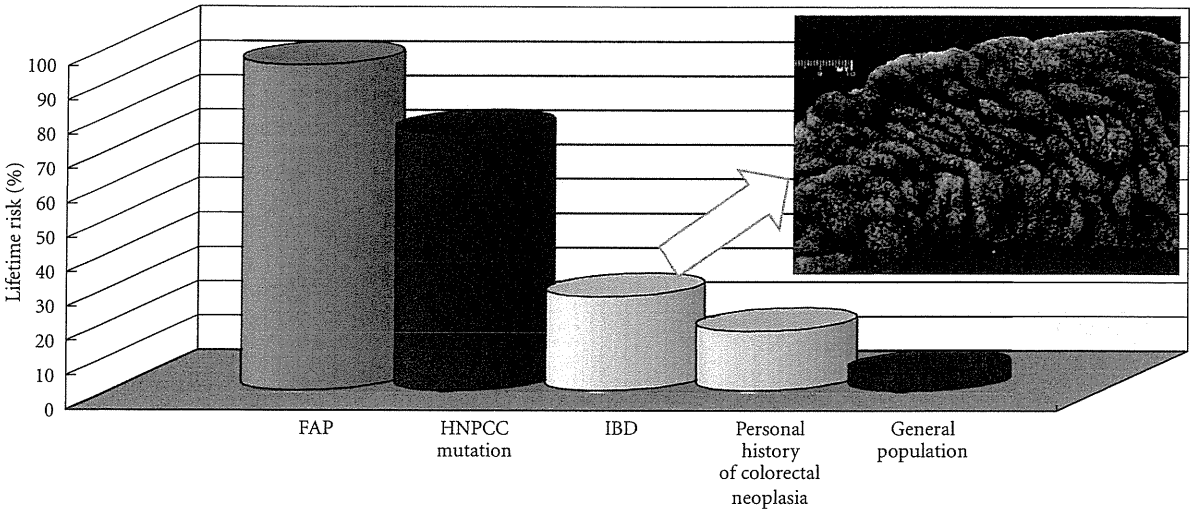


UC patients have been increasing in Japan and Korea

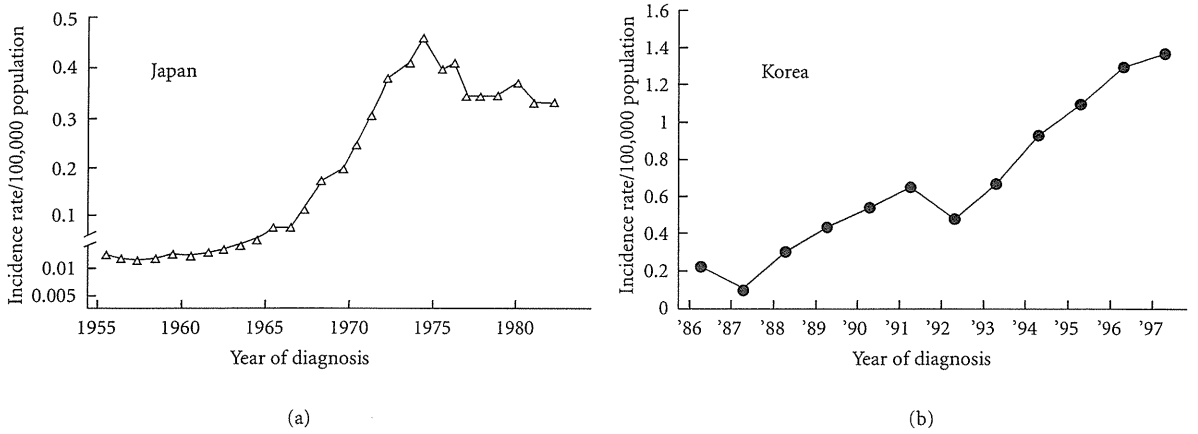


FIGURE 2: Risk of colorectal cancer.

3. Development of an Inflammation-Associated Colorectal Cancer Model

Rats have mostly been employed for an animal colorectal carcinogenesis model, and azoxymethane (AOM), methylazoxymethanol (MAM) acetate, and 1,2-dimethylhydrazine (DMH) have been widely used as colorectal carcinogenic substances (Table 2) [10]. About 30 weeks are required for development of colorectal cancer in about half of rats that are initiated with the colonic carcinogens. On the other hand, in experiments and studies using mice, multiple administrations of similar colorectal carcinogens are required and it takes a long term of 40 weeks or longer to develop colorectal cancer [11]. Therefore, I tried to develop a novel mouse model that would develop colorectal cancer in a short term in the inflamed colon [12]. To settle the issue of the influence of peroxisome proliferator-activated receptor (PPAR) agonists on colorectal carcinogenesis, which has been a topic on the journal *Nat Med* since 1998 [13–15], we confirmed that colitis inducing dextran sodium sulfate (DSS), employed in an experiment using rats with aberrant crypt foci (ACF) as a biological marker (Figure 4) [9, 16–18], had tumor promoter activity to accelerate development of ACF and hypothesized that a combination of DSS and AOM would induce colorectal cancer in a short-term period in mice as well [19].

Since DSS is a nongenotoxic carcinogen [20], male ICR mice were divided into three groups that received different administration patterns: DSS → AOM, AOM during DSS administration, and AOM → DSS (Figure 5). In the groups of DSS → AOM and AOM → DSS, there was a one-week interval between the treatments [12]. DSS was given at the concentration of 2% in drinking water (distilled water) for one week and AOM was administered intraperitoneally once at a low dose of 10 mg/kg body weight, which could not induce colorectal tumors, namely, the low-dose initiation. Interestingly, many colorectal tumors (tubular adenomas

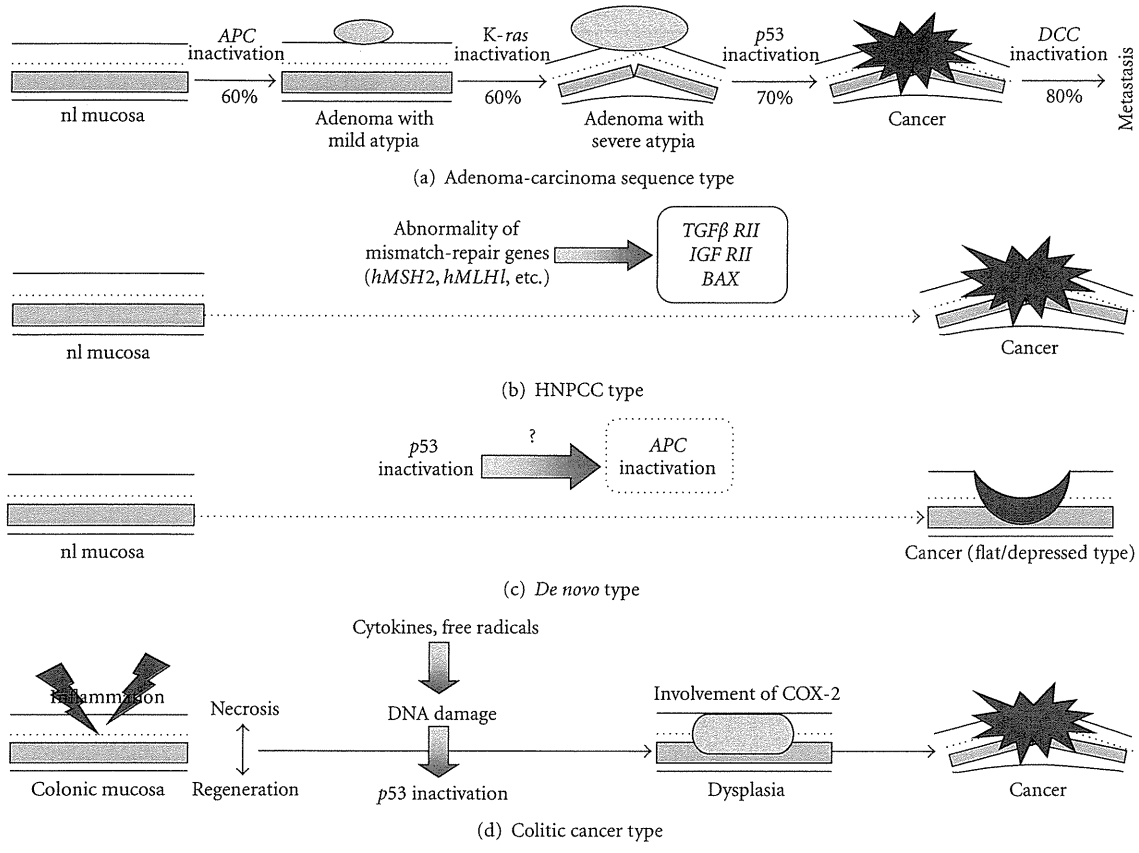


FIGURE 3: Carcinogenic steps of four types of human colorectal cancer.

