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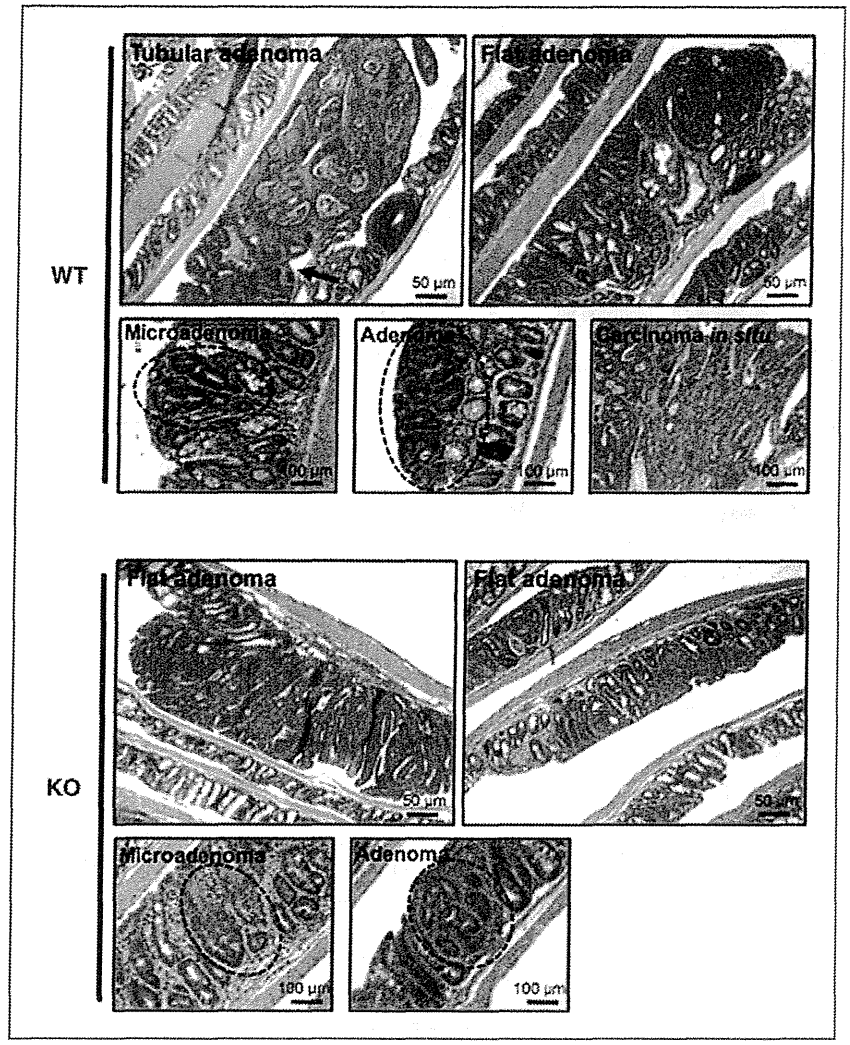

Figure 2. Evaluation of colonic lesions. Tumors in WT mice exhibit tubular or flat adenoma, whereas *mPGES-1* KO mice only develop flat or slightly raised adenomas. Microadenomas and adenomas were found in the colons of both genotypes. Carcinomas *in situ* were identified only in the WT colons. Lesions are delineated by a dotted line. $n = 14$ in WT and $n = 19$ in KO mice. Scale bars as indicated.

microadenomas showed slight, moderate, or severe nuclear atypia without evidence of expansion and invasive growth, characteristic of AOM-induced tumors occurring in this model (refs. 7, 12; Fig. 2). The morphology of the adenomas was similar between genotypes, comprised largely of microscopically tubular structures. However, tumors in WT mice were considerably larger, with frequent compression of surrounding normal crypts. In several cases, there was evidence for moderately differentiated tubular carcinomas *in situ* with invasive growth (Fig. 2, WT). Analysis of crypt dynamics revealed that within the normal colonic epithelium, the absence of *mPGES-1* did not affect overall crypt length, nor alter the size of the proliferative compartment in comparison to the WT colons (Supplementary Fig. S1).

mPGES-1 status does not affect tumor markers

The profound suppression in tumor growth observed in the *mPGES-1* KO mice raised the possibility that PGE_2 levels may directly affect cell turnover within the tumor epithelium. To evaluate this possibility, colon tumors were examined immunohistochemically for PCNA staining (proliferation), and cleaved caspase-3, which detects an earlier stage of apoptosis in cells that have not yet undergone major morphologic changes (14). A total of 10 colons from each genotype were selected for immunohistochemical analyses. Representative adenomas from a WT and *mPGES-1* KO colon are shown in Figure 3A. Intense PCNA staining was present throughout the tumor epithelium, regardless of *mPGES-1* genotype and independent of tumor size (Fig. 3A, PCNA). Cleaved caspase-3 immunostaining

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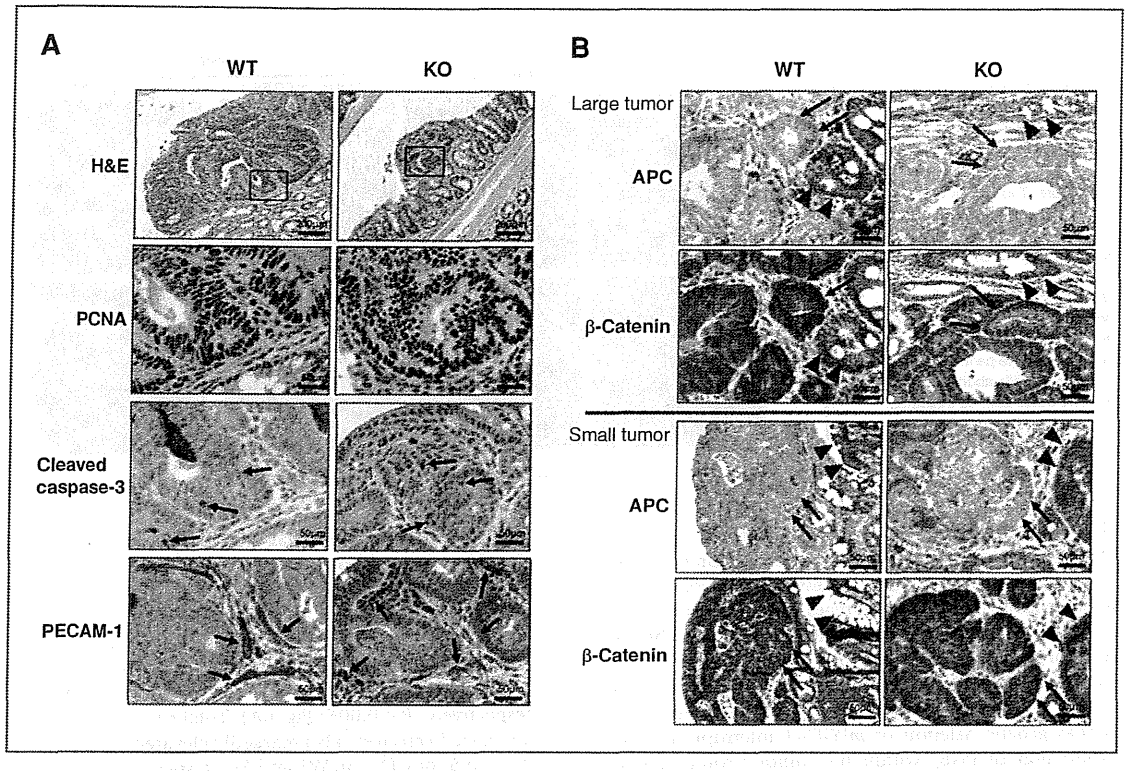

Figure 3. Immunohistochemical analysis of colon tumor markers. A, representative serial sections of colons showing staining for PCNA, cleaved caspase-3, and PECAM-1, where positive staining is indicated by the arrows. Boxed areas in H&E sections were enlarged to show the positive staining. Scale bars as indicated. B, representative images of large and small tumors from each genotype stained for APC and β-catenin. Tumor cells from either genotype lack expression of APC (arrows) compared with adjacent normal crypts (arrowheads), independent of tumor size. Similarly, β-catenin staining is increased in the tumor cells (arrows) compared with adjacent normal cells (arrowheads), independent of genotype or tumor size. $n = 10$ per group. Scale bars as indicated.

revealed few apoptotic cells within the normal crypts (data not shown), and their frequency was unaffected by *mPGES-1* status, nor affected by tumor size (Fig. 3A, cleaved caspase-3). These observations suggest that *mPGES-1* status does not influence cell turnover in the AOM colon tumor model.

