast, prostate, colon, and lung (134). Lycopene is an antioxidant whose physical quenching rate constant for singlet oxygen is almost twice as much as that of beta carotene (135, 136). However, under certain conditions, it can act as a potential pro-oxidant (137). It has been reported that the IGF-1 stimulated cell growth was reduced by physiological concentrations of lycopene in endometrial, mammary (MCF-7) and lung (NCI-H226) cancer cells (66, 138). Lycopene treatment was also associated with an increase in membrane-associated IGFBPs (138). Two studies in humans have also shown that higher intake of cooked tomatoes or lycopene were significantly associated with lower circulating levels of IGF-1 and higher levels of IGFBP-3 (139). In an animal study, lycopene supplementation reduced local prostatic IGF-1 expression in the Dunning prostate cancer model (140).

Previously, we have demonstrated that lycopene supplementation in smoke-exposed ferrets inhibits lung squamous metaplasia by up-regulating IGF-binding protein 3 (72), a potent inhibitor of both the PI3K/Akt/PKB and the MAPK signaling pathways (141). It also regulates the bioactivity of IGF-1 by sequestering it away from its receptor in the extracellular milieu, thereby inhibiting the mitogenic and anti-apoptotic action of IGF-1 (Figure 3). Furthermore, the changes in IGFBP-3 as a result of lycopene supplementation in the plasma of ferrets were associated with increased apoptosis and decreased cell proliferation in the lungs of smoke-exposed ferrets. Interestingly, smoke-induced phosphorylation of BAD, which has the potential to be a chemopreventive or a therapeutic target because of its central position between growth factor signaling pathways and apoptosis (142), was prevented by lycopene supplementation in the lung tissue of ferrets after 9 weeks of treatment. Our observation is in agreement with a previous study that reported that IGFBP-3 could inhibit both the PI3K/Akt/PKB and the MAPKs signaling pathways in non-small cell lung cancer (141). This is because the PI3K appears to mediate survival factor-induced phosphorylation of BAD Ser 136, whereas the MAPK are thought to mediate survival factor-induced phosphorylation of BAD Ser 112. Although the mechanism by which lycopene increases the level of IGFBP-3 remains to be elucidated, these results demonstrate the importance of IGFBP-3 in the regulation of smoke-induced lung lesions, proliferation and apoptosis, suggesting that IGFBP-3 is a molecular target of lycopene for the prevention of lung cancer.

It has been reported that the oxidative cleavage product of lycopene, (E, E, E)-4 methyl-8-oxo-2,4,6 nonatrienal, caused a dose dependent reduction of viability in HL-60 cells, induced DNA fragmentation, and increased the number of apoptotic cells in a time and dose dependent manner (143). Recently, we have shown that the ferret carotene 9', 10'-monooxygenase enzyme can cleave *cis* isomers of lycopene at the 9',10'- double bond to produce

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apo-10'-lycopenoids (144). Furthermore, we have also shown that one of these products, apo-10'-lycopenoic acid, mediates the chemopreventive activity of lycopene by inhibiting cell proliferation and by modulating the activation of RAR beta in human lung cancer cells (145). These studies support the notion that the metabolic products of lycopene may exert biological functions in cancer prevention. However, the molecular properties of lycopene and its metabolites need more investigation and knowledge of their metabolic pathway, dose effects, tissue specificity and possible adverse effects with tobacco smoking and alcohol drinking need to be addressed.

5. SUMMARY

In summary, nutritional intervention to protect tissues against smoke-borne chemical carcinogens and oxidative free radical damage is an appropriate way to modify cigarette smoke-induced cancer risk caused due to the strong addictive power of nicotine. While the risk for lung cancer in smokers persists for many years after smoking cessation, passive smokers (those exposed to environmental cigarette smoke, smoker's spouse and children) are also at an increased risk of lung cancer. Beneficial effects of carotenoid-rich fruits and vegetables on lung cancer risk have been found in many epidemiological studies, however, whether lower dose carotenoid supplements in individuals with high risk could afford benefits against lung cancer is still unknown. Although the metabolism and molecular biological properties of carotenoids remain to be determined through further study, laboratory studies have demonstrated that the oxidative cleavage products of beta carotene which are formed in large quantities in the cell (as a result of supplementation with high-dose beta carotene in the highly oxidative conditions of the smoke-exposed lung) enhance catabolism of retinoic acid by their induction of cytochrome P450 enzymes. These lower retinoic acid levels then enhance smoke-induced phosphorylation of the MAPK and down-regulate MKP-1 expression, thereby promoting lung carcinogenesis. On the other hand, low dose beta carotene supplementation, particularly combined with other antioxidants, inhibits tobacco smoke-induced phosphorylation of JNK, ERK and p38 MAPK by increasing MKP-1 and retinoic acid levels in the lung tissue, thereby preventing the formation of smoke-induced precancerous lesions. In addition, lycopene supplementation protects against smoke-induced lung carcinogenesis by up-regulating IGFBP-3, a molecular target that interrupts the signal transduction pathway of IGF-1, thereby lowering the risk of lung cancer. In considering the efficacy and complex biological functions of carotenoids in human lung cancer prevention, it appears that combining provitamin A carotenoids (beta carotene, alpha carotene and beta cryptoxanthin) with other antioxidants would be a particularly useful approach for chemoprevention. Antioxidants such as ascorbic acid and alpha tocopherol, limit the formation of oxidative cleavage products of carotenoids in smoke-exposed lungs and enhance retinoid signaling by blocking the activation of MAPK. Combining provitamin A carotenoids with ascorbic acid and/or alpha tocopherol, would be an effective

chemopreventive strategy against lung cancer in current smokers or former smokers. In addition, provitamin A carotenoids combined with non-provitamin A carotenoids (such as lycopene), which target different signaling pathways, could provide complementary or synergistic protective effects against lung cancer.

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Abbreviations: CYP: cytochrome P450 enzymes, ERK: extracellular-signal-regulated protein kinase, IGF-1: insulin-like growth factor-1, IGF-1R: insulin-like growth factor-1 receptor, JNK: Jun N-terminal kinase, MAPK: mitogen-activated protein kinases, MKP-1: MAPK phosphatases-1, PI3K: phosphatidylinositol 3'-kinase, RARE: Retinoic acid response element, IGFBP-3: Insulin like growth factor binding protein – 3.

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