REVIEW

Chemoprevention of Colon Carcinogenesis by Dietary Non-nutritive Compounds

Takuji Tanaka, Hiroyuki Kohno, Hideki Mori

Abstract

In addition to mutagens and/or carcinogens a number of modulators of carcinogenesis are present in our environment. Some of them are contained in our regular foods and therefore dietary factors play a role in the development of some types of cancers including colon cancer. Epidemiological studies have suggested that a diet rich in fruits and vegetables is associated with reduced risk for a number of common cancers. There are still many unknown constituents and/or factors in foods that could either enhance or reduce the possibility of developing cancer. Animal studies of experimental chemical carcinogenesis have indicated that several non-nutritive components in foods, belonging to different chemical groups, protect against certain types of cancers including colonic neoplasms. These chemicals are known as "chemopreventive agents". Many of them are antioxidants and might suppress carcinogenesis through: (I) inhibiting Phase I enzymes; (ii) induction of Phase II enzymes; (iii) scavenging DNA reactive agents; (iv) suppression of hyper-cell proliferation induced by carcinogens; and/or (v) inhibition of certain properties of neoplastic cells. With the continuing increase in the incidence of colon cancer, there is an ever increasing need to determine the most effective means for prevention and to understand the underlying mechanism(s). Previous studies in our laboratory demonstrated protective effects of several naturally occurring products against rat colon tumorigenesis. This article will introduce our recent studies in our search for chemopreventive effects of flavonoids (diosmin and hesperidin) and other phytochemicals in edible plants on rat colon carcinogenesis.

Key words: colon carcinogenesis - chemoprevention - non-nutritives - flavonoids - diet - rats

Asian Pacific J Cancer Prev, **2**, 1-13

Introduction

A number of compounds in diet are known to modulate the development of tumors in experimental animal models (Slaga, 1980). Epidemiological studies also suggest that specific, pharmacologically active agents present in the diet might reduce or increase the relative risk of cancer development. As to colon cancer, marked variations in dietary habits among populations of different cultures and life-styles have been associated with a risk of this malignancy (Reddy, 1986; Weisburger, 1991). Also, there is an inverse correlation between the intake of vegetables/fruits and human colon cancer (Block et al., 1992; Hirayama, 1979; Steinmetz and Potter, 1991; Steinmetz and Potter, 1991). Thus, a relationship between the risk of the development of colon cancer and dietary habits is important (Reddy, 1993; Tanaka, 1997), although the etiology of colon cancer is multifactorial and complex. Among the dietary components, fiber is found to reduce the risk of colorectal cancer development (Fuchs et al., 1999; Giovannucci et al., 1994). Also, green tea could inhibit colorectal tumorigenesis (Dashwood et al., 1999; Weisburger, 1999; Weisburger, 2000; Williams et al., 1999). However, recently several epidemiological data have suggested no effects of dietary fiber or green tea on colon or stomach tumorigenesis (Alberts et al., 2000; Schatzkin et al., 2000; Tsubono et al., 2001).

Prevention of disease is an old and important concept. An essential consideration in cancer research today is that exposure to pharmacologically active chemicals may play an important role in reducing the relative risks resulting from exposure to carcinogenic chemicals. Chemoprevention of cancer might be defined as the deliberate introduction of these selected non-toxic substances into the diet for the purpose of reducing cancer development. Numerous

Takuji Tanaka and Hiroyuki Kohno, First Department of Pathology, Kanazawa Medical University, 1-1 Daigaku, Uchinada-cho, Kahoku-gun, Ishikawa 920-0293, Japan, Fax+81-076-286-6926 E-mail: takutt@kanazawa-med.ac.jp Hideki Mori, First Department of Pathology, Gifu University School of Medicine, Fax 058-265-9005 Email: hidmori@cc.gifu-u.ac.jp

Table 1. Possible Chemopreventive Agents Against Colon Carcinogenesis

Chemopreventive agents	Mechanisms of action
Dietary supplements	
Fiber; wheat bran	Decrease fecal diacylglycerol: decrease PKC activation
Calcium	Bind bile salts; direct antiproliferative effect on cryptal cells
Vitamin D	Normalize differentiation in crypt epithelium
Folic acid	Correct DNA methylation imbalance
Selenium	Antioxidant activity
Allyl sulfides, isothiocyanates, indoles	Induce GST and other detoxifying enzymes
Vitamin C, vitamin E, b-carotene, flavonoids	Scavenging oxygen radicals, preventing DNA damage
Inositol, phytic acid	Modulate transmembrane signaling
Caffeic acid, other plant phenolics	Inhibit nitrosation to form carcinogenic nitrosamines, antioxidants in vivo; reduce AA metabolism
w-3 fatty acids	Reduce AA metabolism, thereby reducing PG activity
Conjugated linoleic acid	Alter membrane phospholipids; decrease cryptal cells proliferation: induce epithelial differentiation
Drugs	
Sulindac and related NSAIDs	Block PG activity; induce apoptosis
Aspirin	Block PG activity
Specific COX-2 inhibitors	Block COX-2 activity
DFMO	Inhibit ODC
N-Acetyl-L-cysteine	Increase DNA repair capability
Oltipraz	Induce GST and other detoxifying enzymes

PKC, protein kinase C; GST, glutathione S-transferase; AA, arachidonic acid; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; COX-2, cyclooxygenase-2; DFMO, DL-α-difluoromethylornithine.

epidemiological studies on the relationship between diet and carcinogenesis have demonstrated a protective effect of the consumption of fruits and vegetables against various forms of cancers (Block et al., 1992; Hebert et al., 1993; Steinmetz and Potter, 1996). Potential chemopreventive agents are to be found both among nutrients and non-nutrients in diet. Epidemiological and experimental studies have revealed that a number of micronutrients may have cancer preventive properties in several organs including large bowel (Micozzi, 1989). Examples are vitamins A, C, and β-carotene, selenium, and calcium. We have demonstrated cancer chemopreventive ability of two xanthophylls without provitamin A activity in the rat colon and oral cavity (Tanaka et al., 1995; Tanaka et al., 1995). Most of these compounds are antioxidants which could serve as an explanation for their mode of action. The well-known non-nutritive chemopreventives in colon tumorigenesis is dietary fiber, a variety of ingestible carbohydrates (Weisburger et al., 1993). Since the modifying effects of the major dietary factors on rodents colon carcinogenesis resulted in heterogeneous (Angres and Beth, 1991), we focus on other non-nutritive inhibitors derived from vegetables and fruits in experimental colon carcinogenesis. Wattenberg also suggested that some minor non-nutrients in the diet have protective effects on colon tumorigenesis (Wattenberg, 1983). In 1985, he roughly classified chemopreventive agents into blocking and suppressing agents based on the time period that agents appear to have activity in animal models of carcinogenesis (Wattenberg, 1985). Since then, several naturally occurring compounds and synthetic chemicals have been intensively investigated for their chemopreventive ability on chemically-induced malignant epithelial neoplasms including colon carcinoma. These include the inorganic and organic selenium salts, phenolic antioxidants, non-steroidal anti-inflammatory drugs (NSAIDs), ornithine decarboxylase (ODC) inhibitors, etc. (Table 1). Our group also found several natural or synthetic chemopreventive agents against colon carcinogenesis (Table 2). Indeed food chemists and natural product scientists have identified hundreds of

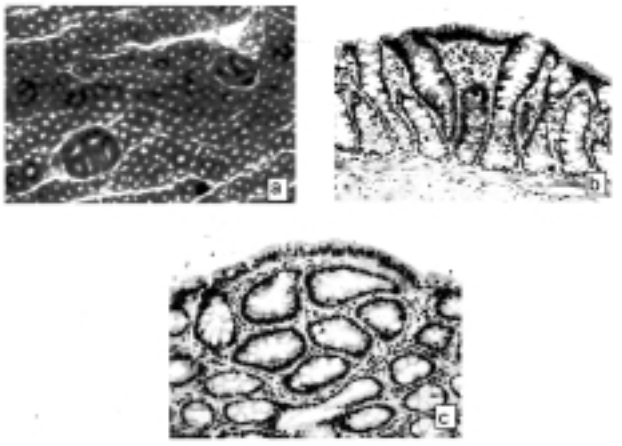


Figure 1. ACF in Rat Colon Exposed to AOM. (a) ACF in Methylene Blue-Stained Colonic Mucosa; (b) ACF in a Histological Section Stained with Hematoxylin and Eosin; and (c) Aberrant Crypts Show Weakly Postive for PPARγ Antibody.