In contrast to our previous findings, whereby deletion of *mPGES-1* was found to disrupt neovessel growth within small intestinal tumors in $Apc^{\Delta 14/+}$ mice (4), *mPGES-1* deletion did not directly affect PECAM-1 staining within and adjacent to AOM-induced colon tumors (Fig. 3A, PECAM-1). Even in the smallest colon lesions examined in the *mPGES-1* KO mice, PECAM-1 staining within the tumor stroma and surrounding colonic mucosa showed the presence of well-formed vascular structures (arrows; Fig. 3A, PECAM-1). The difference in PECAM-1 staining between these 2 studies, however, may result from the dissimilar experimental systems employed including differing genetic backgrounds and distinct mechanisms of tumor initiation.

We next examined the possibility that disruption of PGE_2 formation may directly impact Wnt signaling, an effect that was shown earlier in adenomatous polyposis coli (APC)-deficient DLD-1 colon cancer cells (15). Surprisingly, loss of APC protein, with increased cytoplasmic staining and nuclear localization of β-catenin, was equivalent within tumors regardless of *mPGES-1* genotype or tumor size (Fig. 3B). The loss of APC protein is consistent with previous findings showing that AOM-induced tumors do not express the full-length protein (16, 17). Taken together, these data suggest that the cancer suppression associated with reduced PGE_2 formation is not related to aberrant cell turnover or dysregulated β-catenin signaling in transformed epithelial cells.

mPGES-1 is expressed in the stroma in the colon

To define the localization of mPGES-1, immunofluorescence imaging was done on colonic mucosa prepared from mice harboring tumors. In the WT colons, we identified increased expression of mPGES-1 localized primarily at the apical surface of the tumor stroma (Fig. 4). Positive staining was also found within the stroma immediately adjacent to the tumors (ref. 18; Fig. 4, arrows). Similar to

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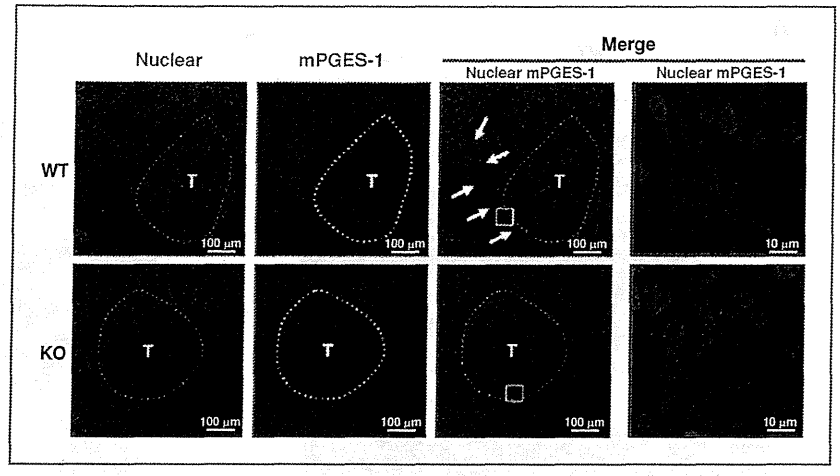

Figure 4. Immunolocalization of mPGES-1 in tumor stroma. Immunofluorescence detection of mPGES-1 in tumor and adjacent normal colonic mucosa after AOM exposure. T identifies tumor area (delineated by dotted line). Arrows in the merged images indicate the presence of mPGES-1–expressing cells at the apical surface of the tumor and also within the adjacent tumor stroma in WT mice. mPGES-1 was localized to the perinuclear region of stromal cells abutting the tumor. Scale bars as indicated.

that reported by Murakami and colleagues (19), mPGES-1 staining was confined to the perinuclear region of the cells (Fig. 4). In addition, the expression of mPGES-1 was observed within multiple cell types including macrophages and fibroblasts (ref. 20; Supplementary Fig. S2). The location of mPGES-1 indicates that the primary source of inducible PGE_2 originates within the tumor stroma (21). Therefore, an alternative possibility for tumor suppression is that genetic deletion of *mPGES-1* interrupts localized production of PGE_2 within the tumor stroma, eliciting effects that extend into the epithelial compartment.

The presence of mucosal ulcerations in *mPGES-1* KO mice

Further evaluation of colon histology in the *mPGES-1* KO mice revealed the presence of synchronous, localized mucosal ulcerations affecting up to 15% of the colonic epithelium. These cryptal lesions developed independently of AOM treatment and were characterized histologically by the presence of regenerative atypia (Fig. 5A). Mucosal ulcerations within crypt abscesses resembled the active phase of ulcerative colitis. Regenerative crypts adjacent to the ulcerated areas, as well as infiltrating immune cells, were positive for Ki-67, indicating active cell proliferation (Fig. 5A, Ki-67). However, these regenerative crypts did not share other molecular features typically associated with neoplasia. For example, APC expression was largely retained compared with the extensive loss of APC protein observed in dysplastic adenomatous crypts in the *mPGES-1* KO mice (Fig. 5B, APC). Importantly, we found no evidence for β-catenin activation within these regenerative crypt lesions, with plasma membrane staining observed in all cases examined (Fig. 5B, β-catenin). Although cyclinD1, a key β-catenin target, showed intermittent nuclear staining within these epithelial structures (Fig. 5B, cyclinD1), the normal status of APC expression and β-catenin localization within these colonic structures support their nonneoplastic nature.

Reduced frequency of $CD4^+FoxP3^+$ Tregs in the draining MLNs of the *mPGES-1* null mice

The mild and localized chronic inflammation observed within the colons of *mPGES-1* KO mice was further substantiated by the presence of macroscopically inflamed MLNs, with a significant expansion of total lymphocytes (1.6 ± 0.3 vs. 7.4 ± 1.5 in WT and KO, respectively; $P < 0.006$; Fig. 6A). Total numbers of $CD4^+$ and $CD8^+$ cells were also markedly elevated (0.9 ± 0.2 vs. 2.9 ± 0.5 for $CD4^+$ in WT and KO, respectively; $P < 0.004$ and 0.2 ± 0.04 vs. 0.8 ± 0.2 for $CD8^+$ in WT and KO, respectively; $P < 0.01$, respectively; Fig. 6A), presumably a direct result of the ongoing localized inflammation. In the spleen, however, there were no significant differences in the total numbers of both $CD4^+$ and $CD8^+$ cells (Fig. 6A). Correspondingly, serum PGE_2 concentrations were moderately ($P < 0.05$) lower in the *mPGES-1* KO than in WT mice (Supplementary Fig. S3A). Moreover, the concentration of a panel of pro- and anti-inflammatory cytokines in the serum was mostly unchanged between genotypes, confirming the localized effect associated with *mPGES-1* deletion (Supplementary Fig. S3B). The only exception was a significant decrease in IL-1α in *mPGES-1* KO mice, which was recently shown to be regulated by PGE_2 (22) and might be indicative of a stronger chronic inflammatory response.

We next investigated the immunoregulatory mechanisms that may underlie colonic inflammation in the *mPGES-1* KO mice. PGE_2 has been shown *in vitro* to enhance the differentiation of naive $CD4^+$ T cells into FoxP3-positive Tregs that have the potential to suppress effector T-cell function (23). Furthermore, Tregs play an important regulatory role in gastrointestinal immunity (24). As shown in Figure 6B, in the MLNs of *mPGES-1* KO mice, the frequency of CD4 FoxP3 double-positive cells was reduced by 55% compared than in WT mice (21.1 ± 1.1 vs. 11.7 ± 1.3 in WT and KO, respectively; $P < 0.0004$). Importantly, this effect was not systemic, as