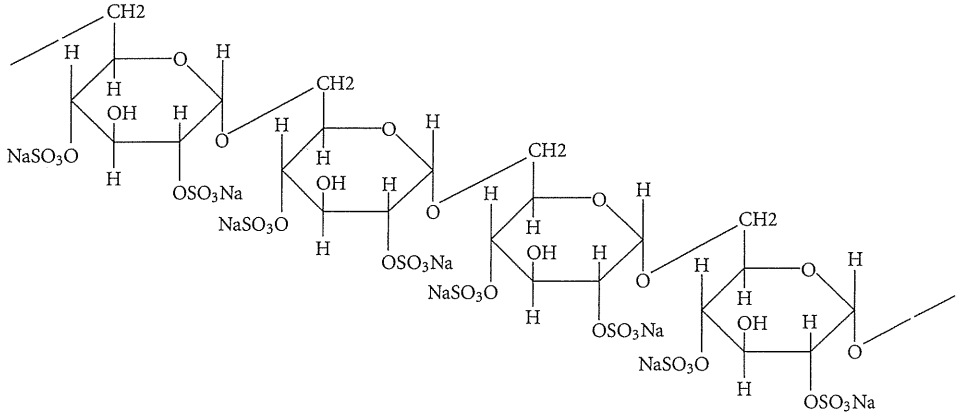


FIGURE 4: Chemical structure of dextran sulfate sodium (DSS), a sulfated polysaccharide, and its biological activities. DSS (1–5% in drinking water or diet) induces colitis in rodents. Treatment with DSS (1% in diet) after DMH exposure produces colonic adenocarcinoma [44]. The tumorigenicityof DSS is non-genotoxic effects [20]. Cycle treatment with 3% DSS (MW 54,000, 7 days) and distilled water (14 days) produces colonic tumors [45]. DSS increases the number of ACF induced by AOM [19].

and tubular adenocarcinomas) developed in the distal colon, where DSS could induce severe colitis, of mice in the group of AOM → DSS. On the other hand, mice of other groups (the DSS → AOM and the AOM during DSS administration groups) did not develop colorectal tumors. The findings confirm potent tumor-promotion activity of DSS (Figure 6). At the same time, the results reconfirmed importance of inflammation in colorectal carcinogenesis [12]. In addition, accumulation of β-catenin in the nuclei of colorectal adenocarcinoma cells was observed.

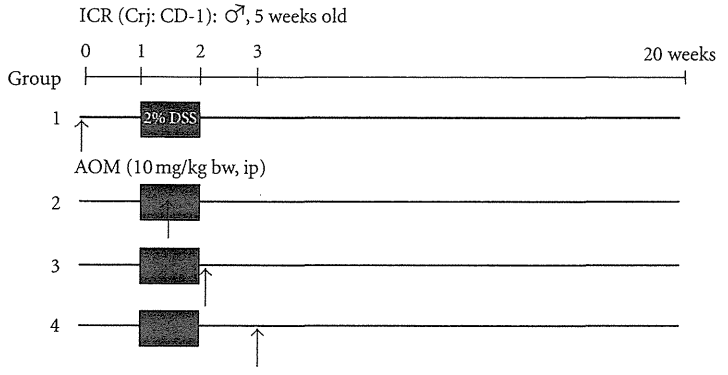


FIGURE 5: Experimental protocol to develop an inflammation-associated mouse colon carcinogenesis model, to develop a new inflammation-related mouse colon carcinogenesis model [12].

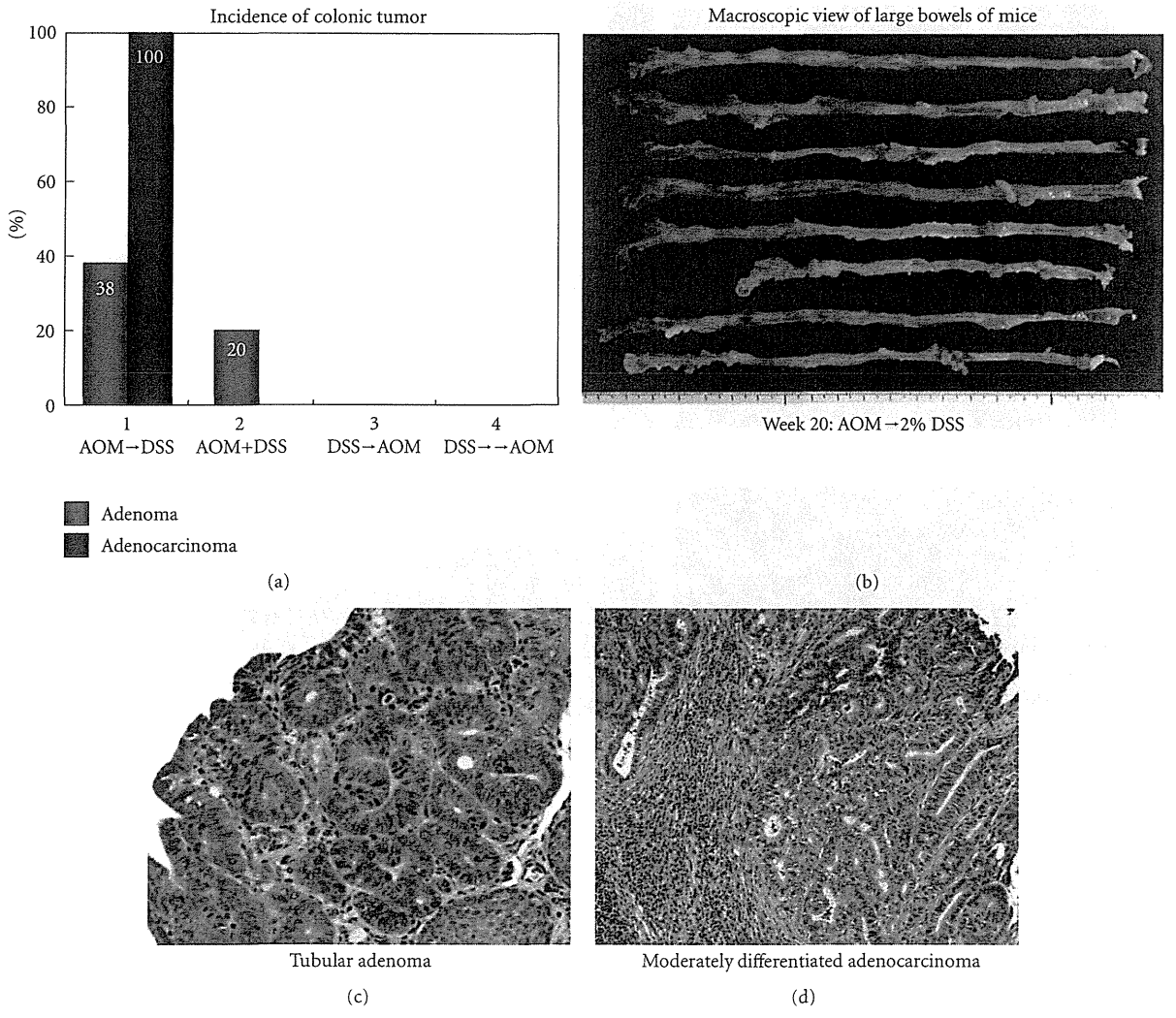


FIGURE 6: Macroscopic view, incidence, and histopathology of colonic tumors in the groups of mice that received four different treatment schedules of AOM and DSS.