Table 2. Natural and Synthetic Chemopreventive Agents Against Colon Carcinogenesis

	Agents	Carcinogens	Year
Natural compounds	Chlorogenic acid	MAM acetate	1986
	Magnesium hydroxide	MAM acetate, DMH	1989
	Flavoglaucin	AOM	1991
	Shikonin	AOM	1992
	Gingerol	AOM	1992
	Protocatechuic acid	AOM	1992
	Benzyl isothiocyanate	AOM	1994
	Benzyl thiocyanate	AOM	1994
	Astaxanthin	AOM	1994
	Canthaxanthin	AOM	1995
	Hesperidin	AOM	1995
	Diosmin	AOM	1995
	Costunolide	AOM	1994
	S-Methyl methane	AOM	1995
	thiosulfonate	AOM	1995
	1'-Acetoxychavicol acetate	AOM	1995
Synthetic compounds	*p*-Methoxybensenselenol	AOM	1985
	Disulfiram	AOM	1995
	Indomethacin	1-Hydroxyanthraquinone	1991
	KYN-54	AOM	1992
	DFMO	AOM	1995
	Mofarotene	AOM	1995

MAM acetate, methylazoxymethnaol acetate; DMH, dimethylhydrazine: AOM, azoxymethane; KYN-54, 5-hydroxy-4-(2-phenyl-(E)-ethenyl)-2(5H)-furanone; DFMO, DL-α-difluoromethylornithine.

"phytochemicals" that are being evaluated for the prevention of cancer (Huang et al., 1994; Am, Inst. Cancer Res., 1996). Among the non-nutrients dietary components believed to exert a chemopreventive effect are flavonoids, polyphenolic derivatives of benzo(γ)pyrone that are widely distributed in edible plants (Formica and Regelson, 1995). There are several major classes of flavonoids, which may occur as glycosides or aglycones. Total dietary intake of flavonoids has been estimated as high as 1 g/day, equivalent to 50,000 ppm in diet (Pierpoint, 1986), although more recent studies have indicated that intake varies widely (Hertog et al., 1995).

It has been proposed that aberrant crypt foci (ACF, Fig. 1a and b) being present in carcinogen-treated colons of rodent and in the colons of humans with a high risk for colon cancer could be employed to study modulators of colon carcinogenesis (Table 3) (Bird, 1995; Kawamori et al., 1995; Pereira et al., 1994), since ACF are putative precursor lesions for colon cancer in rodents (Bird, 1995) and humans (Pretlow et al., 1991). ACF possess several biological aberrations including gene mutations and amplification (Bird, 1995). Also, alteration (decreased) of hexosaminidase activity is found in ACF. Tsukamoto et al. found down-regulation of both hexosaminidase-α and -β in ACF (Tsukamoto et al., 2001). ACF also have increased cell proliferation activity compared to surrounding normal crypts (Pretlow et al., 1994; Yamashita et al., 1994). We recently have confirmed their results (Table 4). Certain chemopreventive compounds are reported to reduce such hyper-cell proliferation in ACF (Li et al., 1998; Zheng et al., 1997) and to inhibit c-myc expression induced by methylazoxymethanol (MAM) acetate (Wang et al., 1993). For demonstrating the inhibitory action of compounds in colon carcinogenesis, we have used two experimental animal bioassays: (1) a 5-week short-term bioassay of ACF for screening natural compounds, which are present in vegetables and fruits, with possible chemopreventive ability (Fig, 2) and (2) a long-term rat colon carcinogenesis model for evaluating their inhibitory effects against colon carcinoma development (Fig. 3). In these bioassays, several biochemical and morphologic biomarkers are used (Table 5). Cell proliferation plays an important role in multistage carcinogenesis (Cohen and Ellwein, 1990; Lipkin, 1991; Pegg, 1988; Tanaka, 1992). ODC and polyamines are intimately involved in normal cellular proliferation and are likely to play a role in carcinogenesis including colon tumorigenesis (LaMuraglia et al., 1986; Luk et al., 1986). 5'-Bromodeoxyuridine (BrdU)-labeling index, proliferating cell nuclear antigen (PCNA)-labeling index, and silver-stained nucleolar regions (AgNORs) number are also known to be proliferation biomarkers (Tanaka, 1997).

Current data suggest that the balance between the Phase I carcinogen-activating enzymes and the Phase II detoxifying enzymes is critical to determining an individual's risk for cancer (Wilkinson and Clapper, 1997). Human deficiencies in Phase II enzyme activity, specifically glutathione *S*-

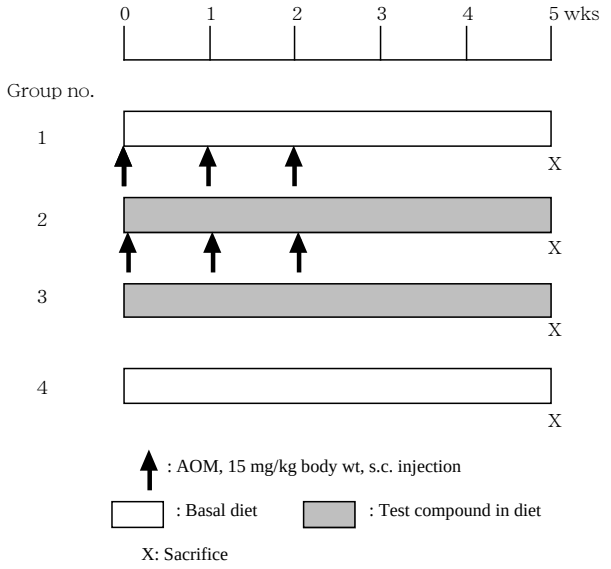


Figure 2. Experimental Design for Screening Chemopreventive Agents Against Colon Tumorigenesis (a pilot study). At Sacrifice, AFC and Cell Proliferation Biomarkers' Expression are Measured. AOM Injection are Done Once a Week for 2 (20 mg/kg bw) or 3 times (15 mg/kg bw).

Table 3. Compounds Tested for Inhibition of ACF

Anti-inflammatories/Analgesics including NSAIDs	Piroxicam, Sulfasalazine, Ibuprofen, Ketoprofen, Indomethacin, COX-2 inhibitors, etc.
Anti-helminthics	Levamisole, Oltipraz, etc.
Organosulfur compounds	Diallyl sulfide, Sodium thiosulfate, Mesna, etc.
Minerals	Sodium selenite, Sodium molybdate, Calcium, etc.
β-Glucosidase inhibitors	Potassium glucarate, Calcium glucarate, β-Sitosterol, etc.
Phenolic antioxidants	Ellagic acid, Rutin, Propyl gallate, Butyl hydroxyanisole, Curcumin, Quercetin, Nordihydroguaiaretic acid, etc.
Indoles/Isothio-compounds	Benzyl isothiocyanate, Indole-3-carbinol, Phenylethylisothiocyanate, etc.
Vitamins	Ascorbyl palmitate, Follic acid, Vitamin D_3, etc.
Epicatechins	Catechin, etc.
Differentiation agents	Dehydroepiandrosterone, Sodium butyrate, 18β-Glycyrrhetinic acid, Fluocinolone acetonide, Inositol hexaphosphate, etc.
Others	Silymarin, Arginin, Purpurin, d-Mannitol, Sodium cromolyn, Rebaudioside A, Liquiritin, Phyllodulcin, Hydrangenol, Oleanolic acid, Costunolide, Soyasaponin A2, etc.

NSAIDs, non-steroidal anti-inflammatory drugs.

transferase (GST), have been identified and associated with increased risk for colon cancer (Szarka et al., 1995). Therefore, Phase II detoxifying enzymes, such as GST and quinone reductase (QR), might be useful as a biomarker for chemopreventive studies.

The present report will introduce our recent data demonstrating chemopreventive properties of two flavonoids diosmin (DIO, Fig. 4a) and hesperidin (HPD, Fig. 4b) (Tanaka et al., 1997) and other antioxidative natural products 1'-acetoxychavicol acetate (ACA, Fig. 4c) (Tanaka et al., 1997; Tanaka et al., 1997) and auraptene (AUR, Fig. 4d) (Tanaka et al., 1998; Tanaka et al., 1997), which are present in vegetables and fruits, in colon carcinogenesis.

Screening of Possible Chemopreventive Agents Against Colon Tumorigenesis Ability Using a 5-week Short-term Bioassay of ACF

As the first bioassay for pilot studies, we investigated the modifying effects on test compounds DIO, HPD, ACA, and AUR on the development of ACF. ACF could be induced by weekly subcutaneous injections of azoxymethane (AOM, 15 mg/kg body weight, 3 times; or 20 mg/kg body weight, 2 times) and test chemicals in the basal diet at various dose levels were administered to male F344 rats for 5 weeks, starting 1 week before AOM dosing (Fig. 2). At the end of the study, ACF were counted and expression of several biomarkers was examined. The biomarkers assayed included