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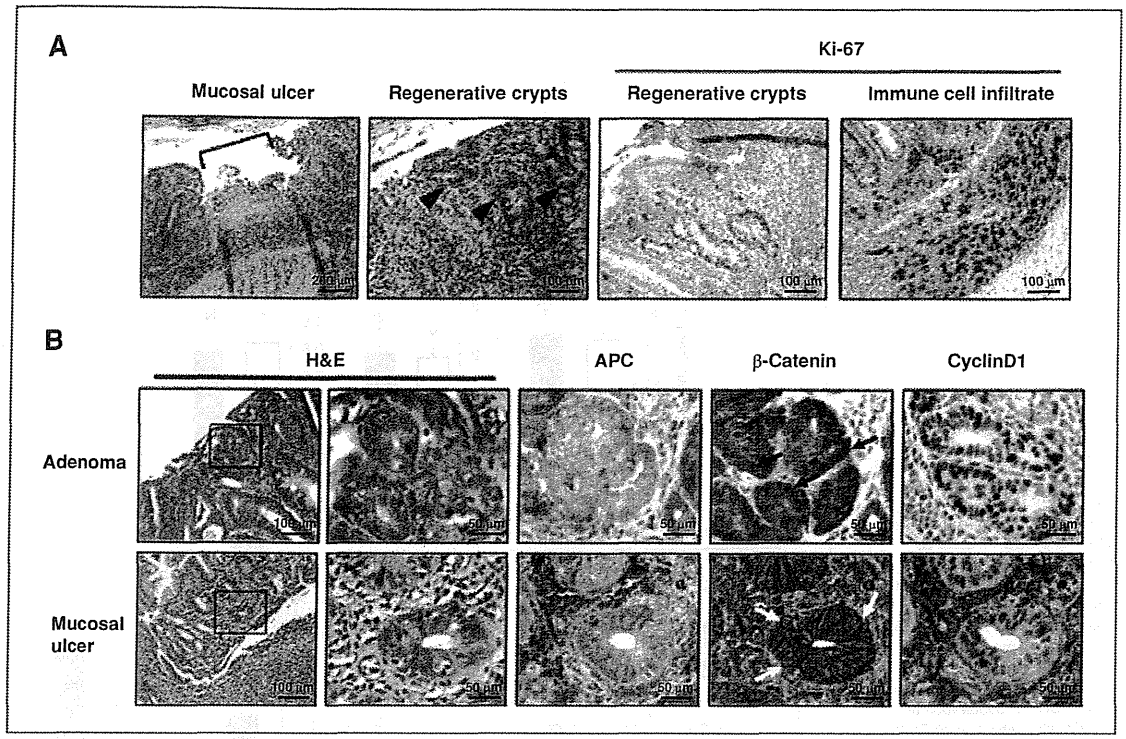

Figure 5. Histologic features of localized colonic ulcerations. A, a representative localized ulceration in the colon of a *mPGES-1* KO mouse containing regenerative crypts (arrowheads). Highly proliferative cells within the regenerative crypts are detected by Ki-67 staining. Intense Ki-67 staining is also seen in infiltrating immune cells within the colonic mucosa. B, comparison of tumor-related markers in serial sections of tumors and mucosal ulcers in *mPGES-1* KO mice. Enlarged areas are identified by the box. Scale bars as indicated.

the composition of Tregs within the spleen was unaffected by genotype (Fig. 6B).

We also examined the possibility that a population of myeloid-derived suppressor cells (MDSC), immunomodulatory cells that are often increased in tumor-bearing mice (25), may be expanded in *mPGES-1* KO mice in response to the localized mucosal ulcerations. As anticipated, increased levels of GR-1 CD11b double-positive MDSCs were found in the MLN (0.03 ± 0.02 vs. 0.20 ± 0.03 in WT and KO, respectively; $P < 0.00004$) and spleen (2.08 ± 0.40 vs. 8.37 ± 2.76 in WT and KO, respectively; $P < 0.01$; Fig. 6C). However, the frequency of MDSCs was modest in comparison to changes found in other mouse tumor models. For example, in some cases, the spleen can harbor up to 40% of MDSCs within the T-cell population, depending of course on the underlying pathology (26). The present findings suggest that limited expansion of MDSCs in *mPGES-1* KO mice, most likely attributed to reduced PGE_2 levels (27), contributes to the enhanced inflammatory state that is present within the colon and that may ultimately impede colon tumor progression.

On the basis of the reduced levels of Tregs and the moderate effect on MDSCs, we postulated that additional immunoregulatory mechanisms might also contribute to tumor suppression. Thus to evaluate this possibility, we harvested cells from the spleens and MLNs of untreated WT and *mPGES-1* KO mice. The total number of cells harvested from the MLN of the *mPGES-1* KO mice was significantly higher in comparison to the WT mice, a result of the localized colonic ulcerations (Supplementary Fig. S4). In addition, the number of $CD4^+$ and $CD8^+$ cells was also markedly higher in the *mPGES-1* KO MLN (Supplementary Fig. S4), consistent with our findings in tumor-bearing mice (Fig. 6A). Following a 4-hour stimulation with PMA/ionomycin, the ability of $CD4^+$ cells to produce IL-10 or IFN-γ was slightly impaired in the *mPGES-1* KO group (Supplementary Fig. S4). These observations suggest that *mPGES-1* deficiency may affect the production of regulatory T type 1 cells (Tr1), another type of immunoregulatory cell present within the gut mucosa (28).

Discussion

Elevated prostanoid production in the colon plays a key role in cancer pathogenesis and efforts have been made to suppress this pathway, primarily via inhibition

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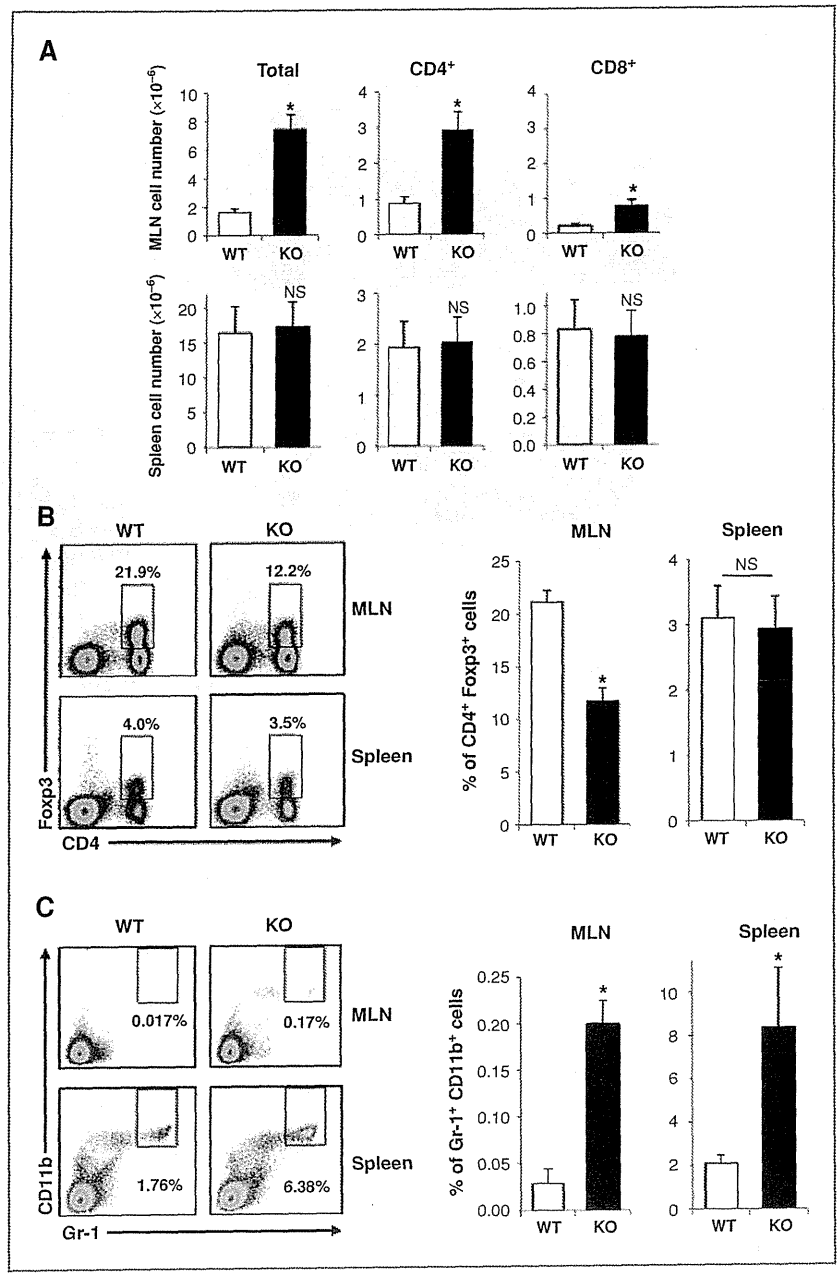

Figure 6. Flow cytometry of cells in MLNs and spleens. A, total numbers of cells in MLN and spleen, including the number of $CD4^+$ and $CD8^+$ T cells. B, representative FACS analysis showing the frequency of CD4 FoxP3 double-positive Tregs. Quantification of FACS analyses is expressed as means ± SEM, $n = 9$ per group. C, representative FACS analysis showing frequency of Gr-1 CD11b double-positive MDSCs. Quantification of FACS analysis is expressed as means ± SEM, $n = 8$ in WT and $n = 6$ in KO mice. *, $P < 0.05$; NS, not significant compared with WT mice.

of COX-2 activity. Although long-term COX-2 inhibition can be effective, it has also been associated with a number of adverse effects, notably cardiovascular and gastrointestinal (GI) toxicities (29). Evidence from several tumor models provides the rationale for the development of alternative chemoprevention targets within the arachidonic acid pathway including the terminal PGE_2 synthase, mPGES-1 (3). In the present study, we provide evidence that suppression of inducible PGE_2 production through genetic deletion of *mPGES-1* effectively reduces colon cancer development. We also go on to show that suppressing inducible PGE_2 formation

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influences cancer development in part by promoting an effective immune response to the tumor.

