Carotenoids and Cancer: Biological Functions

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Abstract

Carotenoids in foods could reduce the risk of different disorders such as many forms of cancer. The carotenoids are usually augmented in lipophilic compartments. The main biochemical functions of carotenoids are related to the conjugated double bonds in their structures responsible for various colors. Several statistical investigations indicated an association between the use of carotenoids in diets and a decreased incidence of cancer types, suggesting the antioxidant properties of these compounds as an important factor. In this line, several studies have represented different mechanisms of action for carotenoids including up-regulation of gap junctional communication (GJC), stimulation of detoxifying enzymes and prevention of cell proliferation, as well as antioxidant activities and their conversion to vitamin A. Preclinical studies demonstrated that the carotenoids have effective anti-tumor effects both *in vitro* and *in vivo*, suggesting their potential preventive or therapeutic roles. Briefly, the reports suggested that carotenoids are safe, but they may exert carcinogenic effects under high doses. Furthermore, antioxidant activities of carotenoids are the most important anti-cancer properties among other biological functions. In this mini-review, we describe the major biological activities of carotenoids against a wide variety of tumor cells and tissues. Indeed, the anti-tumor effects of some carotenoids as well as their mechanisms will be individually evaluated in various types of cancers.

Keywords: Carotenoids; Function; Cancer; Treatment

Abbreviations

AhR: Aryl-Hydrocarbon Receptor; ERE: Estrogen Response Element; ER: Estrogen Receptors; HMG-CoA reductase: 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase; IGF-I: Insulin-Like Growth Factor I; GJC: Gap Junctional Communication; GSH: Glutathione; NO: Nitric Oxide; iNOS: Inducible Nitric Oxide Synthase; PPARc: Peroxisome Proliferator-Activated Receptor Gamma; RAR: Retinoic Acid Receptor; ROS: Reactive Oxygen Species; Rb: Retinoblastoma; TNF-α: Tumor Necrosis Factor; VEGF: Vascular Endothelial Growth Factor

Introduction

Carotenoids contain a group of natural fat-soluble pigments available in various vegetables and fruits. Based on their chemical composition as shown in figure 1, they are classified in two main groups: a) carotenes (e.g. β-carotene, α-carotene, and lycopene containing hydrogen and carbon atoms), and b) xanthophylls (e.g. lutein, zeaxanthin, canthaxanthin, α- and β-cryptoxanthin and astaxanthin containing hydroxy- and keto groups) [1]. Epidemiologic studies have demonstrated a reverse correlation between the incidence of different cancer types, and blood or food carotenoid levels [2,3]. The most promising effects of carotenoids are associated with their role as antioxidants and/or their earlier conversion to vitamin A [4,5]. A variety of carotenoids, acting as antioxidants, can effectively decrease the toxic effects of reactive oxygen species (ROS) generated in different diseases such as aging, cancer and cardiovascular and neurodegenerative diseases. The studies have shown that dietary carotenoids without provitamin A activity such as canthaxanthin, lutein, astaxanthin and lycopene could enhance immune responses significantly higher than β-carotene *in vivo* [5,6].
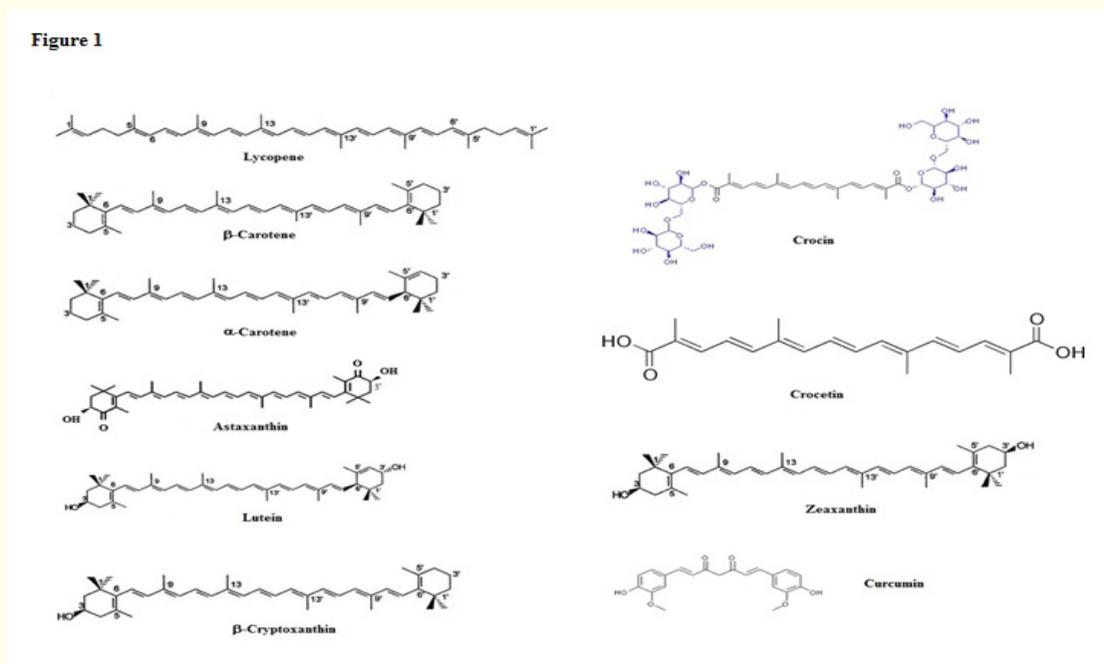
Figure 1: Chemical composition of carotenoids is shown in two main groups: Carotenes containing hydrogen and carbon atoms (e.g. β-carotene, α-carotene and lycopene), and Xanthophylls containing hydroxy- and keto groups (e.g. lutein, zeaxanthin, β-cryptoxanthin, astaxanthin, crocin and crocetin) [https://en.wikipedia.org].