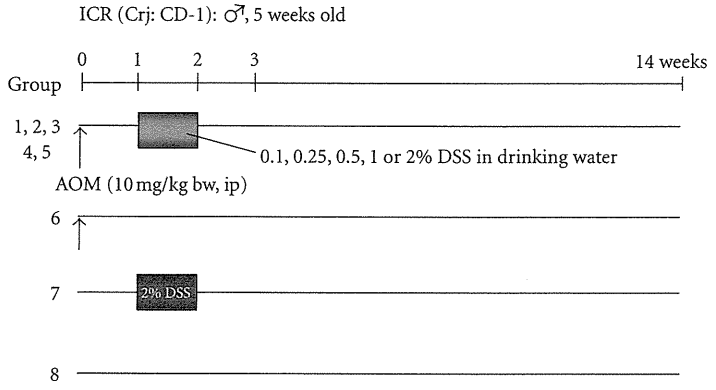


FIGURE 7: Experimental protocol for determining dose-response of DSS in mice initiated with AOM [21].

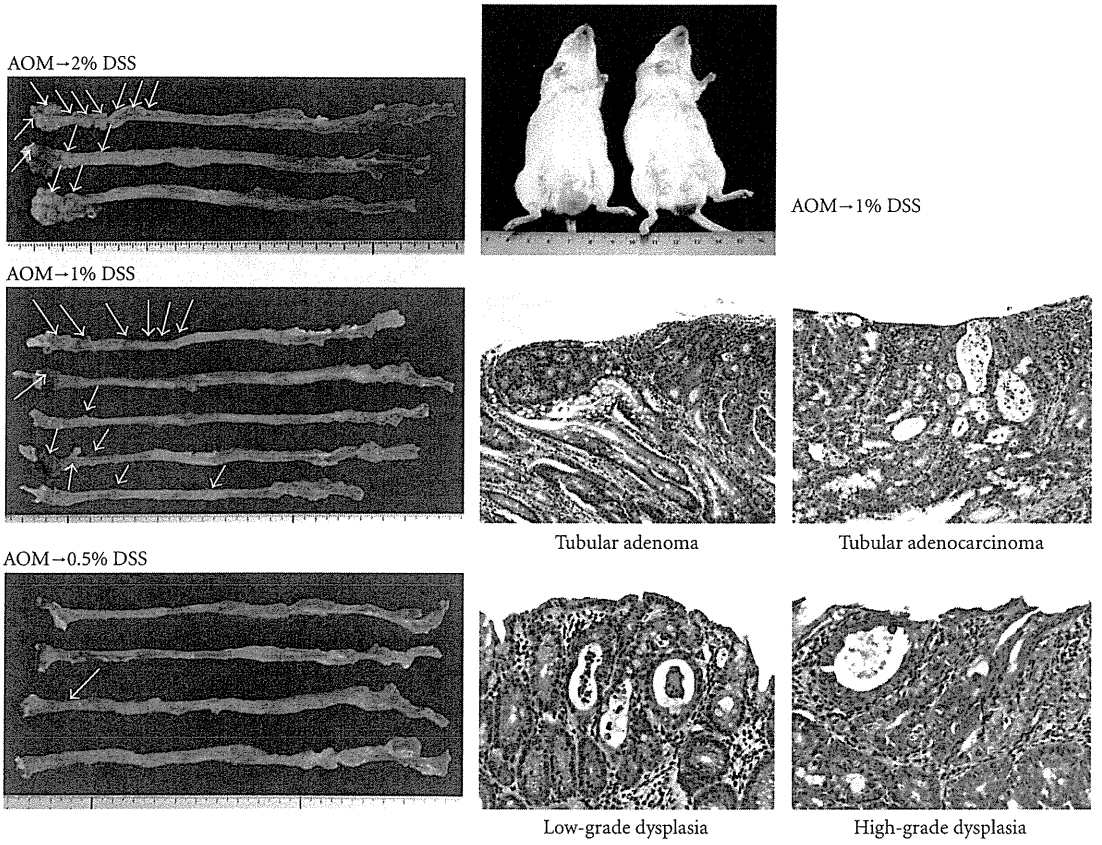


FIGURE 8: Macroscopic view and histopathology of colonic tumors developed in mice that received AOM and DSS (0.5%, 1%, or 2% DSS in drinking water).

Dose dependence of tumor-promotion activity of DSS after a single intraperitoneal administration of AOM (10 mg/kg body weight) was subsequently examined at five doses (0.1%, 0.25%, 0.5%, 1%, and 2%) of DSS (Figure 7) [21]. The findings indicated that tumor-promotion activity DSS was not observed at the concentration 0.25% or lower and only one tubular adenoma developed in a mouse that received AOM and 0.5% DSS. Colorectal tumors were developed in all mice by the treatment with 1% DSS and 2% DSS after AOM initiation and the number of colorectal adenocarcinoma was much greater in the group of mice treated with 2% DSS (Figure 8). The severity of colonic inflammation was determined by the histological inflammation score and immunohistochemical nitrotyrosine-positive reactivity. Both the inflammation score and nitrotyrosine-positive score in inflammatory cells that infiltrated colonic