Remarkably, tumor suppression was so effective that only 1 of 19 *mPGES-1* KO mice (5.3%) developed a colon tumor exceeding 3 mm in size. Despite this protection afforded to the colon, however, *mPGES-1* deletion did not influence the frequency of ACF to the same extent. This latter observation is consistent with the recent findings of Cho and colleagues (30) who reported that within a subset of patients on the Adenoma Prevention with Celecoxib trial, adenoma suppression by celecoxib treatment was not correlated with changes in the density of ACF within the distal colorectum. It is possible that at least for agents that target the COX-2 pathway, ACF do not provide a surrogate marker for colon cancer suppression.

PGE_2 is a pleiotropic molecule that is formed within a variety of cell types and can elicit effects that are both cell- and tissue-context dependent. The precise location of inducible PGE_2 formation, however, remains somewhat controversial. For example, it is broadly accepted that tumor cell–derived PGE_2 promotes tumor growth through an autocrine mechanism. Consistent with this mechanism, mPGES-1 expression has been identified directly within the epithelial cells of colon tumors (31–33). In the present study, however, we found that mPGES-1 was localized primarily within the tumor stroma, indicating that inducible formation of PGE_2 may impair tumor expansion via non–cell autonomous mechanisms. This finding is consistent with the results of Kamei and colleagues (32), who showed that the growth of Lewis lung carcinoma (LLC) tumor cells explanted into an *mPGES-1*–deficient host was markedly impaired in comparison to the growth observed in an *mPGES-1*–competent host. In addition, a number of studies have found COX-2 expression to be confined to the tumor stroma (reviewed in ref. 34).

The depletion of inducible PGE_2 formation is associated with the development of colonic mucosal abnormalities that are reminiscent of ulcerative colitis. The lesions are restricted to the large intestine, and the histologic features of these lesions consist of crypt erosion and an influx of inflammatory cells. Interestingly, Hara and colleagues (35) recently reported that *mPGES-1* KO mice show enhanced susceptibility to dextran sodium sulfate (DSS)-induced ulcerative colitis, confirming a critical role for PGE_2 in maintaining colonic epithelial barrier function under conditions of chemical-induced stress. It is possible that the localized ulcerations present within the colons of the *mPGES-1* KO mice induce a chronic inflammatory condition. We further speculate that this underlying inflammation may actually be a contributing factor in the suppression of colon tumors observed in the present study.

PGE_2 is among the most potent immunoregulatory molecules within the intestinal mucosa. In addition to its modulating effects on normal gut barrier function and mucosal response to pathogens (36), inducible formation of PGE_2 also plays a critical role in the immunosuppression associated with advanced neoplasia (37). Tregs, with the potential to suppress effector T-cell function (38, 39) have been shown to play an important immunomodulatory role within the GI tract (24, 40). Within the tumor microenvironment, PGE_2 has been reported to enhance Treg differentiation by inducing the expression of Foxp3 in naive $CD4^+$ T cells (23, 41, 42). In CRC patients, increased levels of Tregs were identified within the tumors as well as in the regional lymph nodes (43). Moreover, the effect of Tregs on the production of proinflammatory cytokines was reversed by treatment with nonsteroidal anti-inflammatory drugs (NSAID), further evidence for Treg dependence on PGE_2 during tumor evolution (43). Given the direct influence that PGE_2 elicits on FoxP3 expression in T cells, it is entirely possible that *mPGES-1* deficiency may result in a persistent overreactive immune response due to the loss of functional activation of Tregs.

Interestingly, the present study shows that the impaired immunoregulatory response in the *mPGES-1* KO mice, reflected by the spontaneous development of localized ulcerations, was not entirely accounted for by attenuated Treg expansion. *mPGES-1* deletion also modestly affected the levels of MDSCs and Tr1-like cells, suggesting that the absence of inducible PGE_2 formation may disrupt fundamental immunomodulatory mechanisms within the tumor microenvironment. One possibility is that *mPGES-1* deficiency causes a shift in cytokine profiles during tumorigenesis. In fact, Monrad and colleagues (44) recently showed that *mPGES-1*–deficient bone marrow–derived dendritic cells (BMDC) had decreased production of IL-12 in response to lipopolysaccharide (LPS) stimulation. Furthermore, our preliminary data show that MLN cells harvested from the *mPGES-1* KO mice produce higher levels of several cytokines when compared with similarly stimulated WT cells (data not shown). Because the *mPGES-1* genotype did not affect systemic cytokine profiles with the exception of IL-1α, a more detailed analysis of tissue-specific cytokine profiles is warranted.

A number of studies suggest that chronic inflammation may play an important role in the pathogenesis of up to 20% of human cancers (45). In particular, long-standing inflammatory bowel disease (IBD) is considered a significant risk factor for CRC (46). Although the present data appear to contradict these earlier observations, we postulate that the underlying inflammation present in *mPGES-1* KO mice, resulting from the mild and localized colonic ulceration, may actually confer protection against tumor progression by providing a mechanism for active clearance of cancer-initiating cells. Consistent with this hypothesis, an earlier study by Tanaka and colleagues (13) showed that mice administered DSS prior to a single injection of AOM failed to develop colon tumors 20 weeks later. In the present study, *mPGES-1* KO mice did not exhibit clinical signs of severe ulcerative colitis, such as rectal bleeding and excessive weight loss (data not shown), despite the presence of these benign, localized mucosal ulcerations. Of course, the possibility exists that the mild inflammation that is present within the colons

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of the *mPGES-1* KO mice may contribute to subsequent cancer risk. However, without additional genetic hits, these lesions may not have the capacity to progress to cancer (47). Therefore, additional studies to better define the mechanisms by which selective suppression of PGE_2 directly modulates antitumor immunity and contributes to colon cancer suppression are underway. These studies will ultimately enable the development of effective therapeutic strategies for targeting mPGES-1 for cancer prevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Review

Cancer Chemoprevention by Carotenoids

Takuji Tanaka [1,2,*], Masahito Shnimizu [3] and Hisataka Moriwaki [3]

[1] Tohkai Cytopathology Institute, Cancer Research and Prevention (TCI-CaRP), 5-1-2 Minami-Uzura, Gifu 500-8285, Japan

[2] Department of Tumor Pathology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan

[3] Department of Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan

* Author to whom correspondence should be addressed; E-Mail: takutt@toukaisaibou.co.jp; Tel.: +81-58-273-4399; Fax: +81-58-273-4392.

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Abstract: Carotenoids are natural fat-soluble pigments that provide bright coloration to plants and animals. Dietary intake of carotenoids is inversely associated with the risk of a variety of cancers in different tissues. Preclinical studies have shown that some carotenoids have potent antitumor effects both *in vitro* and *in vivo*, suggesting potential preventive and/or therapeutic roles for the compounds. Since chemoprevention is one of the most important strategies in the control of cancer development, molecular mechanism-based cancer chemoprevention using carotenoids seems to be an attractive approach. Various carotenoids, such as β-carotene, α-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin, fucoxanthin, canthaxanthin and astaxanthin, have been proven to have anti-carcinogenic activity in several tissues, although high doses of β-carotene failed to exhibit chemopreventive activity in clinical trials. In this review, cancer prevention using carotenoids are reviewed and the possible mechanisms of action are described.

Keywords: carotenoids; xanthophylls; cancer chemoprevention; mechanisms

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Abbreviations

ABCA1, ATP-binding cassette transporter 1; AFB_1, aflatoxin B_1; Akt, protein kinase B; AMD, age-related macular degeneration; AOM, azoxymethane; AP-1, activator 1; ARE, antioxidant response element; CAR, constitutive androstane receptor; Cdks, cyclin-dependent kinases; CHRP, β-cryptoxanthin- and hesperidin-rich powder; CMO-1, β-carotene 15,15'-monooxygenase; COM2, β-carotene 9',10'-monooxygenase; COX, cyclooxygenase; CUSM, citrus unshiu segment membrane; CVD, cardiovascular disease; CYP, cytochrome P450; DMH, 1,2-dimethylhydrazine; EGF, early growth response gene; ERK, extracellular signal-regulated kinase; GJIC, gap junctional intercellular communication; GSK3β, glycogen synthase kinase 3β; GSTs, glutathione *S*-transferases; HDL, high-density lipoproteins; HO-1, heme oxygenase-1; IGF, insulin growth factor; IGFBPs, IGF binding proteins; IL, interleukin; LDL, low-density lipoproteins; MJ, satsuma mandarin (*Citrus unshiu* Marc) juice; MMP, matrix metalloproteinases; NF-kB, nuclear factor kappaB; 4-NQO, 4-nitroquinoline 1-oxide; NQO1, NAD(P)H:quinone oxidoreductase; Nrf2, NF-E2-related factor 2; OH-BBN, *N*-butyl-*N*(4-hydroxybutyl)nitrosamine; PPARs, peroxisome proliferator-activated receptors; PSA, prostate-specific antigen; RAR, retinoic acid receptor; ROS, reactive oxygen species; RXR, retinoid X receptor; SXR/PXR, steroid and xenobiotic receptor/pregnane X receptor; TCF/LEF, transcription factors T cell factor/lymphoid enhancer factor; TNF, tumor necrosis factor; TRE, TPA response element; UV, ultraviolet; VDR, vitamin D3 receptor.