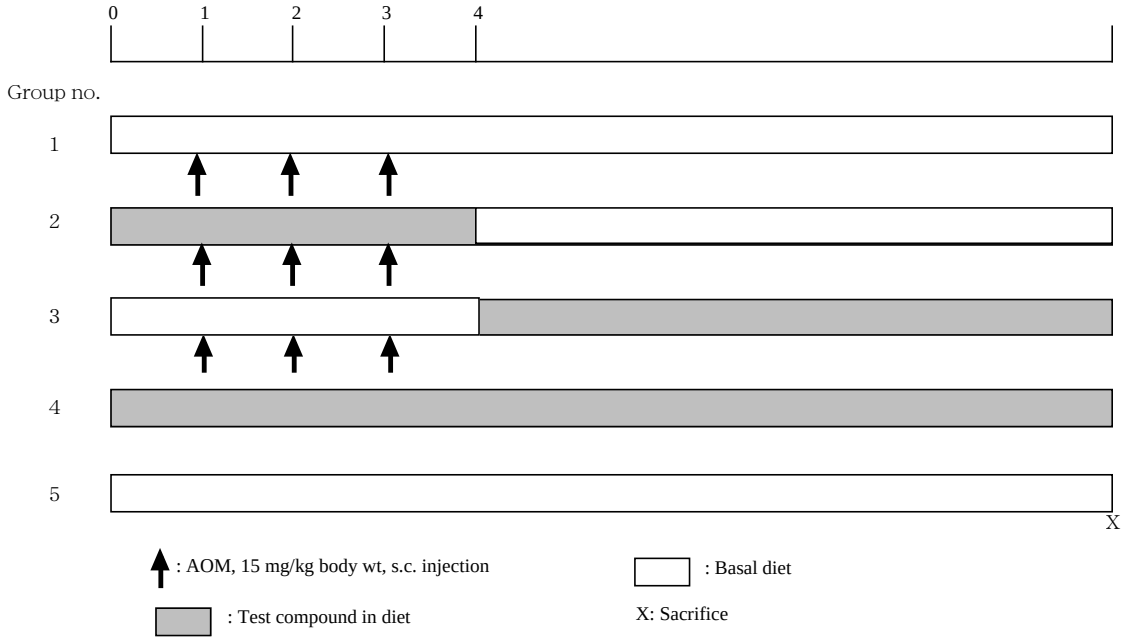


Figure 3. Experimental Design for Detecting Chemopreventive Agents Against Colon Carcinogenesis. At Sacrifice, Colonic Neoplasms and Various Cell Proliferation Biomarkers' Expression are Measured. AOM Injections are Done Once a Week for 2 (20 mg/kg bw) or 3 times (15 mg/kg bw).

Table 4. Proliferative Activity of Colonic Pathological Lesions Induced by AOM in Rats

Lesions	BrdU-labeling index (%)	PCNA-positive nuclei (%)	AgNORs number (/nucleus)
Normal crypts (without AOM)	5.9	18	1.18
Normal appearing crypts (with AOM)	7.9	20	1.68
ACF	18.6	31	2.97
Adenoma	21.1	33	3.07
Adenocarcinoma	28.3	58	3.78

AOM, azoxymethane; BrdU, 5'-bromodeoxyuridine, PCNA, proliferative nuclear antigen; AgNORs, silver-stained nucleolar organizer regions.

ornithine decarboxylase (ODC) activity and polyamine level in the colonic mucasaY, number of (AgNORs) protein/nucleus in the colonic crypts, and/or activities of GST and QR in the colonic mucosa (Table 5).

Evaluation of Chemopreventive Ability of Selected Compounds Using a Long-term Rat Colon Carcinogenesis Model

Based on the results in the pilot studies, the second bioassay for evaluating the chemopreventive effects of compounds, which have been screened by a short-term pilot study, on colon carcinogenesis was conducted. Male F344 rats were given subcutaneously injections of AOM (15 mg/kg body weight, weekly, 3 times; or 20 mg/kg body weight, 2 times) to induce colonic adenocarcinoma (Fig. 3). For "initiation" feeding, oral administration of these compounds in the diets was begun 1 week before the AOM exposure and continued for 4 or 3 weeks, and for "post-initiation" feeding, experimental diets containing test compounds, beginning 1 week after the last dosing of AOM, were given for 28 weeks (DIO and HPD) or for 32 weeks (ACA and AUR). Biomarkers used were as follows: polyamine level, activities of ODC, GST, and QR and in the colonic mucosa, number of AgNORs protein/nucleus in the colonic crypts, and/or level of aldehydic lipid peroxidation products, malondialdehyde (MDA) and 4-hydroxy-2(E)-nonenal (4-HNE), in the colonic mucosa (Table 5).

Inhibition of Colon Carcinogenesis by DIO and HPD

Two flavonoids DIO and HES are present in citrus fruits:

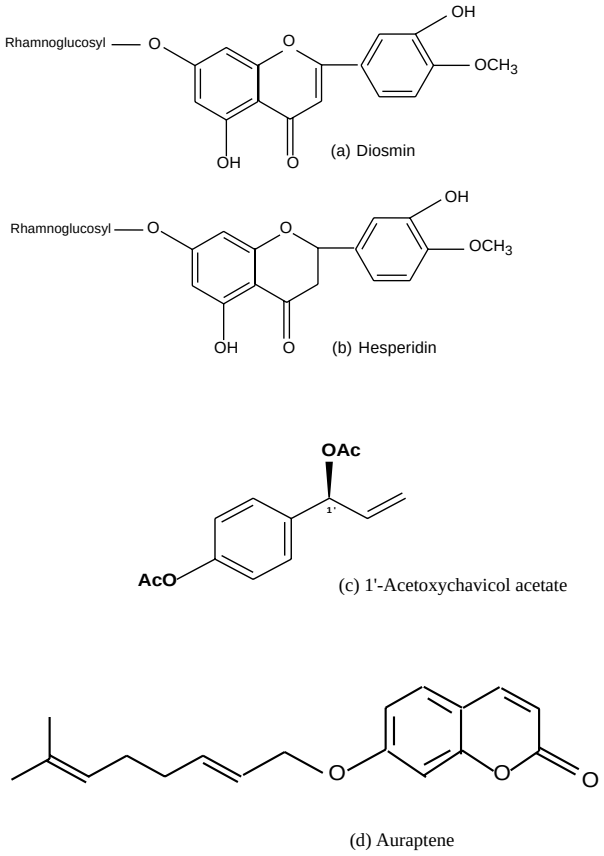


Figure 4. Chemical Stuctures of (a) Diosmin, (b) Hesperidin, (c) L'-acetoxychavicol Acetate, and (d) Auraptene

0.036 mg DIO and 34.707 mg HPD/g fresh mass of Citrus unshu Marc. (Nogata et al., 1994). These have several biological activities including antioxidant property, anti-inflammatory effect, and inhibition of prostaglandin (PG) synthesis (Tanaka et al., 1997). Since alteration of PGs biosynthesis could modulate colon carcinogenesis (Reddy, 1992), these compounds were suspected to affect colon tumorigenesis.

In the pilot study using ACF enumeration, male F344 rats received subcutaneous injections of AOM (15 mg/kg body weight) once a week for 3 weeks. They were also fed the diets contained 0.1% DIO, 0.1% HPD, and 0.09% DIO plus 0.01% HPD, respectively, for 5 weeks, starting 1 week before the first injection of AOM. Two compounds, both alone (0.1% DIO or 0.09% HPD in diet) or in combination

Table 5. Biomarkers Used for Detecting Chemopreventive Compounds Against Colon Carcinogenesis

Proliferation biomarkers	BrdU-labeling index, PCNA-labeling index, AgNORs number, etc.
Biochemical biomarkers	ODC activity, Polyamine levels, GST activity, QR activity, MDA, 4-HNE
Histological biomarkers	ACF, Adenoma, Adenocarcinoma

BrdU, 5'-bromodeoxyuridine; PCNA, proliferative nucleaqr antigen; AgNORs, silver-stained nucleolar regions; ODC, ornithine decarboxylase; GST, glutathione S-transferase; QR, quinone reductase; MDA, malondialdehyde; 4-HNE, 4-hydroxy-2(E)-nonenal; ACF, aberrant crypt foci.

Table 6. Incidence of ACF and Colonic Mucosal ODC Activity in Rats Treated with AOM plus DIO (0.1%), HPD (0.1%), or DIO (0.09%) + HPD (0.01%): a Pilot Study

Treatment	No. of ACF/rat (% inhibition)	ODC activity (pmol $^{14}CO_2$/h/mg protein)
AOM	193 ± 27	23.4 ± 11.1
AOM / DIO (0.1% in diet)	108 ± 22* (44%)	5.1 ± 1.1***
AOM / HPD (0.1% in diet)	96 ± 13* (50%)	4.9 ± 0.8***
AOM / DIO (0.09% in diet) + HPD (0.01% in diet)	52 ± 10** (73%)	6.1 ± 2.0***

*P<0.01, **P<0.001, and ***P<0.05

(0.1% DIO plus 0.09% HPD in diet), significantly inhibited the occurrence of ACF induced by AOM (44%-73% reduction) as shown in Table 6. The combination regimen inhibited all sizes of ACF. Dietary administration of these compounds also suppressed colonic mucosal ODC activity (Table 6). In the subsequent long-term bioassay, "initiation" (4 weeks) or "post-initiation" feeding (28 weeks) of two test compounds (both alone and in combination) effectively reduced the incidence and multiplicity of colonic adenocarcinoma induced by AOM (Table 7). Expression of biomarkers for cell proliferation, such as BrdU-labeling index and ODC activity in the colonic mucosa was also suppressed by these treatment (Table 7). These data indicate that dietary administration of two flavonoids DIO and HPD, both alone and in combination, during either the initiation or post-initiation phase, significantly inhibited AOM-induced colon carcinogenesis. Such effects may be partly due to suppression of cell proliferation in the colonic crypts. Other mechanisms of action, such as inhibition of PGs biosynthesis, induction of Phase II enzymes (Boutin et al., 1993), and antioxidant property, are also considered. Recently, Ciolino et al. (Ciolino et al., 1998) suggested that chemopreventive effect of diosmin may be due to the potent inhibitory activity of diosmetin on metabolic activation. The combined regimen (0.09% DIO plus 0.01% HPD) used in the study is based on the constituents of the drug "Daflon", which is used for the treatment of venous insufficiency in Europe (Labrid, 1994). Given the considerable interest in this drug as a possible chemopreventive agent, it would be intriguing to know whether chronic use of "Daflon" could reduce the risk for colon cancer in patients with venous insufficiency. The tumor data in the study, however, did not reflect any beneficial effect from DIO and HPD administered together as opposed to when these compounds were given individually.