The immuno-enhancement properties by non-provitamin A carotenoids were due to their relative activity or the type of immune response induced by them as compared to β-carotene [5]. Generally, their mechanisms in chemo-prevention of cancer contain inhibition of cell proliferation, regulation of cell growth and cell cycle progression, anti-oxidant activity, modulation of carcinogen metabolism, stimulation of cell-to-cell gap junction communication, immune modulation, enhancement of cell differentiation, and apoptosis [7]. In this narrative mini-review, we explain the effects of carotenoids against many forms of cancers along with their mechanisms of action. Indeed, each carotenoid could influence some types of cancers. Furthermore, *in vitro* and *in vivo* anti-tumor effects of curcumin extracted from turmeric powder are shown in several trials. As known, curcumin has a different structure from the carotenoids, but due to its hydroxy- and keto groups and the same functions, it has been extensively involved in carotenoids. Generally, anti-tumor effects of main carotenoids as well as curcumin will be individually described against various types of cancers.

Breast Cancer

Lycopene, β-carotene, crocin and crocetin carotenoids were used to heal breast cancer. The studies showed that cell treatment with lycopene inhibited insulin-like growth factor I (IGF-I)-stimulated cell cycle progression from G_1 to S phase and reduced phosphorylation of retinoblastoma protein (pRb). These actions were related to a decrease in cyclin D_1 and p21$^{CIP1/WAF1}$ levels, but no change in the levels of cyclin E and CDK2/ CDK4 in MCF-7, and ECC-1 cell lines [8]. The carotenoids (lycopene or β-carotene) suppressed estrogen-induced transactivation of the estrogen response element (ERE) reporter gene that was mediated by both estrogen receptors (ER_a and Er_b), and consequently cancer cell proliferation induced by either E_2 or genistein in both T47D and ECC-1 cells. Indeed, carotenoids could induce trans-activation of the antioxidant response element and/or activate other transcription systems such as peroxisome proliferator-activated receptor gamma (PPARc), Aryl-hydrocarbon receptor (AhR), and retinoic

acid receptor (RAR) [9]. Lycopene also stimulated strong activation of the ERK1/2, with cyclin D_1 suppression and p21 upregulation in ER/ PR positive MCF-7, HER2-positive SK-BR-3 and triple-negative MDA-MB-468 cell lines. The highest growth inhibition was detected in MDA-MB-468, when the sub-G_0/ G_1 apoptotic population was significantly enhanced along with cleavage of poly (ADP-ribose) polymerase (PARP). A decrease in cell population was observed in S and G_2/M phases regardless of subtypes of breast cancer [10]. Another study showed that the reduction in cyclin D_1 levels using lycopene may have two main results: a) a decrease in CDK4 kinase activity due to reduction in cyclin D ± CDK4 complexes; b) inhibition of CDK2 activity because of retention of p27 in cyclin E ± CDK2 complexes. These events are responsible for a decrease in phosphorylation of pRb and inhibition of G_1/S transition [11]. In triple negative cells, lycopene repressed the phosphorylation of Akt followed by up-regulation of pro-apoptotic Bax without affecting anti-apoptotic Bcl-xL. Indeed, the increased expression of Bax in MDA-MB-468 cells was responsible for the lycopene-induced apoptosis that was not observed in SK-BR-3 and MCF-7 cells [10]. On the other hand, administration of lycopene, genistein, and their combination to 7,12-Dimethyl Benz(a)anthracene (DMBA)-treated animals significantly decreased Bcl-2 expression and significantly enhanced Bax, caspase 3, and caspase 9 expressions. Both lycopene and genistein reduced breast tumor development, but their combination (lycopene + genistein) showed stronger effects in rats [11]. Generally, the biochemical mechanisms affecting the anti-tumor activities of lycopene are unclear. It seems that lycopene and its derivatives may interact with a nuclear receptor (RAR_α) in ER-positive cells and with RARβ in ER-negative cells leading to high expression of BRCA1, D11-BRCA1, BRCA2, and D12-BRCA2 genes in ER-positive cells in contrast to their low expression in ER-negative cells [12]. On the other hand, the data demonstrated that β-carotene significantly enhanced the expression levels of peroxisome proliferator-activated receptor c (PPAR-) in MCF-7 breast cancer cells involved to regulate differentiation and apoptosis. Beta-carotene also augmented the expression of cyclin-dependent kinase inhibitor p21$^{WAF1/CIP1}$ and reduced the expression of prostanoid synthesis rate-limiting enzyme, cyclooxygenase-2. Beta-carotene could stimulate the production of intracellular reactive oxygen species (ROS) leading to mitochondrial dysfunction, cytochrome C release, and cell apoptosis. These functions induced by β-carotene could be suppressed by us-

ing the reduced glutathione (GSH) [13]. Furthermore, other studies showed that crocin carotenoid stimulates apoptosis in tumor tissue. Indeed, it suppressed the growth of tumors and blocked the cell cycle by down-regulation of cyclin D1. Treatment with crocin significantly inhibited overexpression of cyclin D1 in both mRNA and protein levels as well as p21^{Cip1} in a p53-dependent manner as observed in bladder cancer T24 cell line (TCCB) and BALB/c xenograft tumor [14]. Crocetin carotenoid also prevented the proliferation of MDA-MB-231 breast cancer cells in a similar approach with crocin suggesting that the effects of both carotenoids depend on their polyene backbone [15]. Moreover, a significant increase in expression levels of Bax and cytosolic cytochrome c as well as a decrease in bcl-2 expression was observed after treatment with crocin [16].

Neuroblastoma

The data indicated that lycopene is a superior inhibitor than α- and β-carotene in cancer cells (e.g. neuroblastoma) likely due to its specific structure, i.e. the further number of conjugated double bonds and/or the lack of p-ionone rings. Moreover, α-carotene was more effective than β-carotene in inhibiting the proliferation of the human neuroblastoma cell line [17].