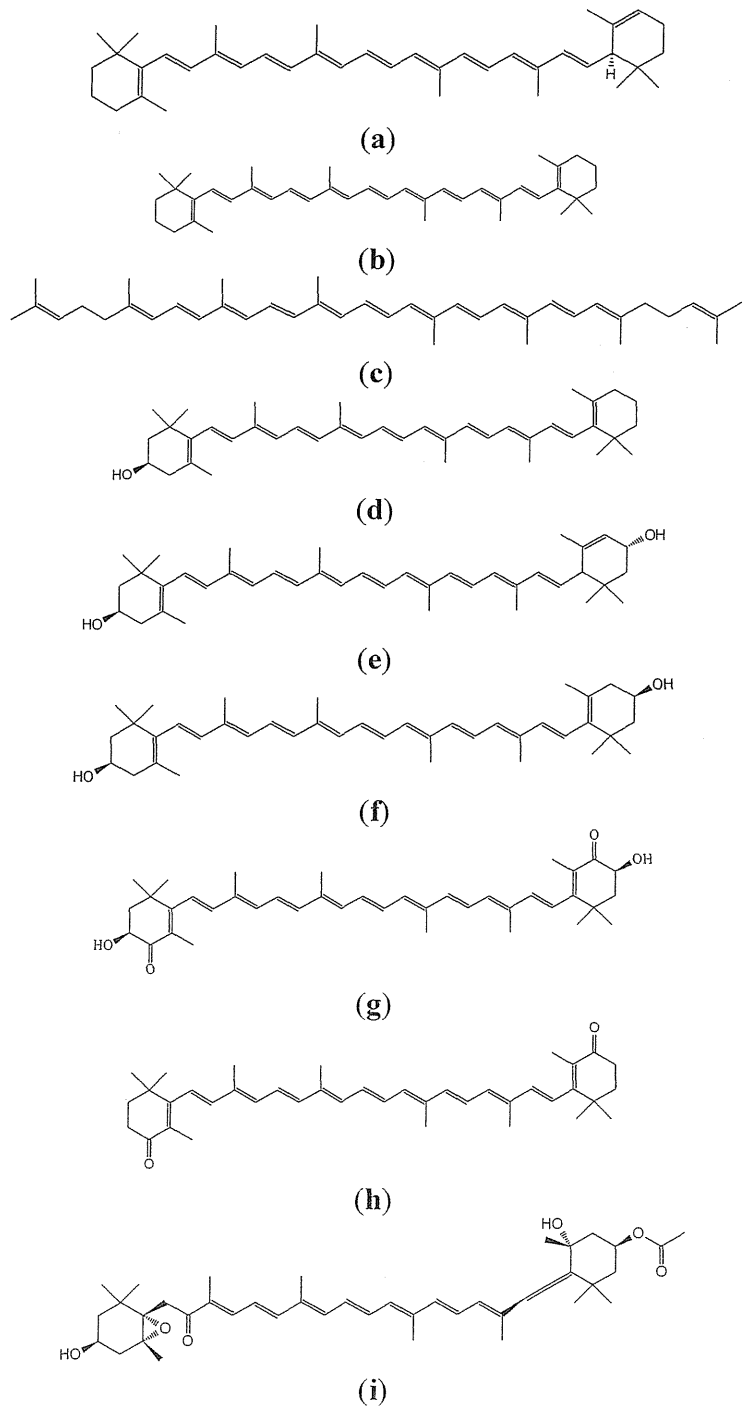
1. Introduction

To date, the cancer problem and the failure of conventional chemotherapy to achieve a reduction in the mortality rates for common epithelial malignancies such as carcinomas of the lung, colon, breast, prostate and pancreas, indicates a critical need for new approaches to control cancer development [1,2]. One of these approaches is chemoprevention, which is a pharmacological approach to intervention with the objective of arresting or reversing the process of multi-step carcinogenesis. The carcinogenic process may be driven by mutation(s), and followed by subsequent alterations in phenotypic, epigenetic and genetic events. Pharmacologic modulation of these regulatory pathways, involving the effective use of drugs, micronutrients and non-nutrients that block mutational damage of DNA, thus offers great potential for cancer prevention.

There is a clear link between dietary intake or dietary habits and cancer development in man [3–5]. Dietary risk factors have ranked higher than smoking and much higher than pollution or occupational hazards in their association with death due to cancer [6]. However, a number of compounds naturally occurring in foods, particularly antioxidative compounds in plants, have shown promise as potential chemopreventive agents [2,6–8]. These phytonutrients include the yellow, orange and red carotenoid pigments that have recently been investigated. Epidemiologically, vegetable and fruit consumption has constantly been associated with a reduced incidence of a variety of cancers [7–9], and dietary carotenoid intake from these sources has similarly been correlated with a reduced cancer risk [10–12]. However, several recent large-scale intervention trials failed to find any chemopreventive effects due to long-term supplementation with β-carotene, the most abundant dietary carotenoid [13–15]. In contrast, several naturally occurring carotenoids other than β-carotene have exhibited chemopreventive

and/or anti-cancer activities [16–19]. Foodstuffs contain various carotenoids. Vegetables contain carotenoids such as α-carotene (Figure 1a), β-carotene (Figure 1b), lycopene (Figure 1c), β-cryptoxanthin (Figure 1d), lutein (Figure 1e), zeaxanthin (Figure 1f), capsanthin and crocetin. Citrus fruits contain β-cryptoxanthin and marine carotenoids include astaxanthin (Figure 1g), β-carotene, zeaxanthin, canthaxanthin (Figure 1h), fucoxanthin (Figure 1i) and lycopene.

Figure 1. Chemical structures of (**a**) α-carotene; (**b**) β-carotene; (**c**) lycopene; (**d**) β-cryptoxanthin; (**e**) lutein; (**f**) zeaxantin; (**g**) astaxanthin; (**h**) canthaxanthin and (**i**) fucoxanthin.



In this brief review, cancer prevention by means of carotenoids (Table 1), are summarized and the possible mechanisms of action are also described.

Table 1. Sources, function, and effects of different carotenoids.

Carotenoids	Dietary Sources	Function	Effects
α-Carotene	Yellow-orange vegetables (carrots, sweet totatoes, pumpkin) and Dark-green vegetables (broccoli, green beans, spinach)	Provitamin A activity; Anti-oxidant	Immune- enhancement; Stimulate cell to cell communication; Decreases risk of some cancers
β-Carotene	Green leafy vegetables and orange and yellow fruits and vegetables (carrots, apricots, spinach, sweet potetoes, pumpkin, pepper, kale, cantaloupe)	Provitamin A activity; Antioxidant	Immune-enhancement; Decreases risk of some cancers and some cardiovascular events; high-dose supplementation may increase the risk of lung cancer among smokers
Lycopene	Tomatoes, water melon, apricot, peaches	Anti-oxidant	Decreases risk of some cancers and some cardiovascular events, diabetes, and osteoporosis
β-Cyptoxanthin	Orange fruits (mandarin orange and papaya, *etc.*), corn, peas, and egg yolks	Provitamin A activity; Anti-oxidant	Anti-inflammatory effects; Inhibits risks of some cancer and cardiovascular events; Immune enhancement
Lutein/Zeaxanthin	Dark green leafy vegetables (spinach, kale), red peppers, maize, tomatoes, corn, and egg yolks	Anti-photosensitizing agent and photosynthetic pigment; Acts as antioxidants and blue light filters	Decrease age-related macular degeneration, cataract, and risk of cardiovascular disease and certain cancers
Astaxanthin	Green algae, salmon, trout, crustacea	Antioxidant; Coloration	Prevent certain cancers, cataract, diabetes, and inflammatory neurodegenerative and cardiovascular diseases
Canthaxanthin	Salmon, crustacea	Antioxidant; Coloration	Immune enhancement; Decreases risk of some cancers
Focoxanthin	Brown algae, heterokonts	Antioxidant	Anti-cancer, anti-allergic, anti-obese, anti-inflammatory, and anti-osteoporotic activities