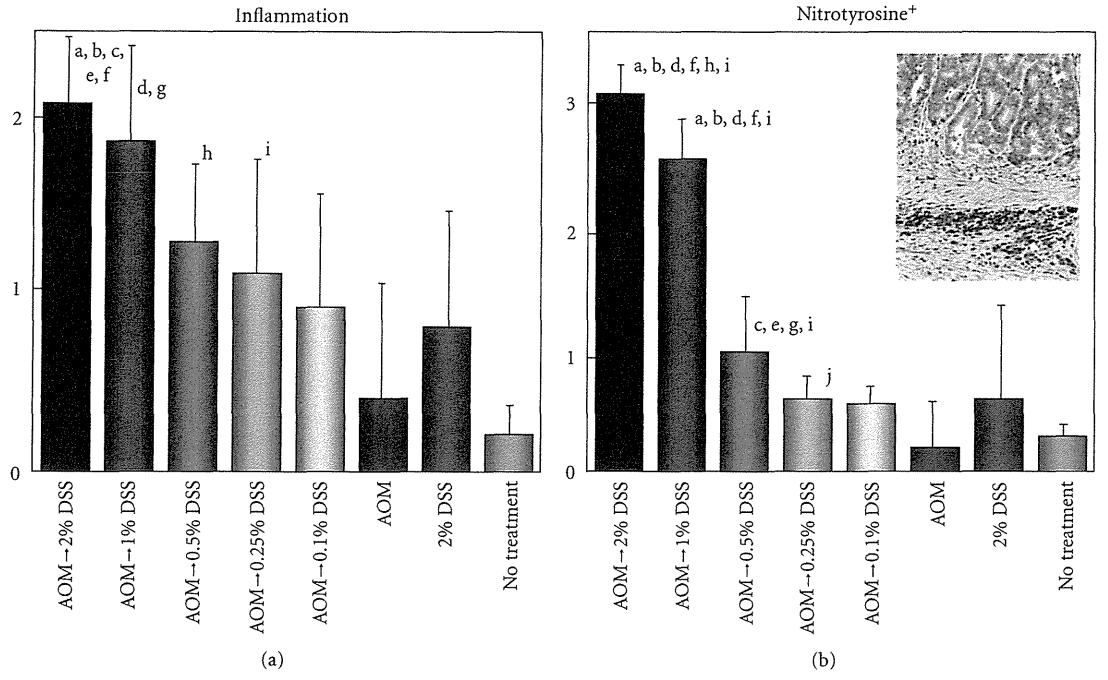


FIGURE 9: Inflammation and nitrotyrosine-positive scores in the colon of mice that received AOM and/or DSS (0.1%, 0.25%, 0.5%, 1%, or 2% DSS in drinking water). (a) Significantly different: a ($P < 0.05$), versus AOM → 0.5% DSS group; b ($P < 0.05$), versus AOM → 0.1% DSS group; c ($P < 0.01$) and d ($P < 0.05$), versus AOM alone group; e ($P < 0.05$), versus 2% DSS alone group; and f ($P < 0.001$), g ($P < 0.005$), h ($P < 0.01$), and i ($P < 0.05$), versus no treatment group. (b) Significantly different: a ($P < 0.001$), versus AOM → 0.5% DSS group; b ($P < 0.001$) and c ($P < 0.05$), versus AOM → 0.25% DSS group; d ($P < 0.001$) and e ($P < 0.01$), versus AOM → 0.1% DSS group; f ($P < 0.001$) and g ($P < 0.05$), versus AOM alone group; h ($P < 0.005$), versus 2% DSS alone group; and i ($P < 0.001$) and j ($P < 0.05$), versus no treatment group.

mucosa were higher in mice that received higher doses of DSS after AOM, suggesting that inflammation and nitrosation were involved in the tumor-promotion activity of DSS (Figure 9).

Time-course observation during AOM/DSS-induced mouse colorectal carcinogenesis was conducted to determine when colonic tumors occur in the inflamed colon of mice that received 2% DSS after the AOM initiation [22]. Male ICR mice were initiated with a single intraperitoneal injection of AOM (10 mg/kg body weight) and followed by one week administration with 2% DSS in drinking water. Our time-course observation revealed that colorectal adenoma and adenocarcinoma developed three and four weeks after AOM administration, respectively, and the numbers increased in a time-dependent manner during the follow-up period up to 14 weeks (Figure 10). Interesting finding of this study was that the high inflammation score and high nitrotyrosine-positive score lasted until five to six weeks after the cessation of DSS administration (Figure 11). Since mucosal ulcer caused by DSS administration was microscopically repaired at this point, persistence of the high nitrotyrosine-positive score, rather than the high inflammatory score, is intriguing as well as strong iNOS expression and weak PPARγ expression in the colonic mucosa at five and 10 weeks after the AOM administration (Figure 12).

Instead of AOM, experiments with DMH [23] or a heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) [24] as an initiator (colonic carcinogene) and followed by DSS treatment showed similar results described previously (Figure 13). Histopathologically, adenocarcinoma induced by DMH/DSS showed severer atypia and more aggressive biological natures than that induced by AOM/DSS. As noticed in the cancers induced by AOM/DSS, the adenocarcinoma cells developed in the inflamed colon of mice that received DMH and DSS were positive for COX-2, iNOS, and β-catenin (Figure 14). Mutation patterns of the *β-catenin* gene were slightly among the adenocarcinomas that were induced by the different treatment regimens: AOM/DSS, codon 32–34, 37, and 41; DMH/DSS, codon 32, 34, 37, and 41; and PhIP/DSS, codon 32 and 34 (Figure 15). However, these mutations were restricted in the codon region (32–34, 37, 41, and 45) that played an important role in degradation of β-catenin protein.

There was a report of a difference in sensitivity of DSS-induced colitis among the species of mice [25]. To investigate whether the species differences influence inflammation-associated colorectal carcinogenesis, the sensitivity for different species of mice (Balb/c, C57BL/6N, C3H/HeN, and DBA/2N) were subjected to AOM/DSS-induced colorectal carcinogenesis [26]. The sensitivity to

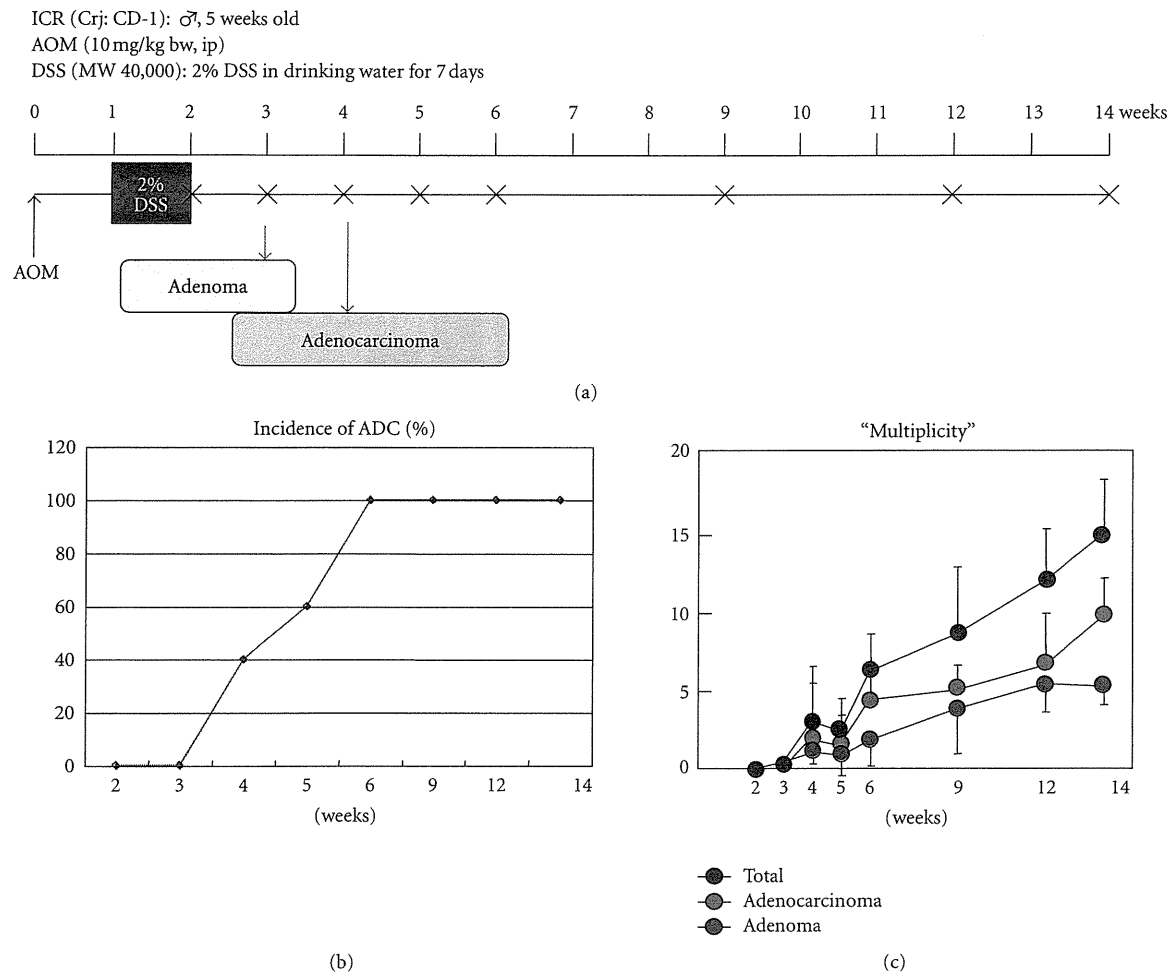


FIGURE 10: Experimental protocol of time-course observation of AOM/DSS-induce inflammation-associated colorectal carcinogenesis and tumor development (incidence and multiplicity) during the study [11].

TABLE 2: Animal models of colorectal carcinogenesis and inflammatory bowel disease. HCAs: heterocyclic amines.