Inhibition of Colon Carcinogenesis by ACA

ACA is present in seeds or a rhizome of Languas galanga (Zingiberaceae), used as a ginger substitute and a stomachic medicine in Thailand. The compound has been reported to suppress tumor promoter-induced Epstein-Barr virus activation in vitro (Kondo et al., 1993). ACA is known to reduce superoxide anion production by inhibiting the xanthine oxidase and NADPH oxidase system (Noro et al., 1988) and this activity has been suggested to be partly responsible for its cancer chemopreventive effects (Pence and Reiners, 1987).

In the pilot study, male F344 rats were given three weekly subcutaneous injections of AOM (15 mg/kg body weight) and fed the diet containing 0.01% or 0.02% ACA for 5 weeks, starting 1 week before the first dosing of AOM. Dietary feeding of ACA at the both dose levels caused significant

Table 7. Incidence and Multiplicity of Colonic Adenocarcinoma, and Cell Proliferation Biomarkers' Expression in Rats given AOM plus DIO (0.1%), HPD (0.1%), or DIO (0.09%) + HPD (0.01%): a Long-Term Study

Treatment	Incidence (% inhibition)	Multiplicity (% inhibition)	BrdU-labeling index (%)	ODC activity (pmol $^{14}CO_2$/h/mg protein)
AOM	71%	0.71 ± 0.46	11.5 ± 2.4*	59.7 ± 12.2*
AOM / DIO (0.1% in diet)	21%* (70)	0.28 ± 0.56* (61)	6.9 ± 1.6*	39.5 ± 2.7*
AOM / HPD (0.1% in diet)	5%* (93)	0.05 ± 0.22* (93)	6.4 ± 1.5*	25.1 ± 5.1*
AOM / DIO (0.09% in diet) + HPD (0.01% in diet)	19%* (73)	0.19 ± 0.39* (73)	5.5 ± 2.9*	23.1 ± 3.0*
AOM → DIO (0.1% in diet)	5%* (93)	0.05 ± 0.22* (93)	5.6 ± 2.0*	15.5 ± 3.1*
AOM → HPD (0.1% in diet)	15%* (79)	0.15 ± 0.36* (79)	3.9 ± 0.8*	13.1 ± 1.6*
AOM → DIO (0.09% in diet) + HPD (0.01% in diet)	5%* (93)	0.05 ± 0.32* (93)	4.3 ± 1.1*	18.7 ± 2.9*
DIO (0.1% in diet)	0%	0	2.5 ± 1.1	7.6 ± 5.8
HPD (0.1% in diet)	0%	0	2.6 ± 0.1	8.4 ± 5.3
DIO (0.09% in diet) + HPD (0.01% in diet)	0%	0	2.7 ± 0.3	6.5 ± 4.7
Nonein diet)	0%	0	2.9 ± 0.1	6.9 ± 5.6

*P<0.05

Table 8. Incidence of ACF and Colonic ODC Activity in Rats Treated with AOM and/or ACA (in diet): a Pilot Study

Treatment	No. of ACF/rat (% inhibition)	ODC activity (pmol $^{14}CO_2$/ h/mg protein)
AOM	118 ± 28	65.4 ± 48.6
AOM / ACA (0.01% in diet)	70 ± 10* (41%)	29.9 ± 12.4
AOM / ACA (0.02% in diet)	74 ± 11* (37%)	4.0 ± 1.6*
ACA (0.02% in diet)	0	11.9 ± 12.4
None	0	4.9 ± 3.5

*P<0.05

reduction in the frequency of ACF (41% inhibition by 0.1% ACA feeding and 37% inhibition by 0.02% ACA feeding) (Table 8). Feeding of ACA also suppressed expression of cell proliferation biomarkers, such as colonic mucosal ODC activity (Table 8), AgNORs number, and polyamine level (blood). Subsequent long-term study for evaluating the chemopreventive ability of ACA when fed at dose levels of 0.01% and 0.05% during the initiation (4 weeks) or post-initiation phase (34 weeks) demonstrated dose-dependent inhibition in the incidence and multiplicity of colonic adenocarcinoma induced by AOM (Table 9) . ACA feeding resulted in low activity of ODC (Table 9) and polyamine content in the colonic mucosa. In addition, GST and QR activities in the liver and colon were significantly elevated in rats gavaged with ACA (Table 10). These finding suggest possible chemopreventive ability of ACA against colon tumorigenesis and the effect may be due to its suppression of cell proliferation in the colonic mucosa and its induction of detoxifying enzymes GST and QR. More recently, ACA has been reported to inhibit nitric oxide (NO) production, apparently mediated by modulation of several transcription factors (Ohta et al., 1998). Since excessive production of NO at inflammatory sites is causatively involved in the process of multistage carcinogenesis (Ohshima and Bartsch, 1994), such activity also contributes to the anticarcinogenic properties of ACA.

Table 10. GST and QR Activities of Liver and Colon in Rats Gavaged with ACA

Dose of ACA (mg/kg bw)	GST-CDNB (mU/mg protein)		QR (mU/mg protein)	
	Liver	Colon	Liver	Colon
0	808 ± 134	145 ± 10	142 ± 23	506 ± 36
50	931 ± 144	156 ± 8*	187 ± 144*	538 ± 32
100	1174 ± 164*	162 ± 6*	227 ± 50*	583 ± 43*
200	1190 ± 146*	166 ± 14*	296 ± 68*	633 ± 95*

*P<0.05

Table 9. Incidence and Multiplicity of Colonic Adenocarcinoma and Colonic mucosal ODC Activity in Rats Given AOM and/or Dietary ACA: a Long-Term Study

Treatment	Incidence (% inhibition)	Multiplicity	ODC activity (pmol $^{14}CO_2$/h/mg protein)
AOM	71%	0.71 ± 0.46	60.3 ± 10.0*
AOM / ACA (0.01% in diet)	33%*(54)	0.39 ± 0.59 (45)	41.1 ± 8.9
AOM / ACA (0.02% in diet)	16%*(77)	0.21 ± 0.41* (70)	24.5 ± 5.8*
AOM → ACA (0.01% in diet)	39%(45)	0.50 ± 0.69 (30)	40.2 ± 8.1
AOM → ACA (0.02% in diet)	5%*(93)	0.05 ± 0.22* (93)	36.4 ± 5.5*
ACA (0.02% in diet)	0	0	7.1 ± 15.8
None	0	0	6.7 ± 4.6

*P<0.05

Inhibition of Colon Carcinogenesis by AUR

A known coumarin, AUR, is present in certain orange peels: 0.04% in Citrus natsudaidai Hayata, 0.01-0.02% in grapefruit and 100 mg/100 ml in grapefruit juice. Antiplatelet action of this compound has been reported (Teng et al., 1992), but the other biological properties are not known. Recently, antitumor promoting effect of AUR on mouse skin carcinogenesis has been found and AUR could suppress superoxide generation induced by 12-*O*-tetradecanoylphorbol-13-acetate (Murakami et al., 1997). Therefore, possible chemopreventive effect of AUR on colon carcinogenesis was examined in rats.