2. Definition of Carotenoids

Carotenoids, which belong to the chemical group known as isoprenoid polyenes, are lipid-soluble, yellow-orange-red pigments found in all higher plants and some animals. The carotenoids can be categorized as follows: (a) vitamin A precursors that do not pigment such as β-carotene; (b) pigments with partial vitamin A activity such as cryptoxanthin, β-apo-8'-carotenoic acid ethyl ester; (c) non-vitamin A precursors that do not pigment or pigment poorly such as violaxanthin and neoxanthin; and (d) non-vitamin A precursors that pigment such as lutein, zeaxanthin and

canthaxanthin. Due to the numerous conjugated double bonds and cyclic end groups, carotenoids present a variety of stereoisomers with different chemical and physical properties. The most important forms commonly found among carotenoids are geometric (*E*-/*Z*-). A double bond links the two residual parts of the molecule either in an *E*-configuration with both parts on opposite sites of the plane, or a *Z*-configuration with both parts on the same side of the plane. Geometrical isomers of this type are interconvertible in solution. This stereoisomerism exerts a marked influence on the physical properties. Isomers differ not only in their melting points, solubility and stability, but also in respect to absorption affinity, color and color intensity. Animals cannot synthesize carotenoids, so their presence in the body is due to dietary intake of foods such as pink salmon flesh. The plumage of many birds owes its color to carotenoids. Plant, algae, fungal and synthetic (nature-identical) carotenoids are permitted as colorants in food products, but not animal carotenoids.

Carotenoids owe their name to carrots (*Daucus carota*), and xanthophylls (originally phylloxanthins) are derived from the Greek words for yellow (*xanthos*) and leaf (*phyllon*). Together with anthocyanins, carotenoids are the most complex class of natural food colorants with over 750 different structures identified.

3. Absorption, Metabolism, and Bioavailability of Carotenes and Xanthophylls

Carotenoids, being mostly fat soluble, follow the same intestinal absorption path as dietary fat. Carotenoids are released from food matrices and solubilized in the gut. This is carried out in the presence of fat and conjugated bile acids. For carotenoid absorption, as little as 3~5 g of fat in a meal is sufficient [20,21]. Absorption is affected by the same factors that influence fat absorption. Thus, the absence of bile or any generalized malfunction of the lipid absorption system, such as diseases of the small intestine and pancreas, will interfere with the absorption of carotenoids. Chylomicrons are responsible for the transport of carotenoids from the intestinal mucosa to the bloodstream via the lymphatics for delivery to tissues. Carotenoids are transported in the plasma exclusively by lipoproteins. Oxygen functionalized carotenoids are more polar than carotenes. Thus, α-carotene, β-carotene and lycopene tend to predominate in low-density lipoproteins (LDL) in the circulation, whereas high-density lipoproteins (HDL) are major transporters of xanthophylls such as cryptoxanthins, lutein and zeaxanthin [22,23]. The delivery of carotenoids to extrahepatic tissues is accomplished through the interaction of lipoprotein particles with receptors and the degradation by lipoprotein lipase.

Although no less than forty carotenoids are usually ingested in the diet, only six carotenoids and their metabolites have been found in human tissues, suggesting selectivity in the intestinal absorption of carotenoids [24,25]. In contrast, thirty-four carotenoids and eight metabolites are detected in breast milk and serum of lactating mothers [26]. Recently, facilitated diffusion in addition to simple diffusion has been reported to mediate the intestinal absorption of carotenoids in mammals. The selective absorption of carotenoids may be due to uptake to the intestinal epithelia by means of facilitated diffusion and an unknown mechanism of excretion into the intestinal lumen. It is well known that β-carotene can be metabolized to vitamin A after intestinal absorption of carotenoids, but little is known about the metabolic transformation of non-provitamin A xanthophylls. The enzymatic oxidation of the secondary hydroxyl group leading to keto-carotenoids would occur as a common pathway of xanthophyll metabolism in mammals [24].

4. Distribution and Nature of Certain Carotenoids

Numerous studies have reported that carotenoids have the potential to prevent cancers, diabetes, and inflammatory and cardiovascular disease (CVD). Some of these carotenoids are listed below.

4.1. Hydrocarbone Carotenoids

Under EU legislation, plant carotenoids may be derived from edible plants, carrots, vegetable oils, grass, alfalfa and nettle. However, according to U.S. legislation carotenes may only be derived from carrots. A good source of plant carotenoids is the mesocarp of oil palm (*Elaeis guineensis*) fruits, which contains an oil rich in carotenes. After separation of the carotenes from the palm fruit oil, which is used for making detergents, the carotenes are suspended in vegetable oil at a concentration of 30%. The predominant carotenes are α- and β-carotene in the ratio 2:3. Other carotenes, including phytoene, phytofluene, ζ-carotene, γ-carotene and lycopene, which are all precursors in the biosynthesis of α- and β-carotene, are present in smaller amounts. Due to heat treatment of the oil palm fruit used in obtaining the oil, a complex mixture of geometric isomers is formed, with only 60% of α- and β-carotene as the *trans*-forms. Synthetic β-carotene is predominantly *trans*-β-carotene. The presence of β-carotene and *cis*-isomers of α- and β-carotene in palm fruit carotenes means that synthetic β-carotene is more orange than palm fruit carotenes, which is more yellow. Carotene from *B. trispora* is also mainly *trans*-β-carotene, with approximately 3% of other carotenoids. Carotene from *D. salina* also primarily consists of β-carotene with 5–6% of other carotenoids (α-carotene, lutein, zeaxanthin and β-cryptoxanthin); according to legislation, the content of *trans*isomers coming from this source should be in the range 50–71%. This means that its color shade would be between that of oil palm carotenes and synthetic β-carotene. Besides being used as colorants, carotenes are also used for nutritional purposes, such as provitamin A agents or as dietary supplements.

β-Carotene is the major source of vitamin A as a provitamin A carotenoid. Two metabolic pathways exist for its conversion to vitamin A, and they are known as the central cleavage pathway and the excentric cleavage pathway. For provitamin A carotenoids, central cleavage is the main pathway leading to the formation of vitamin A [27,28]. β-Carotene, α-carotene, and β-cryptoxanthin are cleaved symmetrically at their central double bond by β-carotene 15,15'-monooxygenase (CMO1), formerly called β-carotene 15,15'-dioxygenase. An alternative excentric cleavage pathway was also reported [29,30] and confirmed by molecular identification of an excentric cleavage enzyme, β-carotene 9',10'-monooxygenase (CMO2) in mice, humans, and zebrafish [31]. CMO2 has the ability to catalyze the asymmetric cleavage of β-carotene to produce β-apo-10'-carotenal and β-ionone [31]. Apo-β-carotenals can be precursors of vitamin A *in vitro* and *in vivo*, by further cleavage enzyme, CMO1 [32]. They can also be oxidized to their corresponding apo-β-carotenoic acids, which undergo a process similar to β-oxidation of fatty acids, to produce retinoic acid [33]. The coexistence of these two cleavage pathways reveals a greater complexity of β-carotene metabolism in organisms and raises a potential link between effects from β-carotene and/or its metabolites and anti-carcinogenesis. Common non-synonymous single-nucleotide polymorphisms (SNPs) exist in the human CMO1 gene and alter β-carotene metabolism [34,35].

4.2. Lycopene

Being a precursor in the biosynthesis of β-carotene, lycopene can be expected to be found in plants containing β-carotene, albeit usually at very low and sometimes undetectable concentrations. The best-known sources of lycopene are tomatoes, watermelon, guava and pink grapefruit. Lycopene may also be produced synthetically and by *B. trispora*. Lycopene is permitted as a food colorant in the EU and was also approved for use as a food supplement in the USA in July 2005. The only permitted source is tomatoes (*Lycopersicon esculentum*, Lycopersicon, meaning wolf peach). Besides lycopene, tomato oleoresin also contains appreciable amounts of β-carotene, phytoene and phytofluene. In solution, lycopene appears orange and not bright red as in the tomato. Lycopene is very prone to oxidative degradation, much more so than β-carotene.

Carotenoids absorb light, transfer energy to chlorophyll in the process of photosynthesis and protect against photo-oxidative damage [36,37]. In man, carotenoids function primarily as dietary sources of provitamin A. However, lycopene lacks the β-ionone ring structure required to form vitamin A and has no provitamin A activity. Therefore, lycopene has no known physiological function in man. However, some potential molecular targets in cells have been identified for lycopene. They include molecules that are involved in antioxidant activity, the antioxidant response element (ARE), apoptosis induction, cell cycle arrest, growth factors and signaling pathways, and invasion and metastasis [38–42].