(1) Animal models of colorectal carcinogenesis
(i) *Carcinogen-induced animal models*
Azoxymethane (AOM)
1,2-Dimethyl-hydrazine (DMH)
HCAs: 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)
2-Amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline (MeIQx)
(ii) *Mutant, transgenic, knockout animal models*
Min mouse and $APC^{\Delta 474}$ knockout mouse
(2) Animal models of inflammatory bowel disease
(i) *Chemically and polymer-induced models*
Trinitrobenzene sulfonic acid (TNBS): rat, mouse, rabbit
Dextran sulfate sodium (DSS): rat, mouse, hamster
Carrageenan: mouse, guinea pig, rabbit
(ii) *Microbial-induced models*
Cotton-top tamarins (*Saguinus oedipus*)
(iii) *Mutant mice*
IL-$2^{-/-}$, IL-$10^{-/-}$, TCR-$\alpha^{-/-}$, TCR-$\beta^{-/-}$

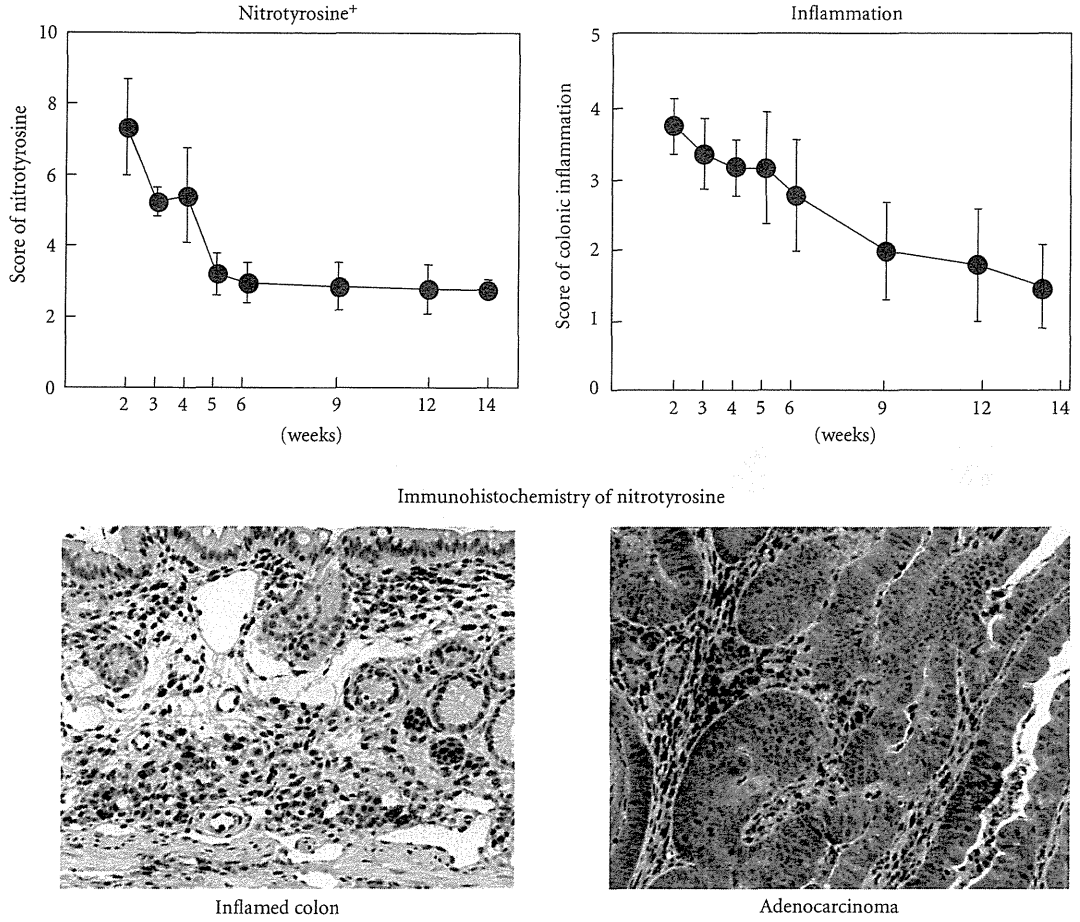


FIGURE 11: Scores of nitrotyrosine-positivity and inflammation in the inflamed colon and colonic tubular adenocarcinoma.

the AOM/DSS-induced colorectal carcinogenesis was as follows: Balb/c > C57BL/6N ≫ C3H/HeN = DBA/2N (Figure 16). The sensitivity was in relation to the nitrotyrosine-positive score estimated by immunohistochemical analysis, suggesting the importance of nitrotyrosine in the AOM/DSS-induced colorectal carcinogenesis [26].

In $Apc^{Min/+}$ mice, known as an animal model for familial adenomatous polyposis (FAP), multiple tumors (tubular adenomas) develop in the small intestine, instead of the large intestine in human FAP, and markedly few tumors develop in the large bowel. However, dysplastic crypts are observed in the colonic mucosa of $Apc^{Min/+}$ mice (Figure 17) [27, 28]. Therefore, DSS possibly enhances the growth of dysplastic crypts, and finally the lesions progress to adenocarcinomas. To investigate whether DSS-induced inflammation in the colonic mucosa would accelerate the growth of dysplastic crypts, $Apc^{Min/+}$ mice were given drinking water containing 2% DSS for one week without the initiation (carcinogen) treatment [29]. Surprisingly, multiple colorectal tumors, which were histopathologically tubular adenomas and adenocarcinomas, developed four weeks after the end of DSS treatment (Figure 18). Immunohistochemistry showed that the developed colorectal adenocarcinomas were positive against β-catenin, COX-2, iNOS, and p53 antibodies (Figure 19), suggesting that these factors were involved in the development of colorectal neoplasms in the $Apc^{Min/+}$ mice by the DSS treatment, in addition to oxidative stress and nitrosative stress. The findings suggested that DSS-induced inflammation in the large bowel of $Apc^{Min/+}$ mice exerts powerful tumor-promotion and/or progression effects on the growth of dysplastic crypts, which had already existed after the birth [27, 28].

Taken together, development of a mouse inflammation-associated colorectal carcinogenesis model was briefly described here, and the model was named as the TANAKA model. This model was possible to induce colorectal tumors in a short-term period in rats as well by similar treatment regimens (AOM/DSS and DMH/DSS) [30, 31]. It is anticipated that use of the TANAKA model will help advance the research on elucidation of the mechanisms of inflammation-associated colorectal carcinogenesis, inhibition of such carcinogenesis, and clarification of the mechanisms of the tumor-promotion ability of DSS. In particular, development of challenging research using Kyoto *Apc* Delta (KAD) rats in Kyoto University will give new insight in the pathogenesis of colorectal cancer development in the inflamed coon [32].