Myeloid Leukemia and Melanoma

Regarding the studies, β-carotene, astaxanthin, and capsanthin could inhibit the proliferation of leukemia K562 cells in a dose- and time-dependent manner, induce cell apoptosis, and regulate cell cycle progression. These carotenoids up-regulated the expression of PPAR   and p21 as well as Nrf2, an important transcription factor in Keap1-Nrf2/EpRE/ARE signaling pathway. In contrast, they down-regulated the expression of cyclin D1 in a dose-dependent manner. Kawada., *et al.* indicated that carotenoids also regulate adipocyte differentiation through changing RXR and peroxisome proliferator-activated receptor gamma (PPAR-γ) activity [18,19]. On the other hand, the results indicated that β-carotene stimulates apoptosis by the activation of caspase-3, caspase-8, caspase-9, and cytochrome c release from mitochondria in human leukemia (HL-60), colon adenocarcinoma (HT-29), and melanoma (SK-MEL-2) cell lines. A dose-dependent decrease in the anti-apoptotic protein Bcl-2 and a dose-dependent increase in the cleaved form of BID (t-BID) were also observed. The data indicated that the activation

of NF-κB plays a major role in apoptosis via β-carotene-induced caspase cascade. The TNF/Fas ligand system/receptor is also involved in the activation of caspase-8 by β-carotene [20,21]. Beta-carotene could significantly inhibit the production of nitric oxide (NO) and tumor necrosis factor (TNF-α), and stimulate apoptosis through suppression of bcl-2 expression and up-regulation of p53 and caspase-3 in B16F-10 melanoma cells. Also, β-carotene could down-regulate the expression of inducible nitric oxide synthase (iNOS) gene in these cells [22].

Colorectal Cancer

In an *in vitro* study, the anti-tumor effects of crocin on three human colorectal cancer cells (HCT-116, SW-480, and HT-29) were evaluated. The results indicated a significant anticancer activity of crocin on HCT-116 as compared to two other cell lines likely due to the presence of wild-type p53 in HCT-116 cells which it was mutated in SW-480 and HT-29 cells [23,24]. In addition, HCT-116 cells showed a significant decrease in S phase and an increase in G2 phase followed by later proliferation. On the other hand, saffron elicited autophagy in a p53-dependent approach. Indeed, the level of LC3-II, a marker of the accumulation of autophagosomes, indicated that saffron stimulated a further conversion from LC3-I to LC3-II in HCT116 p53$^{-/-}$ cells than in HCT116 p53 wild type cells at different times. A higher Beclin 1 protein level interferring with autophagic vesicle formation was also observed in the HCT116 p53$^{-/-}$ cells after saffron treatment. In general, the induction of apoptosis by saffron extract plays an important role in the death of human hepatocellular carcinoma cells and HeLa cells [25]. Dietary administration of Astaxanthin (AX) decreased the IL-1β, IL-6, COX-2, TNF-α, and iNOS mRNA expression, inhibited proliferation, and induced apoptosis in the colonic adenocarcinomas. Indeed, it suppressed significantly several inflammatory events and NF-κB expression. As known, the NF-κB pathway has a main role in anti-apoptotic signaling. Thus, AX demonstrated the chemo-preventive effect on colon carcinogenesis through several pathways, e.g. NF-κB pathway [26].

Gallbladder Adenocarcinoma

Curcumin represented dose-dependent anti-tumor activity against HAG-1 cells, suggesting the cell arrest in G_2/M phase, with progress in the apoptotic cell population (i.e. a high ratio of cells in the G_2/M phase against a low ratio of cells in the G_0/G_1 phase). The sub-G_0/G_1 cell population indicating apoptotic cells was gradually increased 3.1%, 6.8%, 18.4% at 24, 48, and 72 h post-treatment. These results indicated that curcumin reduced the cell proliferation and survival through activation of the MAPK pathway, and inactivation of AKT-mTOR pathway. Curcumin decreased the expression and phosphorylation of anti-apoptotic Bcl-2, but did not influence the expression of pro-apoptotic Bax and anti-apoptotic NF-κB [27]. Other study showed that curcumin prevented NF-κB activation via inhibition of p65 translocation to the nucleus and also IκBα degradation in different cell types [28]. In general, curcumin could suppress the expression of cyclin-D1, IL-6, Bcl-2, Bcl-xL, cyclooxygenase-2 and matrix metallopeptidase-9 genes involved in cell proliferation using inhibition of NF-κB activation [29]. Moreover, curcumin may stimulate apoptosis mainly by inactivation of Bcl-2 through NF-κB signaling-independent mechanism in HAG-1 gallbladder adenocarcinoma cells [30].

Oral Cancer

Astaxanthin suppressed NF-κB and Wnt signaling, and reduced phosphorylation of IκB-α by down-regulating the expression of IKK-β and GSK-3β, thus preventing the nuclear translocation of NF-κB p65, DNA binding and transactivation potential of NF-κB. Indeed, Astaxanthin may inhibit these pathways through inactivation of the upstream signaling kinases Erk/Akt. Astaxanthin also stimulated caspase-mediated mitochondrial apoptosis by down-regulating the expression of anti-apoptotic Bcl-2, p-Bad, survivin, and up-regulating pro-apoptotic Bax and Bad, along with efflux of Smac/Diablo and cytochrome-c into the cytosol, and induced cleavage of poly (ADP-ribose) polymerase (PARP) [31]. On the other hand, a significant reduction in cell viability was observed in curcumin-treated oral carcinoma CAL-27 cells, indicating stimulation of apoptosis and also down-regulation of Notch-1 and NF-κB [32]. In addition, the studies supported the use of crocin as a chemoprophylactic or therapeutic agent for oral squamous cell carcinomas (OSCCs). Crocin could inhibit the synthesis of cellular nucleic acids [33].