4.3. Lutein and Zeaxanthin

Lutein and zeaxanthin are the two major components of the macular pigments of the retina. The macula lutea "yellow spot" in the retina is responsible for central vision and visual activity. Lutein and zeaxanthin are the only carotenoids found in both the macula and lens of the human eye, and have dual functions in both tissues to act as powerful antioxidants and to filter high-energy blue light [43]. Lutein is found in high amounts in human serum [26]. In the diet it occurs in highest concentrations in dark green leafy vegetables (spinach, kale, collard greens and others), corn and egg yolks [44]. Zeaxanthin is the major carotenoid found in corn, orange peppers, oranges and tangerines.

Lutein is also a very common carotenoid and one of the major xanthophylls present in green leafy vegetables. Lutein and zeaxanthin are known to selectively accumulate in the macula of the human retina. They are thought to function as antioxidants [45,46] and as blue light filters [47] to protect the eyes from oxidative stresses such as cigarette smoke and sunlight, which can lead to age-related macular degeneration (AMD) and cataracts. The name lutein is derived from the Latin word for yellow (compare xanthophyll, vide supra). The most interesting source is Aztec marigold (*Tagetes erecta*) in which lutein is primarily found esterified with saturated fatty acids (lauric, myristic, palmitic and stearic acid). Lutein made from Aztec marigold also contains some zeaxanthin (typically less than 10%). Containing only 10 conjugated double bonds, lutein is more yellowish-green than oil palm carotenes.

Zeaxanthin, the principal pigment of yellow corn, *Zeaxanthin mays* L. (from which its name is derived) is the compound that consists of 40 carbon atoms. It also occurs in egg yolks and some of the orange and yellow vegetables and fruits, such as alfalfa and marigold flowers [48]. Zeaxanthin exhibits no vitamin A activity. Zeaxanthin and its close relative lutein play a critical role in the prevention of AMD, the leading cause of blindness [49]. Zeaxanthin is isomeric with lutein; the two carotenols only

differ from each other in terms of the shift of a single double bond, so that in zeaxanthin all double bonds are conjugated. Zeaxanthin is used as a feed additive and colorant in the food industry for birds, swine and fish [50]. The pigment imparts a yellow coloration to the skin of birds and their egg yolk, whereas in pigs and fish it is used for skin pigmentation [51].

4.4. β-Cryptoxanthin

β-Cryptoxanthin is found in human blood together with α-carotene, β-carotene, lycopene, lutein and zeaxanthin. Unlike other abundant carotenoids, β-cryptoxanthin is not found in most fruits or vegetables but only in specific ones, namely hot pepper, persimmon and Satsuma mandarin (*Citrus unshiu* Marc.) [52]. Satsuma mandarin, also known as table orange or Satsuma in Western countries, is one of the most popular citrus fruits in Japan. It is sweet, tasty and rich in vitamin C. It is notable that Satsuma mandarin is one of the most common β-cryptoxanthin rich fruits in the world. The edible part of the Satsuma mandarin contains about 1.8 mg/100 g of β-cryptoxanthin, while the β-cryptoxanthin content is 0.2 mg/100 g in Valencia orange and almost nothing in grapefruits. As β-cryptoxanthin is rarely found in most fruits or vegetables, the serum β-cryptoxanthin concentration in the Japanese population is almost parallel to their consumption of the Satsuma mandarin, and is higher than in western populations [53]. Although the nutritional functions and metabolism of abundant carotenoids, for example β-carotene and lycopene, have been well studied [54,55], those of β-cryptoxanthin have not been examined in detail. Recent reports strongly suggest a significant negative correlation between serum β-cryptoxanthin concentrations and disease morbidity such as liver disorders [56,57], cancer [58,59] and mutagenesis [60], and post-menopausal osteoporosis [61–63]. β-Cryptoxanthin intake is beneficial for human health. The anti-obesity effects of β-cryptoxanthin have recently been reported [64,65]. The major xanthophyll, β-cryptoxanthin, was also reported to decrease the gene expression of interleukin (IL)-1α in mouse macrophage RAW264 cells [66], to promote osteoblastic differentiation of mouse MC3T3 cells [67] and to prevent a decrease of calcium content in the bone of ovariectomized rats [63].

4.5. Astaxanthin

Astaxanthin contains two keto groups on each ring structure as compared with other carotenoids, resulting in enhanced antioxidant properties. This compound occurs naturally in a wide variety of living organisms including microalgae (*Haematococcus pluvialis*, *Chlorella zofingiensis* and *Chlorococcum* sp.), fungi (*Phaffia rhodozyma*, red yeast), complex plants, seafood and some birds such as flamingos and quail; it has a reddish color and gives salmon, shrimp and lobster their distinctive coloration [68]. The microalga *Haematococcus pluvialis* has the highest capacity to accumulate astaxanthin at up to 4–5% of cell dry weight. Astaxanthin has been attributed with the extraordinary potential of protecting the organism against a wide range of diseases. It also has considerable potential and promising applications in the prevention and treatment of various diseases such as cancers, chronic inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, CVD, gastrointestinal and liver diseases, and neurodegenerative diseases [69]. Astaxanthin cannot be manufactured in animals or converted to vitamin A, and therefore must be consumed in the diet. Astaxanthin and canthaxanthin have antioxidant activity, are free radical scavengers, potent quenchers of reactive oxygen species

(ROS) and nitrogen oxygen species, and chain-breaking antioxidants. They are superior antioxidants and scavengers of free radicals as compared with the carotenoids such as β-carotene [70]. Astaxanthin is even called superantioxidant.

4.6. Canthaxanthin

Canthaxanthin was first isolated from the edible mushroom, *Cantharellus cinnabarinus*. In addition, canthaxanthin is said to be produced at the end of the growth phase in several green algae, and also in blue-green algae, as secondary carotenoids instead of, or in addition to, primary carotenoids. It has also been found in bacteria, crustacea and various species of fish including carp (*Cyprinus carpio*), golden mullet (*Mugil auratus*), annular seabream (*Diplodus annularis*) and trush wrasse (*Crenilabrus tinca*). Canthaxanthin is not encountered in wild Atlantic salmon, but represents a minor carotenoid in wild Pacific salmon. It has also been reported in wild trout (*Salmo trutta*). Canthaxanthin is used widely as a drug or as a food and cosmetic colorant (skin tanning), but it may have some undesirable effects on human health. These are mainly caused by the formation of crystals in the *macula lutea* membranes of the retina. This condition is called canthaxanthin retinopathy [71]. It has been shown that this type of dysfunction of the eye is strongly connected with damage to the blood vessels around the locations of crystal deposition.

Canthaxanthin is one of the carotenoids without provitamin A activity, but may have anti-carcinogenic, immune-enhancing, antioxidative activities. The mechanisms by which canthaxanthin may exert anti-tumor activity are associated with its antioxidant properties through radical trapping or chain-breaking processes [72,73], or its enhancement of gap-junction cell to cell communication through upregulation of the gap-junction protein, connexin [74].

4.7. Fucoxanthin

The allenic carotenoid fucoxanthin is one of the most abundant carotenoids, and contributes to nature more than 10% of the estimated total production of carotenoids in nature, especially in the marine environment [75]. Fucoxanthin is a naturally occurring brown- or orange-colored pigment that belongs to the class of non-provitamin A carotenoids. Fucoxanthin acts as an antioxidant under anoxic conditions. The typical antioxidants are usually proton donors (ascorbic acid, α-tocopherol and glutathione). Fucoxanthin, on the other hand, donates an electron as a part of its free-radical quenching function. A combination of these distinct properties is very rarely found among naturally occurring compounds [76,77]. During normal metabolism the body produces heat. Fucoxanthin increases the amount of energy released as heat in fat tissue, a process known as thermogenesis. In a published study it has been reported that fucoxanthin affects multiple enzymes involved in fat metabolism causing an increase in the production of energy from fat [78].

Fucoxanthin is present in *Chromophyta* (*Heterokontophyta* or *Ochrophyta*), including brown seaweeds (*Phaeophyceae*) and diatoms (*Bacillariophyta*) [79]. Based on its unique molecular structure, fucoxanthin has remarkable biological properties similar to neoxanthin, dinoxanthin and peridinin, which make it different to other carotenoids. Fucoxanthin does not exhibit toxicity and mutagenicity under experimental conditions [79–81]. Fucoxanthin may have the ability to increase circulating cholesterol levels in rodents as a common feature [79].