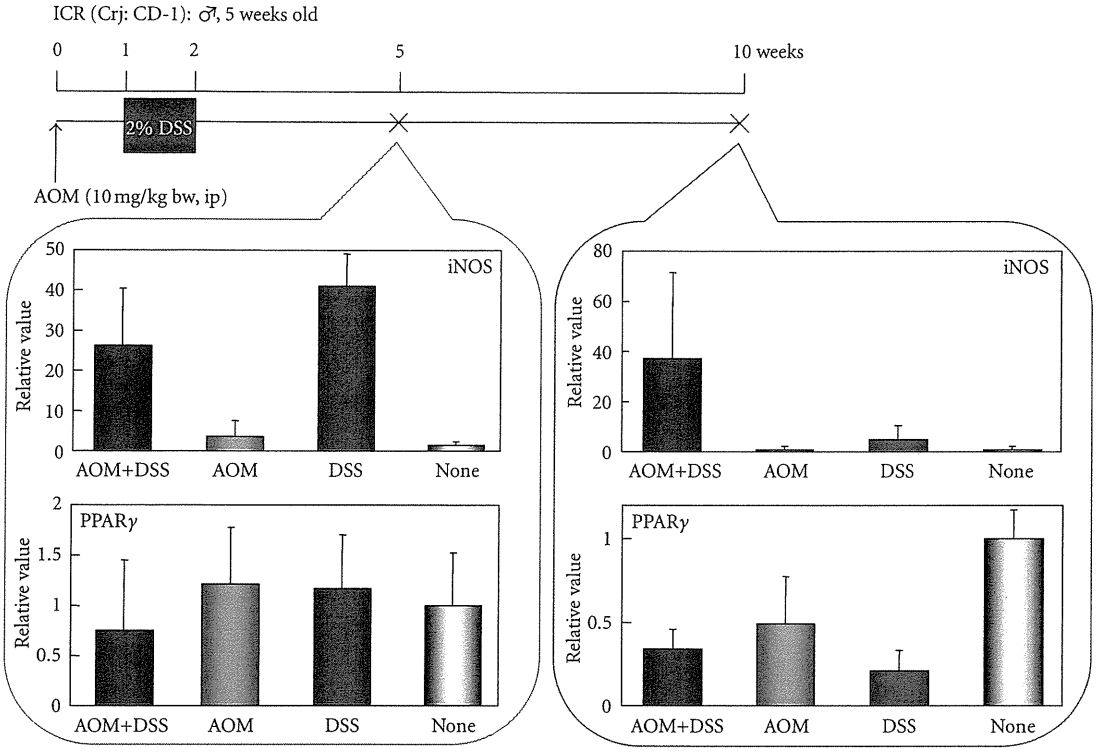
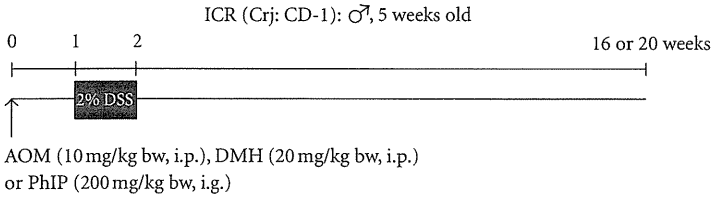


FIGURE 12: Real-time PCR analysis of iNOS and PPARγ in the colonic mucosa of mice that received AOM and DSS at weeks 6 and 10.



Incidence and multiplicity of colonic adenocarcinoma

	AOM (20 weeks)	DMH (20 weeks)	PhIP (16 weeks)
Incidence	100%	100%	56%
Multiplicity	5.6 ± 2.4	5.8 ± 1.8	0.8 ± 1.0

Mutation in exon 3 of *β-catenin* in colonic adenocarcinoma

	AOM (20 weeks)	DMH (20 weeks)	PhIP (16 weeks)
Frequency	77% (10/13)	91% (10/11)	100% (7/7)
Mutated codon	32,33,34	32,34,37,41	32,34

FIGURE 13: DSS is a powerful promoter in colon carcinogenesis in mice initiated with various colonic carcinogens, azoxymethane (AOM), 1,2-dimethylhydrazine (DMH), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) [12, 21–24, 26].

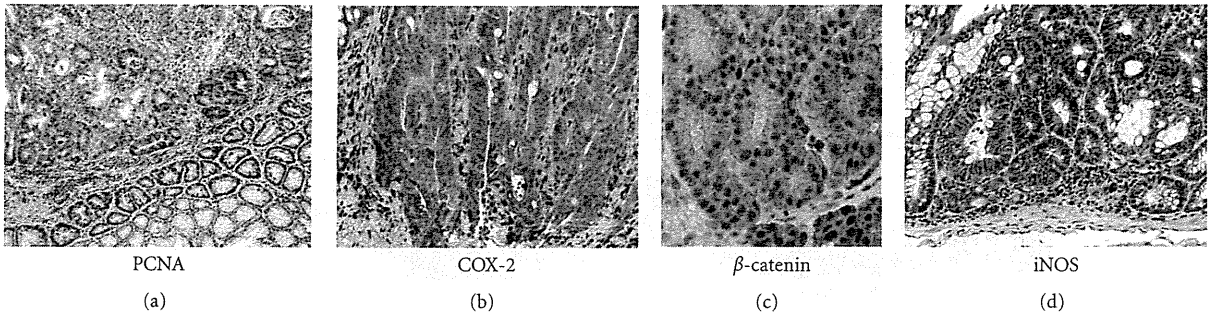


FIGURE 14: Immunohistochemistry of PCNA, β-catenin, COX-2, and iNOS in colonic adenocarcinomas of mice induced by AOM and DSS.

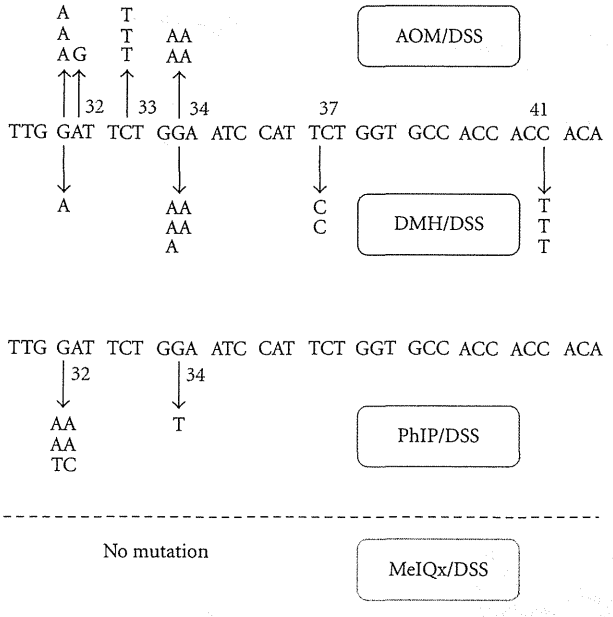


FIGURE 15: Mutations in the GSK-3β phosphorylation consensus motif of the *β-catenin* gene in adenocarcinomas of mice induced by AOM/DSS, DMH/DSS, PhIP/DSS, and 2-amino-3,8-dimethylimidazo-[4,5-*f*]-quinoxaline (MeIQx)/DSS. PhIP and MeIQx are heterocyclic amines.

4. Exploration of Chemopreventive Agents Using an Inflammation-Associated Colorectal Carcinogenic Model and Elucidation of the Mechanisms

Studies on chemoprevention of inflammation-associated colorectal carcinogenesis by several natural and synthetic compounds against have been reported using the AOM/DSS-induced mouse and rat colorectal carcinogenesis models. Several are promising compounds and their clinical application is expected. Representative compounds are auraptene and nobiletin from citrus fruits [33], collinin [33], β-cyclodextrin inclusion compounds of auraptene and 4′-geranyloxyferulic acid [34], tricin [35], melatonin [30], ursodeoxycholic acid [36], COX-2 selective inhibitor nimesulide [37], iNOS selective inhibitors [38], PPAR ligands (troglitazone and bezafibrate) [37], and a lipophilic statin pitavastatin [39]. All these compounds have anti-inflammatory activity and are able to suppress the expression of COX-2, iNOS, and inflammatory cytokines.

5. Conclusions

Animal colorectal carcinogenesis models of our own making with the background of colitis mimicking human UC are introduced, and the exploration of chemopreventive compounds using these animal models is described. In addition, we confirmed upregulation of Wif1, Plat, Myc, and Plscr2 and downregulation of Pparbp, Tgfb3, and PPARγ by comprehensive gene expression analysis in the colonic