Prostate Cancer

The response of androgen-independent cells (PC3) to lycopene was similar to that of androgen-responsive cells (LNCaP), but the maximal growth suppression of PC3 cells was less than that in LN-CaP cells. The data showed that lycopene inhibited mitotic activity in both cells [34]. The anti-tumor effect of Lycopene was caused by changes in mevalonate pathway and in Ras activation [35]. These data confirmed other studies indicating that lycopene is able to suppress intracellular total cholesterol synthesis in cultured macrophages and in human by decreasing the expression of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and also by inactivating Ras [35-37]. Moreover, lycopene decreased the Ras-dependent activation of NF-κB, inhibited production of reactive oxygen species, and reduced the phosphorylation of c-jun N-terminal kinase, extracellular signal-regulated kinase 1/2 and p38. These effects were also associated with an arrest of cell cycle progression and apoptosis induction, a decrease in cyclin D1 and phospho-AKT levels and an increase in p21, p27 and p53 levels and in Bax: Bcl-2 ratio. Lycopene-treated cells indicated a dose-dependent decrease of cyclin D1 and a dose-dependent increase in p53, p21$^{WAF1/CIP1}$ and p27. LNCaP cells therapy with lycopene also reduced AKT phosphorylation in a dose-dependent manner. Altogether, these findings showed a novel insight for the growth-inhibitory effects of lycopene in cancer [35]. In addition, the combination of several carotenoids (e.g. lycopene, phytoene and phytofluene) synergistically inhibited the androgen receptor activity and activated the Electrophile/Antioxidant Response Element transcription system (EpRE/ARE reporter gene) for prostate cancer prevention [38]. A phase II randomized clinical trial showed that patients treated with lycopene had a decrease in the plasma prostate-specific antigen (PSA) level, which is a parameter of *prostate cancer* (Pca) burden. The results suggested that lycopene supplementation may decrease the growth of PCa, likely due to up-regulation of connexin 43 *(Cx43)* [39]. Oral administration of lycopene significantly prevented the growth of DU145 tumors in BALB/c mice suggesting that lycopene can influence the growth of cancer cells and stimulate differentiation or apoptosis in a stage-specific manner as well as its potent antioxidant properties [40]. On the other hand, human prostate cancer LNCaP cells were more sensitive to liposomal curcumin (∼70-80% inhibition of cellular proliferation) than C4-2B cells. Free curcumin could inhibit the cell proliferation only at 10-fold higher doses. In fact, Dipalmitoyl phosphatidylcholine (DPPC) and dimyristoyl phosphatidyl choline (DMPC) liposomal curcumin were more effective inhibitors than free curcumin in prostate cancer cells [41]. Moreover, the nano-and micro-curcumin particles led to a high level of curcumin in the blood which could effectively suppress the growth of tumor in mice. The order of uptake capacity of curcumin and curcumin nanoformulations and subsequently induction of apoptosis was observed as dendrimer-curcumin > cellulose-curcumin > beta-cyclodextrin (CD)-curcumin > PLGA nanoparticles-curcumin > magnetic nanoparticles (MNP)-curcumin > curcumin in prostate cancer cells [42]. For instance, PLGA-curcumin nanoparticles enhanced the expression of tumor suppressor PKD1, inhibited STAT3 and AKT phosphorylation, induced apoptosis through inhibition of MCL-1, Bcl-xL anti-apoptotic proteins, and stimulated PARP cleavage in prostate cancer cells [43]. Transcriptional activities of AP-1 and NF-κB, and expression of CREB binding protein (CBP) were significantly reduced in the curcumin-treated LNCaP and PC-3 cell lines [44]. Another studies showed that high-dose lycopene and β-carotene significantly reduced the expression of proliferating cell nuclear antigen (PCNA) in tumor tissues, enhanced the levels of insulin-like growth factor-binding protein-3 in plasma, and subsequently decreased the growth of prostate tumor cells. Furthermore, the inhibition of the vascular endothelial growth factor (VEGF) by lycopene indicated its role as an anti-angiogenesis agent in comparison with *β*-carotene [45]. Generally, Dietary lycopene, *α*-carotene, *β*-carotene, *β*-cryptoxanthin, lutein, and zeaxanthin could decrease the risk of prostate cancer in a dose-dependent manner [15]. For example, high doses of serum *β*-carotene may augment the risk of prostate cancer in middle-aged men [46].

Pancreatic Cancer

Curcumin decreased the expression of NF-κB-regulated gene products, including cyclooxygenase-2, prostaglandin E2, and interleukin-8 (IL-8), in invasive pancreatic carcinoma. These changes were related to dose- and time-dependent anti-proliferative activity and pro-apoptotic effects [47]. The reports indicated that Gemcitabine is a suitable drug for treatment of pancreatic cancer, but drug resistance is a problem in patient, thus, compounds enhancing the antitumor effects of gemcitabine are required to cure

this cancer. For instance, curcumin inhibited the cell proliferation, increased the apoptosis induced by gemcitabine, and suppressed NF-κB activation in different pancreatic cancer cell lines. Our results showed that their combination was highly effective as compared to curcumin and gemcitabine alone [48]. Indeed, their combination significantly suppressed angiogenesis and down-regulated the expression of Ki-67 in tumor tissues [48]. In addition, nanoparticulate curcumin inhibited the NF-κB regulated gene expression via suppression of IκBa and Akt activation, increased cellular uptake, induced apoptosis, and reduced cell viability effectively higher than native curcumin [49].