In the pilot study, male F344 rats were given three weekly subcutaneous injections of AOM (15 mg/kg body weight) and fed the diet containing 0.01% or 0.05% AUR for 5 weeks, starting 1 week before the first exposure of AOM. Dietary feeding of AUR caused a significant reduction in

Table 11. Incidence of ACF and Expression of Cell Proliferation Biomarkers in Rats Treated with AOM and/or AUR (in diet): a Pilot Study

Treatment	No. of ACF/rat (% inhibition)	Colonic mucosal ODC activity (pmol $^{14}CO_2$/h/mg protein)	No. of AgNORs/ crypt cell nucleus
AOM	157 ± 21	59.6 ± 14.2*	2.09 ± 0.28*
AOM / AUR (0.01% in diet)	93 ± 4* (41%)	28.7 ± 8.7*	1.43 ± 0.38*
AOM / AUR (0.05% in diet)	69 ± 6* (56%)	21.5 ± 8.4*	1.17 ± 0.27*
AUR (0.05% in diet)	0	19.5 ± 9.3	1.16 ± 0.29
None	0	12.4 ± 5.8	1.12 ± 0.23

*P<0.05

Table 12. Incidence and Multiplicity of Colonic Adenocarcinoma in Rats Given AOM and/or Dietary AUR: a Long-Term Study

Treatment	Incidence (% inhibition)	Multiplicity (% inhibition)	ODC activity (pmol $^{14}CO_2$/h/ mg protein)
AOM	59%	0.56 ± 0.48	62.2 ± 10.0*
AOM / AUR (0.01% in diet)	35% (41)	0.40 ± 0.58 (39)	32.3 ± 3.9*
AOM / AUR (0.05% in diet)	20%* (66)	0.20 ± 0.42* (64)	24.1 ± 4.1*
AOM → AUR (0.01% in diet)	24%* (59)	0.24 ± 0.42* (57)	21.2 ± 4.1*
AOM → AUR (0.05% in diet)	20%* (66)	0.20 ± 0.40* (64)	19.2 ± 5.0*
AUR (0.05% in diet)	0	0	7.5 ± 3.4
None	0	0	5.2 ± 1.8

*P<0.05

the frequency of ACF in a dose-dependent manner (Table 11). AUR at the both dose levels also suppressed expression of cell proliferation biomarkers, such as colonic mucosal ODC activity (Table 11), AgNORs number (Table 11), BrdU-labeling index, and polyamine content. Subsequent long-term experiment for evaluating the chemopreventive efficacy of AUR was conducted using male F344 rats. They received AOM and were fed at dose levels of 0.01% and 0.05% during the initiation (4 weeks) or post-initiation phase (34 weeks). At the termination of the study, dietary AUR caused dose-dependent inhibition in the incidence and multiplicity of AOM-induced colonic adenocarcinoma (Table 12). Dietary administration of AUR also suppressed the expression of cell proliferation biomarkers, such as ODC (Table 12) and polyamine content in the colonic mucosa. AUR feeding could reduce the amounts of MDA and 4-HNE (Table 13). Increased levels of the products of lipid peroxidation, including MDA and 4-HNE, were found in colon carcinogenesis (Deschner and Zedeck, 1986; Kang et al., 1988). In a different experiment, gavage of AUR increased GST and QR activities in the liver and colon (Table 14). These findings suggested that the chemopreventive effects of AUR on AOM-induced colon tumorigenesis at the initiation level might be associated, in part, with increased activity of Phase II enzymes, and those at the post-initiation stage might be related to suppression of cell proliferation and lipid peroxidation in the colonic mucosa.

Table 13. Amounts of MDA plus 4-HNE in the Colonic Mucosa of Rats Ttreated with AOM and/or Dietary AUR: a Long-Term Study

Treatment	MDA + 4-HNE (nmol/mg protein) / Relative value
AOM	21.3 ± 8.1* / 760
AOM / AUR (0.01% in diet)	3.1 ± 2.2* / 111
AOM / AUR (0.05% in diet)	2.1 ± 0.8* / 75
AOM → AUR (0.01% in diet)	2.5 ± 1.4* / 89
AOM → AUR (0.05% in diet)	5.1 ± 0.9* / 179
AUR (0.05% in diet)	4.6 ± 2.3 / 164
None	2.8 ± 1.1 / 100

*P<0.05

Table 14. GST and QR Activities of Liver and Colon in Rats Gavaged with AUR

Dose of AUR (mg/kg bw)	GST-CDNB (mU/mg protein)		QR (mU/mg protein)	
	Liver	Colon	Liver	Colon
0	517 ± 125	113 ± 16	127 ± 12	548 ± 69
200	922 ± 146	126 ± 8	214 ± 47*	570 ± 53
400	1266 ± 208*	147 ± 13*	239 ± 23*	680 ± 82*
800	1332 ± 235*	150 ± 20*	298 ± 40*	707 ± 144*

*P<0.05

Discussion

Our recent data on the chemopreventive effects of naturally occurring compounds, DIO, HPD, ACA, and AUR, present in certain vegetables and fruits against AOM-induced colon tumorigenesis are described. All these compounds are antioxidants. In general, plants are complicated mixtures of numerous chemicals, and interactions with their components may affect the effectiveness of the antioxidant. The effectiveness of tested compounds as in vivo antioxidants has been reported, but the metabolic pathway and action of naturally occurring antioxidative compounds is not clear. Flavonoids compounds, which are widely distributed in the plant kingdom and occur in considerable quantities, show a wide range of pharmacological activities other than their antioxidative properties. These compounds have been used to treat various pathological conditions including allergies, inflammation, and diabetes. Experimental data including this report showing their antitumor activities is accumulating; their chemopreventive potential, however, has not been fully proven clinically. Their behavior and fate should be investigated in vivo.

As reported, commonly consumed foods contain non-nutritive compounds capable to inhibit colon cancer in an animal model. The diet provides a rich abundance of these compounds which have the ability to intervene in all phases of carcinogenesis. mechanisms of action include effects of Phase I and Phase II enzymes activities, interception of DNA mutating agents, and influences on cell proliferation and oncogene activation. Each of these mechanisms have been studies in isolation. For explanation of reduced risk for cancer in populations with a greater reliance on fruits and vegetables in the daily diet, future research should focus on potential combinations of foods and the protective components within them.

The association of certain malignancies with chronic inflammation has been recognized for many years (Gordon and Weitzman, 1993). The link between inflammation and subsequent malignancy in visceral sites is known. Examples include large bowel cancer after ulcerative colitis or Crohn's disease (Collins et al., 1987; Gordon and Weitzman, 1993). Central to the concept of inflammation and cancer is the finding that chronic irritation of squamous or glandular epithelium will result in migration of inflammatory cells to the injured site by a mechanism dependent on neutrophil adhesion molecules. These cells, stimulated to produce reactive oxygen species (including superoxide radicals, NO and/or hydroxy radicals) via the respiratory burst and NADPH activation, can function as facilitators in the process of carcinogenesis. There is convincing evidence from animal model systems that prolonged exposure of cells to these products of activated oxygen can result in cell injury and play a role in several stages of carcinogenesis. Recently, up-regulation of COX-2, but not COX-1, gene expression was reported in human colorectal neoplasms (Eberhart et al., 1994). New drugs, specific for inhibition of COX-2, may provide effective tumor prevention with reduced side effects (Oshima et al., 1996; Reddy et al., 1996; Sheng et al., 1997). The elevation of COX-2 expression can protect intestinal epithelial cells from apoptosis (Tsuji and DuBois, 1995). Certain COX-2 inhibitors can induce apoptosis (Hara et al., 1997) and inhibit tumor angiogenesis (Tsuji et al., 1998). Elegant review on chemopreventive ability of NSAIDs including COX-2 inhibitors against colon tumorigenesis has been published in this journal (Wakabayashi, 2000). More recently, synthetic antioxidants have reported to reduce COX-2 expression PG production, and cell proliferation of colorectal cancer cells (Chinery et al., 1998). This may suggest that COX-2 may provide a new chemopreventive target in colorectal malignancies (Rustgi, 1998), if there are the natural products being a specific inhibitor of COX-2 expression in edible plants.

From the evidence mentioned above, our search for chemopreventives against colon cancer focuses on several flavonoids and some other compounds possessing certain biological activities including anti-inflammatory and/or antioxidative properties present in foods. Approximately 2,000 individual members of the flavonoid class have been described and the flavonoids are consumed in rather large amounts through dietary vegetables and fruits.

Other Non-nutritives, Which May Exert Suppressive Effects on Colon Tumorigenesis, in Edible Plants

Our recent studies demonstrated that juices rich in HPD and β-cryptoxanthin could inhibit AOM-induced rat colon tumorigenesis (Kohno et al., 1999; Tanaka et al., 2000). Juices rich in HPD and β-cryptoxanthin also inhibit lung tumorigenesis in mice (unpublished data). Citrus bitter limonoids obacunone and limonin can suppress AOM-induced colon carcinogenesis (Tanaka et al., 2001). In another experiment, a polymethoxyflavonoid nobiletin isolated from Citrus unshiu was able to suppress AOM-induced ACF formation in rats in conjunction with reduction in cell proliferation activity and PGE_2 content of colonic mucosa (Kohno et al., 2001). Thus, citrus fruit is a rich source of cancer inhibiting agents. The rhizomes of Zingiber zerumbet Smith are used for anti-inflammatory folk medicine in Indonesia (Elliott and Brimacombe, 1987). A sesquiterpene zerumbone isolated from the rhizome is potent inhibitor of 12-*O*-tetradecanoyl-13-acetate-induced Epstein-Barr virus activation (Murakami et al., 1999), expression of inducible nitric oxide synthase (iNOS) and COX-2 expression in RAW264.7 macrophages treated with lipopolysaccharide and interferon-γ, and NO/O_2^- generation in leukocytes (unpublished data). We demonstrated that dietary feeding of zerumbone is able to suppress AOM-induced ACF formation in rats (Tanaka et al., 2001). A polyisoprenylated benzophenone, garcinol (also named camboginol) is present in Guttiferae. Dried rind of G. indica ('Kokum') containing garcinol (2-3%, w/w) is used as a garnish for curry and in traditional medicine in India. We have recently found the inhibitory effects of garcinol on AOM-induced ACF (Tanaka et al., 2000). On-going long-term experiments will provide the data showing that these compounds could modify (possibly inhibit) colonic carcinoma development. Ferulic acid (FA), widely found in bran from rice, wheat and barley, vegetables, and other edible plants, is able to inhibit chemically-induced colon carcinogenesis (Kawabata et al., 2000). Recently, Tsuda's group newly synthesized from the parent compound FA by adding a geranyl chain. They tested the chemopreventive efficacy of EGMP and FA in AOM-induced ACF, since the compound is more potent antioxidant than FA. They concluded that both compounds are effective in reducing ACF formation and the effect of EGMP is more potent than FA (Han et al., 2001).