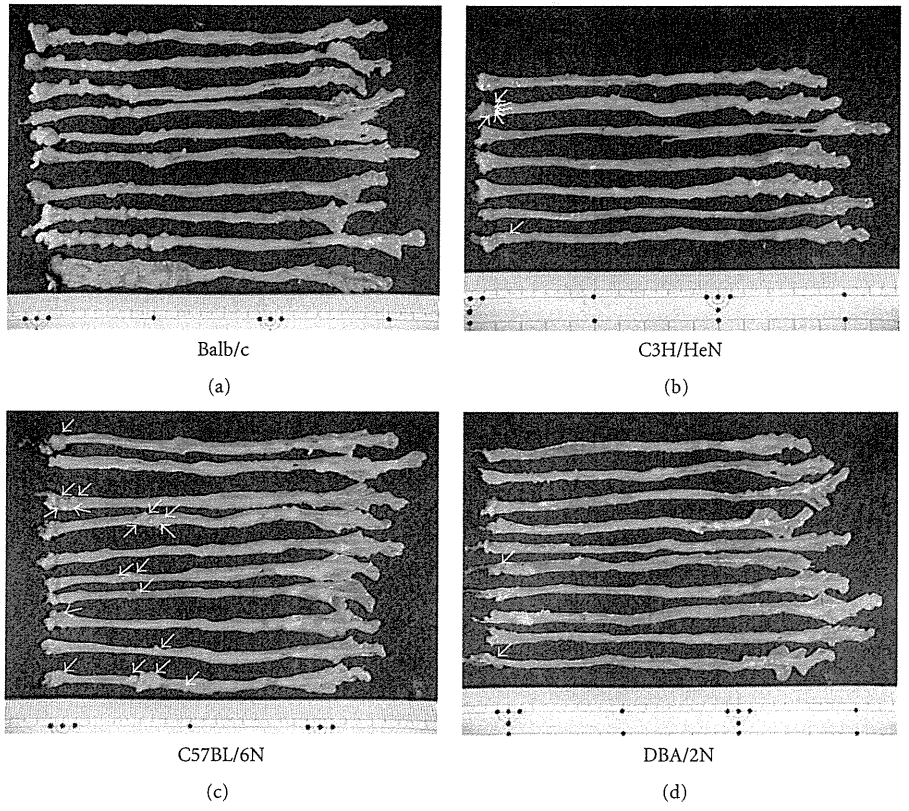


FIGURE 16: Macroscopic view of large bowel of four strains (Balb/c, C57BL/6N, C3H/HeN, and DBA/2N) of mice that received AOM and DSS.

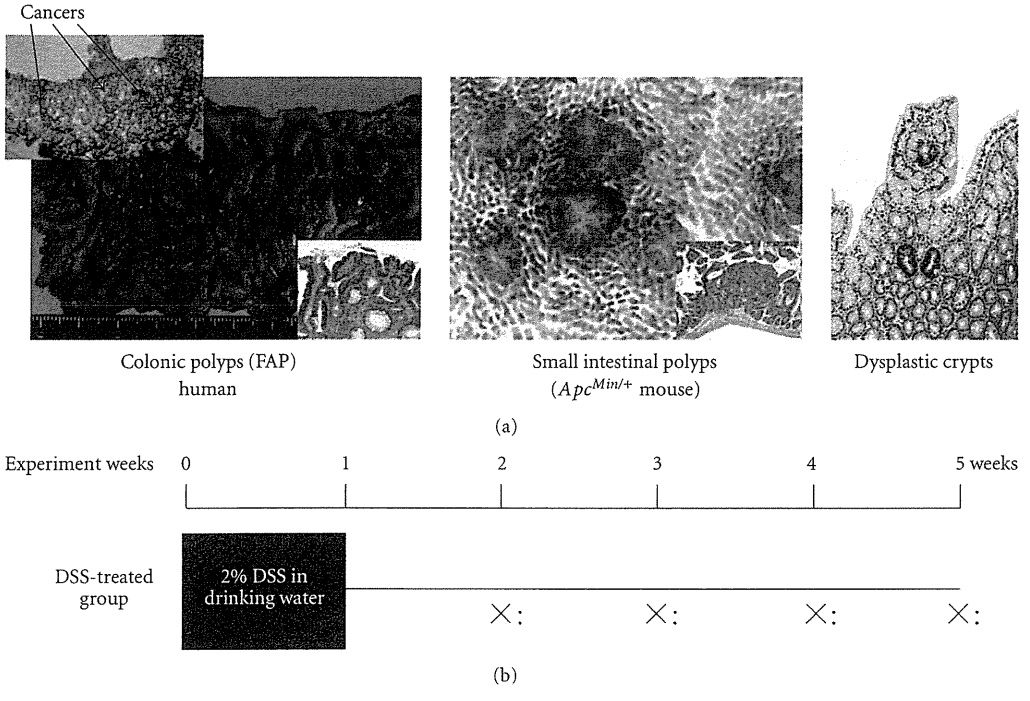


FIGURE 17: Colonic polyps in a familial adenomatous polyposis (FAP) patient and small intestinal polyps in an $APC^{Min/+}$ mouse (a). Experimental protocol for determining whether DSS promotes the growth of colonic dysplastic crypts in $APC^{Min/+}$ mice (b) [29].

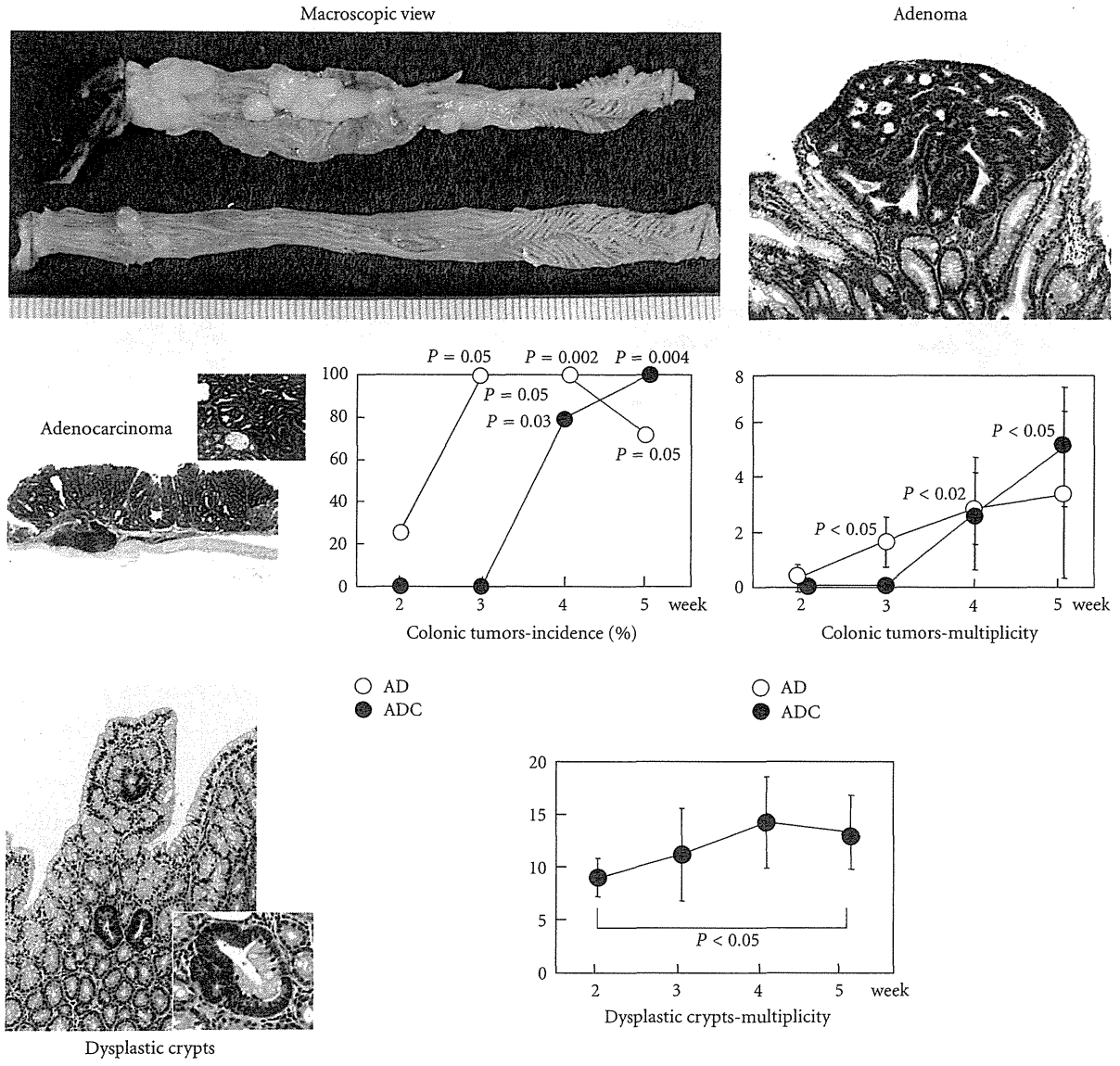
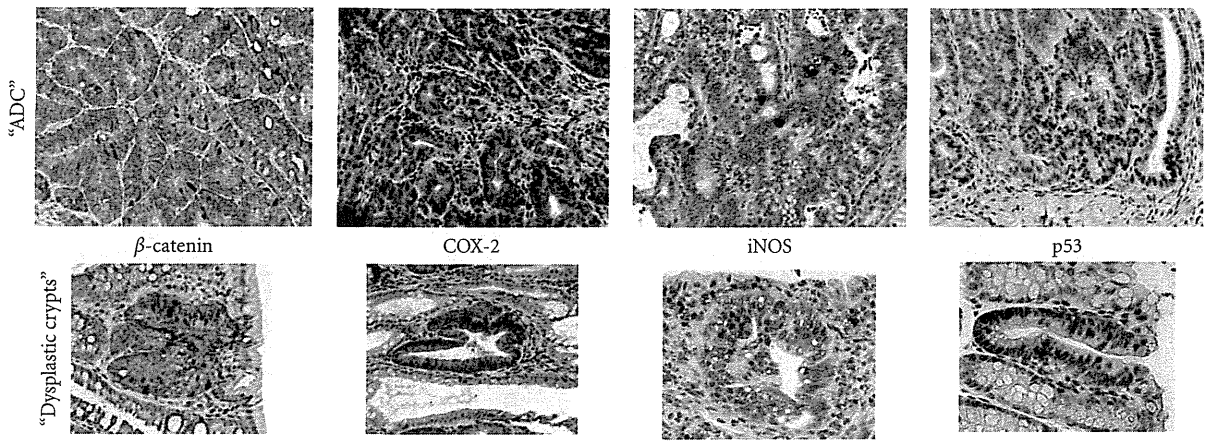