Cervical Cancer

The findings showed a significant inverse relationship between risk of cervical cancer and α-carotene [50]. Crocin, crocetin and dimethylcrocetin (DMC) carotenoids derived from natural saffron are responsible for its color [51,52]. The cytotoxic and apoptogenic effects of saffron extract and crocin in TC-1 malignant cells were more than COS-7 non-malignant cells [53]. Moreover, the survival rate for mice treated with crocin was 100 % as compared to groups receiving DNA vaccine alone (∼66.7 %) and DNA vaccine along with crocin (∼33.3 %) indicating the high potency of crocin as a chemotherapeutic agent [54]. Moreover, the proliferation of cervical cancer cell line (HeLa), ovarian cancer cell line (SKOV3), and non-small lung cancer cell line (A549) was significantly suppressed by incubation with crocetin in a dose-dependent manner. Crocetin significantly induced G1 arrest through p53-dependent and p53-independent mechanisms along with p21$^{WAF1/Cip1}$ induction in cancer cells. Crocetin activated the caspase cascade to promote apoptosis. These data suggested that the combination of crocetin and vincristine would highly develop the anticancer effect of vincristine [55]. In addition, crocetin suppressed DNA, RNA, and protein synthesis in malignant cells. Among major saffron carotenoids, crocetin showed the highest binding affinity for DNA. The previous studies indicated that crocetin inhibits skin carcinogenesis induced by 12-O-Tetradecanoylphorbol-13-acetate (TPA) [56] and also the generation of cellular reactive oxygen species in lung cancer *in vivo* [55,57]. Treatment with both crocetin and crocin carotenoids showed a significant activation of nuclear factor erythroid 2-related factor 2 (Nrf2) indicating a protective response against oxidative stress in HeLa cells [58,59]. Crocetin significantly decreased the lev-

els of lactate dehydrogenase A (LDHA) expression, one of the targets for chemoprevention in cancer cells, compared to crocin [60]. On the other hand, the studies showed that phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway is associated with the development of cervical cancer. In this line, fucoxanthin could induce apoptosis in HeLa cell line via PI3K/Akt pathway [61]. In addition, crocin liposomal forms showed high cytotoxic effects and induced apoptosis as compared to the crocin in HeLa and MCF-7 cells [62].

Hepatoma

Fucoxanthin decreased cisplatin-induced NF-κB expression and increased the NF-κB-regulated Bax/Bcl-2 mRNA ratio, thus the combination of fucoxanthin and cisplatin could be suggested as a novel therapeutic strategy against human hepatoma cells [63]. A previous report showed that inhibition of human hepatoma HepG2 cell proliferation by fucoxanthin is associated with cell cycle arrest by down-regulation of cyclin D and induction of GADD45A gene expression [64-66]. Fucoxanthin displayed anti-drug resistance potential due to reduced interaction between pregnane X receptor (PXR) and coactivator (SRC-1), inhibiting activation of PXR-mediated CYP3A4 and MDR1 expression [67]. The combination of fucoxanthin, NAI (NF-κB activation inhibitor) and cisplatin synergistically enhanced the ratio of Bax/Bcl-2 mRNA expression, likely associated with inhibition of the NF-κB pathway [63].

Gastric Cancer

Treatment with crocin significantly increased sub-G_1 population, activated caspases, and enhanced the Bax/Bcl-2 ratio in the AGS cells indicating its anticancer effect. Elevation of the Bax/Bcl-2 ratio was observed only in the human gastric carcinoma (AGS)-treated cells and not in the human normal fibroblastic (HFSF-PI3)-treated cells [68]. Regarding to the reports, crocin exerted cytotoxic activities in various human tumors, including acute promyelocytic leukemia cell line (HL-60), hepatocellular carcinoma (KIM-1), HeLa cells, lymphoid leukemia (MOLT-4B) cells, and K562 cells by inducing programmed cell death, and therefore arresting proliferation [68,69]. Similar to crocin, crocetin markedly enhanced the expression of Bax and significantly decreased the anti-apoptotic Bcl-2 expression in the AGS cells in a time-dependent manner; however no significant changes in Bcl-2 and Bax mRNA were observed. There were no detectable changes

in the number of the cells in the G_0/G_1, S, and G_2+M phases after incubation of the tumor cells with crocetin in different times, but it significantly increased the cell population with sub-G_1 DNA content. Crocetin treatment significantly reduced the Bcl-2/Bax mRNA ratio in the AGS cell lines and affected protein kinase C translocation, and c-Jun, c-Fos, and c-Myc gene expression [70]. Crocetin increased caspase activity up to 17 times after 48h of incubation with AGS cells [71]. High concentration of β-carotene also induced apoptosis of AGS cells in parallel with nuclear loss of ataxia-telangiectasia mutated (ATM) protein. In response to DNA damage, ATM activated multiple signaling pathways, such as cell cycle checkpoints, DNA repair, and apoptosis. Indeed, the nuclear level of ATM decreased in the cells treated with β-carotene. Moreover, β-carotene stimulated apoptotic protein expression such as p53 and Bax, and suppressed Bcl-2 expression in gastric cancer cells [34,72].

Lung Cancer

Crocetin has strong antioxidant properties in lung cancer-bearing mice [28]. Its inhibitory mechanisms on malignancy include modulating cell transformation, decreasing the degree of lung cancer growth, and controlling cell proliferation and differentiation. As observed, the levels of polyamines were enhanced in tumor-bearing animals. Using crocetin carotenoid, the levels of polyamine synthesis were decreased, likely due to the inhibition of ornithine decarboxylase activity and reduction of cell proliferation in B(a) P-induced lung carcinogenesis. Indeed, crocetin is an important chemo-preventive agent against B(a)P-induced lung carcinogenesis by protection of the glycoprotein levels in serum and tissues [73]. Treatment of A549 cells with various concentrations of crocetin led to a dose-dependent decrease in nucleic acid and protein synthesis. Two human lung tumor cells (A549 and VA-13) showed similar sensitivity to crocetin-induced inhibition of DNA, RNA and protein synthesis. In contrast, human HeLa tumor cells were significantly more sensitive to the inhibition of nucleic acid synthesis likely due to a direct effect of crocetin on the synthetic reactions and less sensitive to the suppression of protein synthesis [74]. Supplementation with Lycopene prevented the development of lung squamous metaplasia, cell proliferation, and elevated BAD-mediated apoptosis in ferrets [75]. A report demonstrated that tomato-based products rich in carotenoids may significantly decrease the risk of lung cancer [76]. Another study also indicated a significant inhibitory effect of lycopene on development of lung neoplasia in male mice [77-79].