Future Prospectives

Yamada et al., recently, found new possible precursor lesions for colon carcinoma in the whole-mount preparations of the colon in rats exposed to AOM (Yamada et al., 2000; Yamada et al., 2001). The lesions are different in their morphology and location from ACF. In the lesions, accumulation are different is more prominent than did not present a ACF-like appearance (Yamada et al., 2000). Cell proliferation activity estimated by counting the number of AgNORs/nucleus in the lesions is also greater than in ACF (Yamada et al., 2001). Thus, we should estimate chemopreventive efficacy of non-nutritives in edible plants reported using both ACF and these new lesions as biological markers for colon carcinogenesis in future studies. Since ligands for peroxisome proliferator-activated receptors (PPARs) can inhibit AOM-induced ACF, which weakly express PPARγ (Fig. 1c) (Kohno et al., 2001; Tanaka et al., 2001), we are now searching natural compounds acting as a ligand for PPARs. In the near future, we would like to provide promising non-nutritive compounds with less toxicity in Asian edible plants (Tanaka, 1976; Yun, 1999) for use in clinical colon cancer chemoprevention trials. Also, a new

compound with more effective chemopreventive effects can be synthesized from the non-nutritive compound in edible plants, when a small amount of the parent compound can be isolated. Known non-nutritive chemopreventive agents with low dose in combination can be considered to obtain a pronounced chemopreventive effect against colon cancer development in future.

Conclusion

In conclusion, certain flavonoids and other substances with biological activity including antioxidative and/or anti-inflammatory properties, which are present in vegetables and fruits, could exert chemopreventive action in rat colon carcinogenesis as shown here. However, more work needs to be done to better understand the underlying mechanism(s) of action and to confirm their safety for use in humans. Plants are complex mixtures of chemicals. The potential for finding new chemopreventive agents in plants is high. Studies are underway to identify new compounds in edible plants with chemopreventive potential. For screening chemopreventive agents based on different mechanisms, new in vitro culture model might be useful (Mace et al., 1998). The effects of these agents on colon carcinogenesis should be carefully studies to assist the discovery and development of new chemopreventive agents, and to understand carcinogenesis mechanisms. Our goal is to develop chemopreventive agents that could be effective in decreasing the risk of colon cancers in general and/or high-risk populations. The strategy was only partially successful, it could give a significant impact on reduction of colon cancer mortality.

Acknowledgements

Research in our laboratory is supported by a Grant-in-Aid for the Second Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare in Japan; by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare in Japan; by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and in part by a Grant (H2000-4) for Project Research from High-Technology Center of Kanazawa Medical University.

References

Alberts DS, Martinez ME, Roe DJ, et al (2000). Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. *N Engl J Med*, **342,** 1156-62.

Angres G, Beth M (1991). Effects of dietary constituents on carcinogenesis in different tumor models. An overview from 1975 to 1988. In 'Cancer and Nutrition' Eds Alfin-Slater RB and Kritchesvsky D. Plenum Press, New York, pp 337-485.

Bird RP (1995). Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Cancer Lett,* **93,** 55-71.

Block G, Patterson B, Subar A (1992). Fruit, vegetables, and cancer prevention: a review of epidemiological evidence. *Nutr Cancer,* **18,** 1-29.

Boutin JA, Meunier F, Lambert P-H, et al (1993). *In vivo* and *in vitro* glucuronidation of the flavonoid diosmetin in rats. *Drug Metab Dispos,* **21,** 1157-66.

Chinery R, Beauchamp D, Shyr Y, et al (1998). Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production, and proliferation in colorectal cancer cells. *Cancer Res,* **58,** 2323-7.

Ciolino HP, Wang TTY, Yeh GC (1998). Diosmin and diosmetin are agonists of the aryl hydrocarbon receptor that differentially affect cytochrome P450 1A1 activity. *Cancer Res,* **58,** 2754-60.

Cohen SM, Ellwein LB (1990). Cell proliferation and carcinogenesis. *Science*, **249,** 1007-11.

Collins RHJ, Feldman M, Fordtran JS (1987). Colon cancer, dysplasia, and surveillance in patients with ulcerative colitis. *N Engl J Med*, **316,** 1654-8.

Dashwood RH, Xu M, Hernaez JF, et al (1999). Cancer chemopreventive mechanisms of tea against heterocyclic amine mutagens from cooked meat. *Proc Soc Exp Biol Med*, **220,** 239-43.

Deschner EE, Zedeck MS (1986). Lipid peroxidation in liver and colon of methylazoxymethanol treated rats. *Cancer Biochem Biophys*, **9,** 25-9.

Eberhart CE, Coffey RJ, Radhika A, et al (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, **107,** 1183-8.

Elliott S, Brimacombe J (1987). The medicinal plants of Gunung Leuser National Park, Indonesia. *J Ethnopharmacol*, **19,** 285-317.

Formica JV, Regelson W (1995). Review of the biology of quercetin and related bioflavonoids. *Food Chem Toxicol,* **33,** 1061-80.

Fuchs CS, Giovannucci EL, Colditz GA, et al (1999). Dietary fiber and the risk of colorectal cancer and adenoma in women. *N Engl J Med*, **340,** 169-76.

Giovannucci E, Rimm EB, Stampfer MJ, et al (1994). Intake of fat, meat, and fiber in relation to risk of colon cancer in men. *Cancer Res*, **54,** 2390-7.

Gordon LI, Weitzman SA (1993). Inflammation and cancer. *Cancer J*, **6,** 2257-261.

Han BS, Park CB, Takasuka N, et al (2001). A ferulic acid derivative, 3-(4'-geranyloxy-3-methoxyphenyl)-2-propenoate, as a new candidate chemopreventive agent for colon carcinogenesis in the rat. *Jpn J Cancer Res*, **92,** 404-9.

Hara A, Yoshimi N, Niwa M, et al (1997). Apoptosis induced by NS-398, a selective cyclooxygenase-2 inhibitor, in human colorectal cancer cell lines. *Jpn J Cancer Res*, **88,** 600-4.

Hebert JR, Landon J, Miller DR (1993). Consumption of meat and fruit in relation to oral and esophageal cancer: a cross-national study. *Nutr Cancer*, **19,** 169-79.

Hertog MG, Kromhout D, Aravanis C, et al (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med*, **155,** 381-6.

Hirayama T (1979). Diet and cancer. *Nutr Cancer*, **1,** 67-81.

Huang M-T, Osawa T, Ho C-T, Rosen RT, Eds. (1994) 'Food Phytochemicals for Cancer Prevention I and II. Fruits and Vegetables', Am Chem Soc, Washington, DC.

Kang JO, Slater G, Aufses AHJ, et al(1988). Production of ethane

by rats treated with the colon carcinogen, 1,2-dimethylhydrazine. *Biochem Pharmacol*, **37,** 2967-71.

Kawabata K, Yamamoto T, Hara A, et al (2000). Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. *Cancer Lett*, **157,** 15-21.

Kawamori T, Tanaka T, Hara A, et al (1995). Modifying effects of naturally occurring products on the development of colonic aberrant crypt foci induced by azoxymethane in F344 rats. *Cancer Res*, **55,** 1277-82.

Kohno H, Maeda M, Honjo S, et al (1999). Prevention of colonic preneoplastic lesions by the β-cryptoxanthin and hesperidin rich powder prepared from *Citrus Unshiu* Marc. juice in male F344 rats. *J Toxicol. Pathol*, **12,** 209-15.

Kohno H, Yoshitani S, Takashima S, et al (2001). Troglitazone, a ligand for peroxisome proliferator-activated receptor-γ, inhibits chemically-induced aberrant crypt foci in rats. *Jpn J Cancer Res*, **92,** 396-403.

Kohno H, Yoshitani S, Tsukio Y, et al (2001). Dietary administration of citrus nobiletin inhibits azoxymethane-induced colonic aberrant crypt foci in rats. *Life Sci*, in press.

Kondo A, Ohigashi H, Murakami A, et al (1993). 1'-Acetoxychavicol acetate as a potent inhibitor of tumor promoter-induced Epstein-Barr virus activation from *Languas galanga*, a traditional Thai condiment. *Biosci Biotechnol Biochem*, **57,** 1344-5.