FIGURE 18: Macroscopic view and histopathology of colonic tumors and dysplastic crypts in $APC^{Min/+}$ mice that received 2% DSS for one week. Graphs show developments of these lesions during the study (up to 5 weeks).

mucosa of mice that received AOM and DSS [40]. Moreover, proteomics analysis demonstrated that beta-tropomyosin, tropomyosin 1 alpha isoform b, and S100 calcium binding protein A9 were upregulated, while Car1, selenium-binding protein 1, HMG-CoA synthase, thioredoxin 1, 1 Cys peroxiredoxin protein 2, Fcgbp protein, Cytochrome c oxidase subunit Va, and ETHE1 protein were downregulated [41]. Significance of expression of these genes and proteins in inflammation-associated colorectal carcinogenesis remains poorly understood and further detailed analysis is required. Since our recent study demonstrated that NF-κB and Nrf2 were expressed in not only inflammatory cells but also cancer cells in the TANAKA (AOM/DSS) model [34], these molecules may be the targets for cancer chemoprevention against colorectal cancer in the inflamed colon. Moreover, modification of the protocol of the TANAKA model may help us to detect environmental carcinogens [42] and tumor-promoters [43] for the large bowel. Fortunately, the animal models introduced here have attracted attention of young researchers that are doing research on colorectal carcinogenesis, IBD, inflammation, and cancer. It is anticipated that use of these models will advance elucidation of the mechanisms (methylation and microRNA) of inflammation-associated colorectal carcinogenesis, exploration of its suppression and mechanisms, and clarification of the mechanisms of tumor-promotion activity of DSS.



Treatment	*Apc* allelic loss	Gene mutation	
		β-catenin	K-*ras*
DSS	14/14 (100%)	0/14 (0%)	0/14 (0%)
Tap water	2/2 (100%)	0/2 (0%)	0/2 (0%)

FIGURE 19: Immunohistochemistry of β-catenin, COX-2, iNOS, and p53 in the colonic adenocarcinoma and dysplastic crypts developed in male $Apc^{Min/+}$ mice that received 2% DSS (upper panel). *Apc* allelic loss and gene mutations of *β-catenin* and K-*ras* in the colonic adenocarcinoma from male $Apc^{Min/+}$ mice (lower panel).

Conflict of Interests

The author declare that he has no conflict of interests.

Acknowledgments

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Review

Cancer Chemoprevention by Carotenoids

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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.

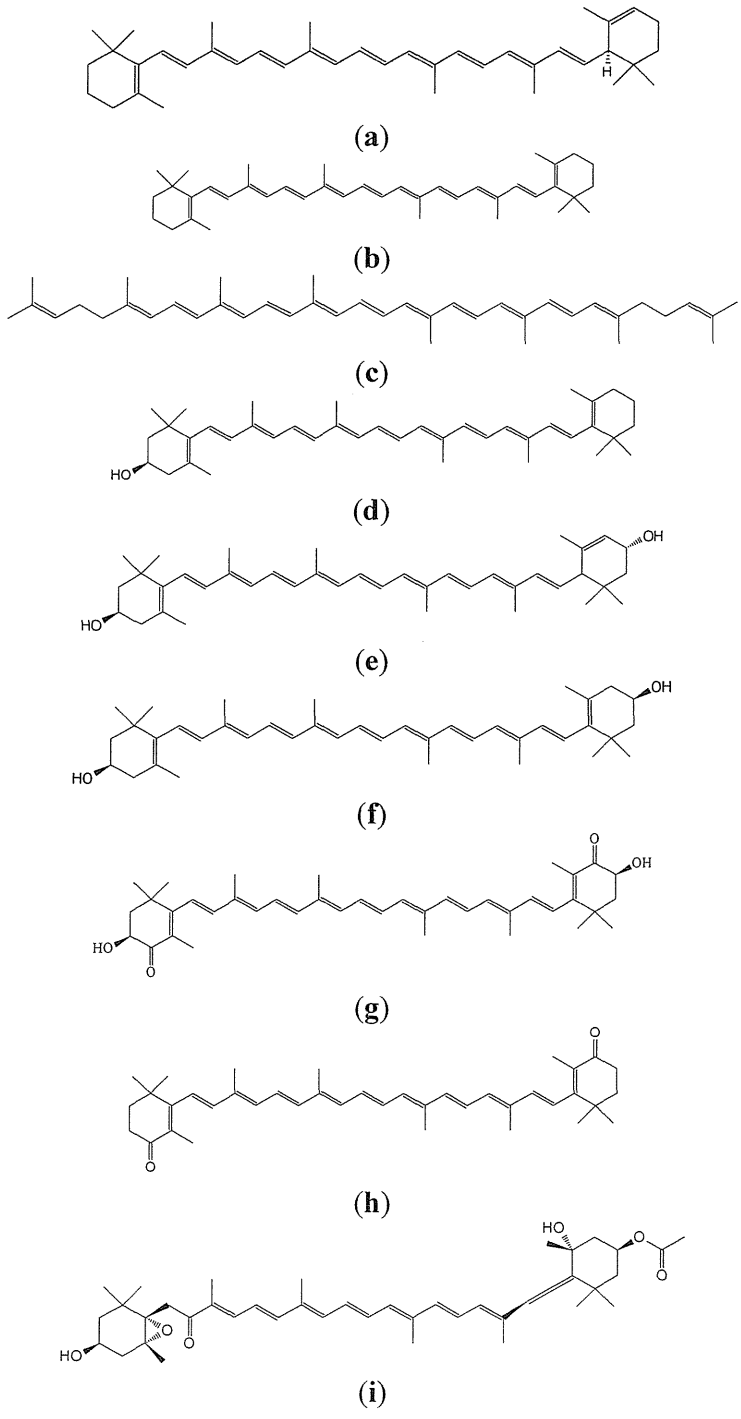
1. Introduction

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].