Conclusion

Regarding to the epidemiological studies, populations that use a diet rich in carotenoids have a lower cancer risk. The experiments strongly suggest that carotenoids are safe, but they may be toxic and exert carcinogenic effects under specific conditions and high doses. Several mechanisms were suggested to cause the anticancer properties of carotenoids such as antioxidant activities. These molecules can stimulate apoptosis in tumor cells. Their ability to modify the expression of transcription factors and/or proteins involved in the apoptotic pathway depends on carotenoid concentration, the effects of other carotenoids, and tumor cell type. There are some problems that are still unsolved. For example, carotenoid levels obtained in experiments are much higher than those in human blood. In addition, a synergistic action of low concentrations of various carotenoids is usually more effective than each carotenoid alone. On the other hand, it is not clear that the observed changes in various cellular pathways are due to direct effects of the carotenoid molecules or are mediated by their derivatives. However, further studies are necessary to determine the effective and active carotenoid derivatives.

Conflicts of Interest

The authors declare that there is no conflict of interest statement.

Bibliography

1. Stahl W and Sies H. "Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans". *Journal of Nutrition* 122.11 (1992): 2161-2166.

2. Tanaka T., *et al*. "Cancer chemoprevention by carotenoids". *Molecules* 17 (2012): 3202-3242.

3. Smith TA. "Carotenoids and cancer: prevention and potential therapy". *British Journal of Biomedical Science* 55.4 (1998): 268-275.

4. Johnson EJ. "The role of carotenoids in human health". *Nutrition in Clinical Care* 5.2 (2002): 56-65.

5. Chew BP and Park JS. "Carotenoid action on the immune response". *Journal of Nutrition* 134.1 (2004): 257S-261S.

6. Hughes DA. "Effects of carotenoids on human immune function". *Proceedings of the Nutrition Society* 58 (1999): 713-718.

7. Bolhassani A., *et al*. "Saffron and natural carotenoids: Biochemical activities and anti-tumor effects". *Biochimica et Biophysica Acta* 1845.1 (2014): 20-30.

8. Nahum A., *et al*. "Lycopene inhibition of IGF-induced cancer cell growth depends on the level of cyclin D1". *European Journal of Nutrition* 45.5 (2006): 275-282.

9. Hirsch K., *et al*. "Lycopene and other carotenoids inhibit estrogenic activity of 17beta-estradiol and genistein in cancer cells". *Breast Cancer Research and Treatment* 104.2 (2007): 221-230.

10. Takeshima M., *et al*. "Anti-proliferative and apoptosis-inducing activity of lycopene against three subtypes of human breast cancer cell lines". *Cancer Science* 105 (2014): 252-257.

11. Sahin K., *et al*. "Inhibitory effects of combination of lycopene and genistein on 7,12-dimethyl benz(a)anthracene-induced breast cancer in rats". *Nutrition and Cancer* 63.8 (2011): 1279-1286.

12. Chalabi N., *et al*. "The effects of lycopene on the proliferation of human breast cells and BRCA1 and BRCA2 gene expression". *European Journal of Cancer* 40.11 (2014): 1768-1775.

13. Cui Y., *et al*. "Beta-carotene induces apoptosis and up-regulates peroxisome proliferator-activated receptor gamma expression and reactive oxygen species production in MCF-7 cancer cells". *European Journal of Cancer* 43.17 (2007): 2590-2601.

14. Ashrafi M., *et al*. Effect of crocin on cell cycle regulators in N-Nitroso-N-Methylurea-induced breast cancer in rats". *DNA and Cell Biology* 34.11 (2015): 684-691.

15. Chryssanthi DG., *et al*. "Inhibition of breast cancer cell proliferation by style constituents of different Crocus species". *Anticancer Research* 27 (2007): 357-362.

16. Lu P., *et al*. "Antitumor effects of crocin on human breast cancer cells". *International Journal of Clinical and Experimental Medicine* 8.11 (2015): 20316-20322.

17. Levy J., *et al*. "Lycopene is a more potent inhibitor of human cancer cell proliferation than either alpha-carotene or β-carotene". *Nutrition and Cancer* 24 (1995): 257-266.

18. Zhang X., *et al*. "Carotenoids inhibit proliferation and regulate expression of peroxisome proliferators-activated receptor gamma (PPARgamma) in K562 cancer cells". *Archives of Biochemistry and Biophysics* 512.1 (2011): 96-106.

19. Kawada T., *et al*. "Carotenoids and retinoids as suppressors on adipocyte differentiation via nuclear receptors". *BioFactors* 13 (2000): 103-109.

20. Palozza P., *et al*. "Mechanism of activation of caspase cascade during beta-carotene-induced apoptosis in human tumor cells". *Nutrition and Cancer* 47.1 (2003): 76-87.

21. Zhuang S., *et al*. "Caspase-8 mediates caspase-3 activation and cytochrome c release during singlet oxygen-induced apoptosis of HL-60 cells". *Experimental Cell Research* 250.1 (1995): 203-212.

22. Guruvayoorappan C and Kuttan G. "β-Carotene down-regulates inducible nitric oxide synthase gene expression and induces apoptosis by suppressing bcl-2 expression and activating caspase-3 and p53 genes in B16F-10 melanoma cells". *Nutrition Research* 27.6 (2007): 336-342.

23. Chau BN and Wang JY. "Coordinated regulation of life and death by RB". *Nature Reviews Cancer* 3 (2003): 130-138.

24. Aung HH., *et al*. "Crocin from Crocus sativus possesses significant anti-proliferation effects on human colorectal cancer cells". *Experimental Oncology* 29.3 (2007): 175-180.