Labrid C (1994). Pharmacologic properties of Daflon 500 mg. *Angiology*, **45,** 524-30.

LaMuraglia GM, Lacaine F, Malt RA (1986). High ornithine decarboxylase activity and polyamine levels in human colorectal neoplasia. *Ann Surg*, **204,** 89-93.

Li H, Kramer PM, Lubet RA, et al (1998). Effect of calcium on azoxymethane-induced aberrant crypt foci and cell proliferation in the colon of rats. *Cancer Lett*, **124,** 39-46.

Lipkin M (1991). Intermediate biomarkers and studies of cancer prevention in the gastrointestinal tract. *Prog Clin Biol Res*, **369,** 397-406.

Luk GD, Hamilton SR, Yang P, et al (1986). Kinetic changes in mucosal ornithine decarboxylase activity during azoxymethane-induced colonic carcinogenesis in the rat. *Cancer Res*, **46,** 4449-52.

Mace K, Offord EA, Harris CC, et al (1998). Development of *in vitro* models for cellular and molecular studies in toxicology and chemoprevention. *Arch Toxicol*, **20 (Suppl),** 227-36.

Micozzi MS (1989). Foods, micronutrients, and reduction of human cancer. In 'Nutrition and Cancer Prevention: Investigating the Role of Micronutrients' Eds Moon TE and Micozzi MS. Marcel Dekker, Inc., New York, pp 213-42.

Murakami A, Kuki W, Takahashi Y, et al (1997). Auraptene, a Citrus coumarin, inhibits 12-*O*-tetradecanoylphorbol-13-acettate-induced tumor promotion in ICR mouse skin possibly through suppression of superoxide generation in leukocytes. *Jpn J Cancer Res*, **88,** 443-52.

Murakami A, Takahashi M, Jiwajinda S, et al (1999). Identification of zerumbone in *Zingiber zerumbet* Smith as a potent inhibitor of 12-*O*-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation. *Biosci Biotechnol Biochem*, **63,** 1811-2.

Nogata Y, Ohta H, Yoza K, et al (1994). High-performance liquid chromatographic determination of naturally occurring flavonoids in *Citrus* with a photodiode-ray detector. *J Chromatogr*, **667,** 59-66.

Noro T, Sekiya T, Katoh (nee Abe) M, et al (1988). Inhibitors of xanthine oxidase from Alpinia galanga. *Chem Pharm Bull.*, **36,** 244-8.

Ohshima H, Bartsch H (1994). Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat Res*, **305,** 253-64.

Ohta T, Fukuda K, Murakami A, et al (1998). Inhibition by 1'-acetoxychavicol acetate of lipopolysaccharide- and interferon-γ-induced nitric oxide production through suppression of inducible nitric oxide synthase gene expression in RAW264 cells. *Carcinogenesis*, **19,** 1007-12.

Oshima M, Dinchuk JE, Kargman SL, et al (1996). Suppression of intestinal polyposis in APC^{*716} knockout mice by inhibition of prostaglandin endoperoxide synthase-2 (COX-2). *Cell*, **87,** 803-9.

Pegg AE (1988). Polyamine metabolism and its importance in neoplastic growth as a target for chemotherapy. *Cancer Res*, **48,** 759-74.

Pence BC, Reiners JJ Jr (1987). Murine epidermal xanthine oxidase activity: correlation with degree of hyperplasia induced by tumor promoters. *Cancer Res*, **47,** 6388-92.

Pereira MA, Barnes LH, Rassman VL, et al (1994). Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. *Carcinogenesis*, **15,** 1049-54.

Pierpoint WS (1986). Flavonoids in the human diet. *Prog Clin Biol Res*, **213,** 125-40.

Pretlow TP, Barrow BJ, Ashton WS, et al (1991). Aberrant crypts: putative preneoplastic foci in human colonic mucosa. *Cancer Res*, **51,** 1564-7.

Pretlow TP, Cheter C, O'Riordan MA (1994). Aberrant crypt foci and colon tumors in F344 rats have similar increases in proliferative activity. *Int J Cancer*, **56,** 599-602.

Reddy BS (1986). Diet and colon cancer: evidence from human and animal model studies. In 'Diet, Nutrition and Cancer: A Critical Evaluation' Eds Reddy BS and Cohen LA. CRC Press, Boca Raton, FL, pp. 47-65, .

Reddy BS (1992). Inhibitors of the arachidonic acid cascade and their chemoprevention of colon carcinogenesis. In 'Cancer Chemoprevention' Eds Wattenberg L, Lipkin M, Boone CW and Kelloff GJ. CRC Press, Boca Raton, FL, pp 153-64.

Reddy BS (1993). Nutritional factors as modulators of colon carcinogenesis. In 'Food, Nutrition and Chemical Toxicity' Eds Parke DV, Ioannides C and Walker R. Smith-Gordon/ Nishimura, London/Niigata, pp 325-36.

Reddy BS, Rao CV, Seibert K (1996). Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. *Cancer Res*, **56,** 4566-9.

Am Inst Cancer Res (1996) 'Dietary Phytochemicals in Cancer Prevention and Treatment' Plenum Press, New York, pp 1-340.

Rustgi AK (1998). Cyclooxygenase-2: The future is now. *Nature Med*, **4,** 773-4.

Schatzkin A, Lanza E, Corle D, et al (2000). Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. *N Engl J Med*, **342,** 1149-55.

Sheng H, Shao J, Kirkland SC, et al (1997). Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest*, **99,** 2254-9.

Slaga TJ (1980). 'Modifiers of Chemical Carcinogenesis: An Approach to the Biochemical Mechanism and Cancer Prevention - A Comprehensive Survey' Raven Press, New York, pp 1-275.

Steinmetz KA, Potter JD (1991). Vegetable, fruits, and cancer. I. Epidemiology. *Cancer Causes Control*, **2,** 325-57.

Steinmetz KA, Potter JD (1991). Vegetable, fruits, and cancer. II. Mechanisms. *Cancer Causes Control*, **2,** 427-42.

Steinmetz kA, Potter JD (1996). Vegetables, fruit, and cancer prevention: a review. *J Am Diet Assoc*, **96,** 1027-39.

Szarka CE, Pfeiffer GR, Hum ST, et al (1995). Glutathine *S*-transferase activity and glutathione *S*-transferase μ expression in subjects with risk for colorectal cancer. *Cancer Res*, **55,** 2789-93.

Tanaka T (1976). 'Tanaka's Cyclopedia of Edible Plants of the World' Keigaku Pub. Co., Ltd., Tokyo, pp 1-924.

Tanaka T (1992). Cancer chemoprevention. *Cancer J*, **5,** 11-6.

Tanaka T (1997). Chemoprevention of human cancer: biology and therapy. *Crit Rev Oncol/Hematol*, **25,** 139-74.

Tanaka T (1997). Effect of diet on human carcinogenesis. *Crit Rev Oncol/Hematol*, **25,** 73-95.

Tanaka T, Kawabata K, Kakumoto M, et al (1998). *Citrus* auraptene exerts dose-dependent chemopreventive activity in rat large bowel tumorigenesis: the inhibition correlates with suppression of cell proliferation and lipid peroxidation and with induction of phase II drug-metabolizing enzymes. *Cancer Res*, **58,** 2550-6.

Tanaka T, Kawabata K, Kakumoto M, et al (1997). *Citrus* auraptene inhibits chemically induced colonic aberrant crypt foci in male F344 rats. *Carcinogenesis*, **18,** 2155-61.

Tanaka T, Kawabata K, Kakumoto M, et al (1997). Chemoprevention of azoxymethane-induced rat colon carcinogenesis by a xanthine oxidase inhibitor, 1'-acetoxychavicol acetate. *Jpn J Cancer Res*, **88,** 821-30.

Tanaka T, Kawamori T, Ohnishi M, et al (1995). Suppression of azoxymethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during the postinitiation phase. *Carcinogenesis*, **16,** 2957-63.

Tanaka T, Kohno H, Murakami M, et al (2000). Suppression of azoxymethane-induced colon carcinogenesis in male F344 rats by mandarin juices in β-cryptoxanthin and hesperidin. *Int J Cancer*, **88,** 146-50.

Tanaka T, Kohno H, Shimada R, et al (2000). Prevention of colonic aberrant crypt foci by dietary feeding of garcinol in male F344 rats. *Carcinogenesis*, **21,** 1183-9.

Tanaka T, Maeda M, Kohno H, et al (2001). Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. *Carcinogenesis*, **22,** 193-8.

Tanaka T, Makita H, Kawabata K, et al (1997). Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally occurring flavonoids, diosmin and hesperidin. *Carcinogenesis*, **18,** 957-65.

Tanaka T, Makita H, Kawamori T, et al (1997). A xanthine oxidase inhibitor 1'-acetoxychavicol acetate inhibits azoxymethane-induced colonic aberrant crypt foci. *Carcinogenesis*, **18,** 1113-8.

Tanaka T, Makita H, Ohnishi M, et al (1995). Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin. *Cancer Res*, **55,** 4059-64.