5. Clinical Trials with Long-Term β-Carotene Supplementation

Epidemiologic studies have shown an inverse relationship between the presence of various cancers and dietary or blood carotenoid levels [82]. However, three [13–15] out of four intervention trials [13–15,83] using high-doses of β-carotene supplements did not show protective effects against cancer or CVD. Rather, the high-dose intervention trials showed an increase in cancer and angina pectoris [13–15,83]. Therefore, carotenoids may promote health when taken at dietary levels, but may have adverse effects when taken high doses by subjects who smoke or who have been exposed to asbestos.

The epidemiologic observations of the possible protective effects of high dietary (not supplemental) β-carotene intakes against cancer, along with what is known about carotenoid biochemical functions, has led to further study of the effect of β-carotene on cancer risk. Long-term large randomized intervention trials were designed to test the efficacy of high doses of β-carotene (20–30 mg/day) in the prevention of cancer (Table 2). As stated above, the results from two trials provided possible evidence of harm from β-carotene supplements in relation to cancer among high-risk individuals such as smokers and asbestos workers [15], but no effect (either beneficial or detrimental) in a generally well-nourished population [84]. Moreover, the Linxian (Chinese) Cancer Prevention Study [83] found that supplementation with β-carotene, vitamin E and selenium led to a significant reduction in total mortality (9%), especially from cancer (13%) and stomach cancer in particular (21%) (Table 2). The positive results of the Chinese study probably reflect the correction of a vitamin A deficiency in the study population. A number of mechanisms have been proposed to account for the association between β-carotene supplementation and lung cancer in smokers and asbestos workers, including an imbalance of other carotenoids or antioxidants, a pro-oxidant activity of β-carotene at the high oxygen tensions found in the lungs, induction of P450 enzymes and the production of damaging β-carotene oxidation products by components of cigarette smoke [85]. The Women's Health Study [86] indicated no statistically significant differences in incidence of cancer, CVD, or total mortality, although the treatment duration is short (a median treatment duration of 2.1 years and a median total follow-up of 4.1 years).

Table 2. β-Carotene supplementation trials.

Studies	Study Designs				Ref. No.
	Population	Intervention	Duration	Cancer outcome	
ATBC	29,133 Finish male smokers (50–69 years of age)	β-carotene, 20 mg/day; vitamin E, 50 mg/day	5–8 years	18% increase in lung cancer; 8% increase in mortality	13
CARET	18,314 men and women and asbestoss workers (45–74 years of age)	β-carotene, 30 mg/day; vitamin A, 25,000 IU	<4 years	28% increase in lung cancer; 17% increase in deaths	15
PHS	22,071 male physicians (40–84 years of age)	β-carotene, 50 mg on alternate days	12 years	No effect of supplementation in incidence of cancer	14

Table 2. *Cont.*

Studies	Study designs				Ref. No.
	Population	Intervention	Duration	Cancer outcome	
Linxian	29,584 men and women, vitamin and mineral deficient (40–69 years of age)	β-carotene, 15 mg/day; selenium, 50 mg/day; α-tocopherol, 30 mg/day	5 years	13% decrease in total cancers; 9% decrease in overall deaths	84
Women's Health Study	39,876 female health professionals (over 45 years of age)	β-carotene, 50 mg on alternate days	4.1 years (2.1 years' treatment and 2.0 years' follow-up)	No effect of supplementation in incidence of cancer	87

The epidemiologic studies reported an inverse relationship between diet and/or blood β-carotene levels and cancer prevention. It is probable that β-carotene serves as a marker of increased fruit and vegetable intake and, therefore, of all components that have cancer prevention potential, for example vitamin C, folic acid, other carotenoids and polyphenols. Alternatively, low-dose dietary levels could have a protective effect against cancer, whereas high-dose β-carotene supplementation could have a cancer stimulating effect.

6. Cancer Chemoprevention by Carotenoids in Preclinical Studies

Cancer chemoprevention is a rapidly expanding discipline that focuses on the discovery and identification of dietary agents and drugs that prevent or inhibit malignant tumor development [4,5]. Since approximately one-third of the overall risk of cancer is attributable to diet, a large number of dietary compounds have been tested to determine their chemopreventive ability using animal carcinogenesis models [87–90]. The higher eukaryotic aerobic organisms, including man, cannot exist without oxygen, yet oxygen represents a danger to their very existence due to its high reactivity. This fact has been termed the paradox of aerobic life [91]. A number of ROS are generated during normal aerobic metabolism such as the superoxide, hydrogen peroxide and the hydroxyl radical. In addition, singlet oxygen can be generated through photochemical events (in skin and eyes), and lipid peroxidation can lead to peroxyl radical formation [92]. These oxidants collectively contribute to aging and degenerative diseases such as cancer and atherosclerosis through oxidation of DNA, proteins and lipids [91–93]. Antioxidant compounds can decrease mutagenesis, and thus carcinogenesis, both by decreasing oxidative damage of DNA and by decreasing oxidant-stimulated cell division [92]. The human body maintains an array of endogenous antioxidants such as catalase and superoxide dismutase; however, exogenous dietary antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and carotenoids play important roles in reducing oxidative damage as well [91–93], and their serum levels have the potential to be manipulated [93]. Major carotenoids with antioxidant activity that have been extensively evaluated with regard to their cancer chemopreventive ability include α- and β-carotenes, β-cryptoxanthin, lycopene, lutein and zeaxanthin.

6.1. α- and β-Carotene

Carotenoids have been studied vigorously to see if these colorful compounds can decrease the risk of cancer. In ecological studies and early case-control studies it appeared that β-carotene was a cancer-protective agent. Randomized controlled trials of β-carotene found that the isolated nutrient was either without effect [14] or actually increased the risk of lung cancer in smokers [13,15]. β-Carotene may be a marker for the intake of fruits and vegetables, but it does not have a powerful protective effect in isolated pharmacological doses. However, there is a large body of literature indicating that dietary carotenoids are cancer preventative. α-Carotene has been found to be a stronger protective agent than its well-known isomer β-carotene [94]. Studies tend to agree that overall intake of carotenoids is more protective than a high intake of a single carotenoid [94]. Hence, a variety of fruits and vegetables is still a better anti-cancer strategy than just using a single vegetable high in a specific carotenoid [94]. The richest source of α-carotene is carrots and carrot juice, with pumpkins and winter squash as a second densest source [94]. There is approximately 1 μg of α-carotene for every 2 μg of β-carotene in carrots. Previous studies in our laboratory have demonstrated the chemopreventive ability of β-carotene against oral carcinogenesis in rats [95].

Several experimental animal studies have shown that α-carotene possesses higher activity than β-carotene in suppressing tumorigenesis in the skin, lung, liver and colorectum [18,96]. In a skin tumorigenesis experiment conducted by Murakoshi *et al.* [18], the incidence of tumor-bearing mice in the positive control group was 69%, whereas those in the groups treated with β- and α-carotene were 13% and 25%, respectively. The average multiplicity (number of tumors/mouse) of tumors in the positive control group was 3.73/mouse, whereas the α-carotene-treated group had 0.13/mouse ($p < 0.01$). β-Carotene treatment also decreased tumor multiplicity (1.31/mouse), but the difference from the positive control group was insignificant ($p < 0.05$). The higher potency of α-carotene relative to β-carotene in the suppression of tumor promotion was further confirmed in their studies [18]. In a mouse lung carcinogenesis model initiated by 4-nitroquinoline 1-oxide (4-NQO) and promoted by glycerol, the average multiplicity of lung tumors per mouse in the positive control group was 4.06/mouse, whereas the α-carotene-treated group had 1.33/mouse ($p < 0.001$). β-Carotene treatment did not show any suppressive effect on tumor multiplicity, which was significantly increased (4.93/mouse, $p < 0.02$). In their liver carcinogenesis experiment [18], male C3H/He mice, which have a high incidence of spontaneous liver tumor development, were treated with drinking water containing 0.05% α- and β-carotene for 40 weeks. The mean number of hepatomas (3.00/mouse; $p < 0.001$) in the mice that received α-carotene was significantly decreased as compared with the untreated control group (6.31/mouse). On the other hand, the β-carotene-treated group only showed a tendency toward a decrease in tumors (4.71/mouse), as compared with the control group [18]. Narisawa *et al.* [96] also demonstrated the protective effects of α-carotene, lycopene and lutein, but not β-carotene, on preneoplastic colorectal adenocarcinoma lesions.

6.2. β-Cryptoxanthin

It is known that certain carotenoids and flavonoids can inhibit cancer development in animal carcinogenesis models [87–90]. β-Cryptoxanthin and hesperidin are such compounds. β-Cryptoxanthin