25. Bajbouj K., *et al*. "The anticancer effect of saffron in two p53 isogenic colorectal cancer cell lines". *BMC Complementary and Alternative Medicine* 12 (2012): 69.

26. Yasui Y., *et al*. "Dietary astaxanthin inhibits colitis and colitis-associated colon carcinogenesis in mice via modulation of the inflammatory cytokines". *Chemico-Biological Interactions* 193.1 (2011): 79-87.

27. Ono M., *et al*. "Antiproliferative and apoptosis-inducing activity of curcumin against human gallbladder adenocarcinoma cells". *Anticancer Research* 33.5 (2013): 1861-1866.

28. Singh S and Aggarwal BB. "Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane)". *The Journal of Biological Chemistry* 270.42 (1995): 24995-25000.

29. Aggarwal S., *et al*. "Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor-kappaB signaling". *International Journal of Cancer* 111.5 (2004): 679-692.

30. Lu Z and Xu S. "ERK1/2 MAP kinases in cell survival and apoptosis". *IUBMB Life* 58.11 (2006): 621-631.

31. Kavitha K., *et al*. "Astaxanthin inhibits NF-kappaB and Wnt/beta-catenin signaling pathways via inactivation of Erk/MAPK and PI3K/Akt to induce intrinsic apoptosis in a hamster model of oral cancer". *Biochimica et Biophysica Acta* 1830 (2013): 4433-4444.

32. Liao S., *et al*. "Inhibitory effect of curcumin on oral carcinoma CAL-27 cells via suppression of Notch-1 and NF-κB signaling pathways". *Journal of Cellular Biochemistry* 112.4 (2011): 1055-1065.

33. Sun J., *et al*. "Crocin inhibits proliferation and nucleic acid synthesis and induces apoptosis in the human tongue squamous cell carcinoma cell line Tca8113". *Asian Pacific Journal of Cancer Prevention* 12.10 (2011): 2679-2683.

34. Ivanov NI., *et al*. "Lycopene differentially induces quiescence and apoptosis in androgen-responsive and -independent prostate cancer cell lines". *Clinical Nutrition* 26 (2007): 252-263.

35. Palozza P., *et al*. "Lycopene induces cell growth inhibition by altering mevalonate pathway and Ras signaling in cancer cell lines". *Carcinogenesis* 31.10 (2010): 1813-1821.

36. Fuhrman B., *et al*. "Hypocholesterolemic effect of lycopene and beta-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages". *Biochemical and Biophysical Research Communications* 233.3 (1997): 658-662.

37. Elson CE., *et al*. "Isoprenoid-mediated inhibition of mevalonate synthesis: potential application to cancer". *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine* 221.4 (1999): 294-311.

38. Linnewiel-Hermoni K., *et al*. "The anti-cancer effects of carotenoids and other phytonutrients resides in their combined activity". *Archives of Biochemistry and Biophysics* 572 (2015): 28-35.

39. Kucuk O., *et al*. "Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy". *Cancer Epidemiology, Biomarkers and Prevention* 10.8 (2001): 861-868.

40. Tang L., *et al*. "Lycopene inhibits the growth of human androgen-independent prostate cancer cells in vitro and in BALB/c nude mice". *Journal of Nutrition* 135.2 (2005): 287-290.

41. Thangapazham RL., *et al*. "Evaluation of a nanotechnology-based carrier for delivery of curcumin in prostate cancer cells". *International Journal of Oncology* 32.5 (2008): 1119-1123.

42. Yallapu MM., *et al*. "Design of curcumin loaded cellulose nanoparticles for prostate cancer". *Current Drug Metabolism* 13.1 (2012): 120-128.

43. Yallapu MM., *et al*. "Anti-cancer activity of curcumin loaded nanoparticles in prostate cancer". Biomaterials 35.30 (2014): 8635-8648.

44. Nakamura K., *et al*. "Curcumin down-regulates AR gene expression and activation in prostate cancer cell lines". *International Journal of Oncology* 21.4 (2002): 825-830.

45. Yang CM., *et al*. "Growth inhibitory efficacy of lycopene and β-carotene against androgen-independent prostate tumor cells xenografted in nude mice". *Molecular Nutrition and Food Research* 55.4 (2011): 606-612.

46. Karppi J., *et al*. "Serum beta-carotene in relation to risk of prostate cancer: the Kuopio Ischaemic Heart Disease Risk Factor study". *Nutrition and Cancer* 64.3 (2012): 361-367.

47. Li L., *et al*. "Nuclear factor-κB and IκB kinase are constitutively active in human pancreatic cells, and their down-regulation by curcumin (diferuloylmethane) is associated with the suppression of proliferation and the induction of apoptosis". *Cancer* 101.10 (2014): 2351-2362.

48. Kunnumakkara AB., *et al*. "Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products". *Cancer Research* 67.8 (2007): 3853-3861.

49. Mohanty C and Sahoo SK. "The in vitro stability and in vivo pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation". *Biomaterials* 31 (2010): 6597-6611.

50. Guo L., *et al*. "Associations between antioxidant vitamins and the risk of invasive cervical cancer in Chinese women: A case-control study". *Scientific Reports* 5 (2015): 13607.

51. Bathaie SZ., *et al*. "Interaction of saffron carotenoids as anti-cancer compounds with ctDNA, Oligo (dG.dC)15, and Oligo (dA.dT)15". *DNA and Cell Biology* 26.8 (2007): 533-540.

52. Bolhasani A., *et al*. "Separation and purification of some components of Iranian saffron". *Asian Journal of Chemistry* 17 (2005): 725-729.

53. Alizadeh F and Bolhassani A. "In vitro cytotoxicity of Iranian saffron and two main components as a potential anti-cancer drug". *SM Journal of Pharmacology and Therapeutics* 1.1 (2015): 1-5.

54. Khavari A., *et al*. "Chemo-immunotherapy using saffron and its ingredients followed by E7-NT (gp96) DNA vaccine generates different anti-tumor effects against tumors expressing the E7 protein of human papillomavirus". *Archive of Virology* 160.2 (2015): 499-508.

55. Zhong YJ., *et al*. "Crocetin induces cytotoxicity and enhances vincristine-induced cancer cell death via p53-dependent and -independent mechanisms". *Acta Pharmacologica Sinica* 32 (2011): 1529-1536.

56. Hsu JD., *et al*. "Suppression of the TPA-induced expression of nuclear-protooncogenes in mouse epidermis by crocetin via antioxidant activity". *Anticancer Research* 19 (1999): 4221-4227.

57. Magesh V., *et al*. "Antitumour activity of crocetin in accordance to tumor incidence, antioxidant status, drug metabolizing enzymes and histopathological studies". *Molecular and Cellular Biochemistry* 287.1-2 (2006): 127-135.

58. Ohta T., *et al*. "Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth". *Cancer Research* 68 (2008): 1303-1309.

59. Wang XJ., *et al*. "Nrf2 enhances resistance of cancer cells to

chemotherapeutic drugs, the dark side of Nrf2". *Carcinogenesis* 29 (2008): 1235-1243.

60. Kim SH., *et al.* "Proposed cytotoxic mechanisms of the saffron carotenoids crocin and crocetin on cancer cell lines". *Biochemistry and Cell Biology* 92.2 (2014): 105-111.

61. Ye G., *et al.* "Fucoxanthin induces apoptosis in human cervical cancer cell line HeLa via PI3K/Akt pathway". *Tumor Biology* 35 (2014): 11261-11267.

62. Mousavi SH., *et al.* "Improvement of cytotoxic and apoptogenic properties of crocin in cancer cell lines by its nanoliposomal form". *Pharmaceutical Biology* 49.10 (2011): 1039-1045.

63. Liu CL., *et al.* "Fucoxanthin enhances cisplatin-induced cytotoxicity via NFkappaB-mediated pathway and downregulates DNA repair gene expression in human hepatoma HepG2 cells". *Marine Drugs* 11.1 (2013): 50-66.

64. Satomi Y and Nishino H. "Implication of mitogen-activated protein kinase in the induction of G1 cell cycle arrest and gadd45 expression by the carotenoid fucoxanthin in human cancer cells". *Biochimica et Biophysica Acta* 1790 (2009): 260-266.

65. Das SK., *et al.* "Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D". *Biochimica et Biophysica Acta* 1780.4 (2008): 743-749.

66. Yoshiko S and Hoyoku N. "Fucoxanthin, a natural carotenoid, induces G1 arrest and GADD45 gene expression in human cancer cells". *In vivo* 21.2 (2007): 305-309.

67. Liu CL., *et al.* "Fucoxanthin attenuates rifampin-induced cytochrome P450 3A4 (CYP3A4) and multiple drug resistance 1 (MDR1) gene expression through pregnane X receptor (PXR)-mediated pathways in human hepatoma HepG2 and colon adenocarcinoma LS174T cells". *Marine Drugs* 10.1 (2012): 242-257.

68. Hoshyar R., *et al.* "Crocin triggers the apoptosis through increasing the Bax/Bcl-2 ratio and caspase activation in human gastric adenocarcinoma, AGS cells". *DNA and Cell Biology* 32.2 (2013): 50-57.

69. Thatte U., *et al.* "Modulation of programmed cell death by medicinal plants". *Cellular and Molecular Biology* 46.1 (2000): 199-214.

70. Ben-Dor A., *et al.* "Effects of acyclo-retinoic acid and lycopene on activation of the retinoic acid receptor and proliferation of mammary cancer cells". *Archives of Biochemistry and Biophysics* 391.2 (2001): 295-302.

71. Bathaie SZ., *et al.* "Anticancer effects of crocetin in both human adenocarcinoma gastric cancer cells and rat model of gastric cancer". *Biochemistry and Cell Biology* 91.6 (2013): 397-403.

72. Karas M., *et al.* "Lycopene interferes with cell cycle progression and insulin-like growth factor I signaling in mammary cancer cells". *Nutrition and Cancer* 36.1 (2000): 101-111.

73. Magesh V., *et al.* "In vivo protective effect of crocetin on benzo(a)pyrene-induced lung cancer in Swiss albino mice". *Phytotherapy Research* 23.4 (2009): 533-539.

74. Abdullaev FI. "Inhibitory effect of crocetin on intracellular nucleic acid and protein synthesis in malignant cells". *Toxicology Letters* 70 (1994): 243-251.

75. Liu C., *et al.* "Lycopene supplementation inhibits lung squamous metaplasia and induces apoptosis via up-regulating insulin-like growth factor-binding protein 3 in cigarette smoke-exposed ferrets". *Cancer Research* 63.12 (2003): 3138-3144.

76. Holick CN., *et al.* "Dietary carotenoids, serum beta-carotene, and retinol and risk of lung cancer in the alpha-tocopherol, beta-carotene cohort study". *American Journal of Epidemiology* 156.6 (2002): 536-547.

77. Kim DJ., *et al.* "Chemoprevention by lycopene of mouse lung neoplasia after combined initiation treatment with DEN, MNU and DMH". *Cancer Letter* 120.1 (1997): 15-22.

78. Yan B., *et al.* "Specific serum carotenoids are inversely associated with breast cancer risk among Chinese women: a case-control study". *British Journal of Nutrition* 115.1 (2016): 129-137.

79. Swanson HM., *et al.* "Lutein, but not other carotenoids, selectively inhibits breast cancer cell growth through several molecular mechanisms". *The FASEB Journal* 30.1 (2016): 34.2.