Tanaka T, Shimizu M, Kohno H, et al (2001). Chemoprevention of azoxymethane-induced rat aberrant crypt foci by dietary zerumbone isolated from *Zingiber zerumbet*. *Life Sci***,** in press.

Teng CM, Li HL, Wu TS, et al (1992). Antiplatelet actions of some coumarin compounds isolated from plant sources. *Thrombosis Res*, **66,** 549-57.

Tsubono Y, Nishino Y, Komatsu S, et al (2001). Green tea and the risk of gastric cancer in Japan. *N Engl J Med*, **344,** 632-6.

Tsuji M, DuBois RN (1995). Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase-2. *Cell*, **83,** 493-501.

Tsuji M, Kawano S, Tsuji S, et al (1998). Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell*, **93,** 705-16.

Tsukamoto T, Fukami H, Yamanaka S, et al (2001). Hexosaminidase-altered aberrant crypts, carrying decreased hexosaminidase α and β subunit mRNAs, in colon of 1,2-dimethylhydrazine-treated rats. *Jpn J Cancer Res*, **92,** 109-18.

Wakabayashi K (2000). NSAIDs as cancer preventive agents. *Asian Pacific J Cancer Prev*, **1,** 97-113.

Wang A, Yoshimi N, Tanaka T, et al (1993). Inhibitory effects of magnesium hydroxide on c-*myc* expression and cell proliferation induced by methylazoxymethanol acetate in rat colon. *Cancer Lett*, **75,** 73-8.

Wattenberg LW (1983). Inhibition of neoplasia by minor dietary constituents. *Cancer Res*, **43,** 2448s-53s.

Wattenberg LW (1985). Chemoprevention of cancer. *Cancer Res*, **45,** 1-8.

Weisburger JH (1991). Causes, relevant mechanisms, and prevention of large bowel cancer. *Semin Oncol*, **18,** 316-36.

Weisburger JH (1999). Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food Chem Toxicol*, **37,** 943-8.

Weisburger JH (2000). Approaches for chronic disease prevention based on current understanding of underlying mechanisms. *Am J Clin Nutr*, **71 (6 Suppl),** 1710S-9S.

Weisburger JH, Reddy BS, Rose DP, et al (1993). Protective mechanisms of dietary fibers in nutritional carcinogenesis. *Basic Life Sci*, **61,** 45-64.

Wilkinson JI, Clapper ML (1997). Detoxication enzymes and chemoprevention. *Proc Soc Exp Bio Med*, **216,** 192-200.

Williams GM, Williams CL, Weisburger JH (1999). Diet and cancer prevention: the fiber first diet. *Toxicol Sci*, **52 (2 Suppl),** 72-86.

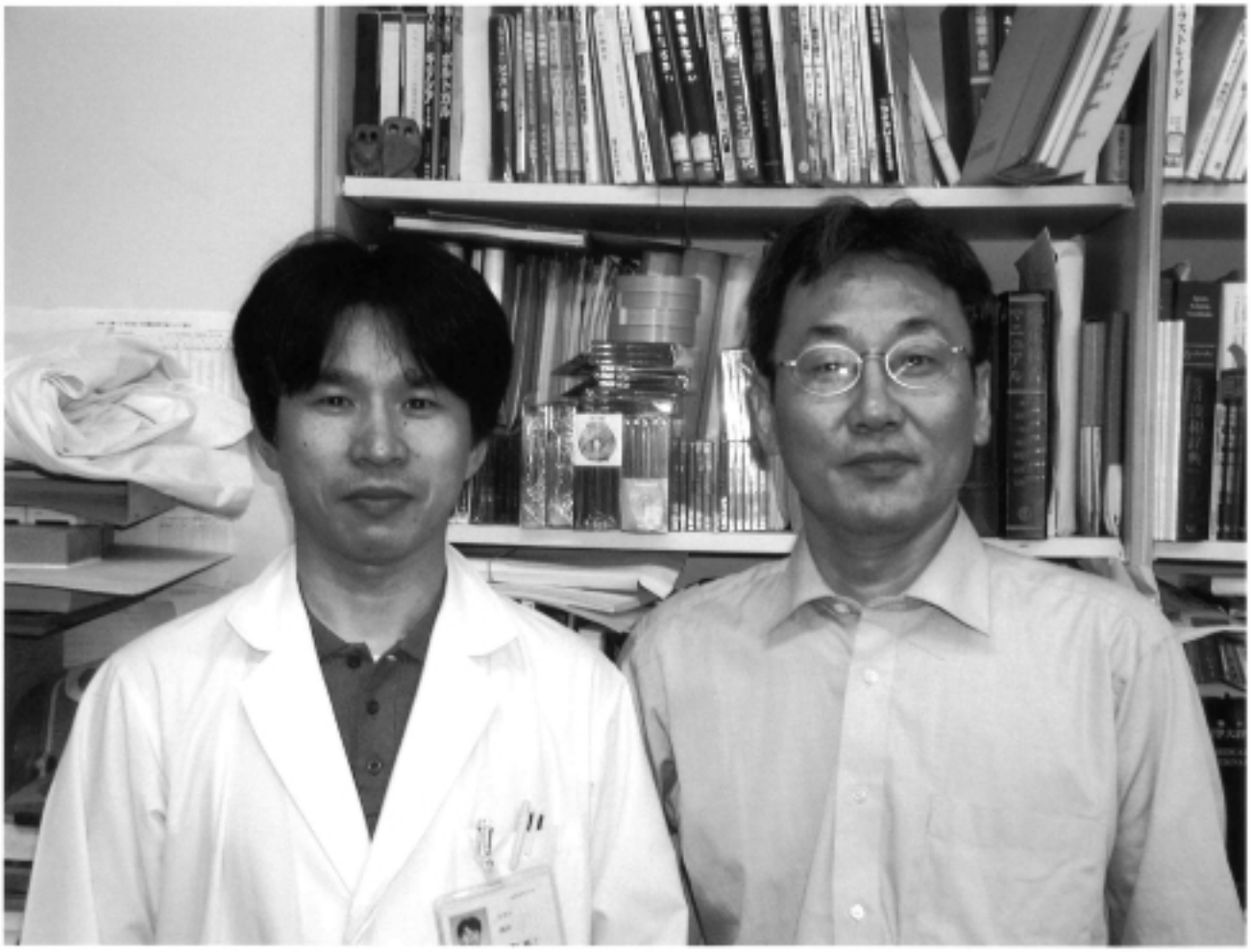
Yamada Y, Yoshimi N, Hirose Y, et al (2000). Frequent β-*catenin* gene mutations and accumulations of the protein in the putative preneoplastic lesions lacking macroscopic aberrant crypt foci appearance, in rat colon carcinogenesis. *Cancer Res*, **60,** 3323-7.

Yamada Y, Yoshimi N, Hirose Y, et al (2001). Sequential analysis of morphological and biological properties of β-catenin-accumulated crypts, probable premalignant lesions independent of aberrant crypt foci in rat colon carcinogenesis. *Cancer Res*, **61,** 1874-8.

Yamashita N, Minamoto T, Onda M, et al (1994). Increased cell proliferation of azoxymethane-induced aberrant crypt foci of rat colon. *Jpn J Cancer Res*, **85,** 692-8.

Yun T-K (1999) Update from Asia: Asian study on cancer chemoprevention. Ann NY Acad Sci, **889**, 157-92.

Zheng Y, Kramer PM, Olson G, et al (1997). Prevention by retinoids of azoxymethane-induced tumors and aberrant crypt foci and their modulation of cell proliferation in the colon of rats. *Carcinogenesis*, **18,** 2119-25.



Personal Profile : Hiroyuki Kohno (left in the photo)

Hiroyuki Kohno was born in Hokkaido, Japan in 1957. He graduated from Faculty of Pharmaceutical Sciences, Hokkaido University, in 1982.
He joined Prof. Shizuo Odashima' group and received the Ph.D. in 1995 from Kanazawa Medical University. He has been a Lecture at the Departments of Pathology and Serology of Kanazawa Medical University since 1995 and his research interests are cancer immunology and chemoprevention using compounds with immune modulation and conjugated fatty acids.

For a Personal Profile of Dr **Hideki Mori** please see APJCP Vol 1, page 216

Personal profile : Takuji Tanaka (right in the photo)

Takuji Tanaka was born in Ehime Prefecture of Japan in 1949. He graduated from Gifu University School of Medicine (M.D.) in 1976, and received the Ph.D in 1982 from Gifu University. He joined Prof. Iwao Hirono's group as a graduate student , Prof. Masayoshi Takahashi's group as an Assistant Professor, and Pro. Hideki Mori' group as a Lecture and an Associate Professor at the First Department of Pathology, Gifu University School of Medicine from 1976 to 1997. He enjoyed cancer chemoprevention stduies with Drs. Gary M. Williams, Bandaru S. Reddy, and John H. Weisburger as a Visiting Scientist at Naylor Dana Institute American Health Foundation (NY) for two years (1983-1985). In 1997, he moved to Kanazawa Medical University as a Professor of the First Department of Pathology. His research interests include cancer chemoprevention using natural compounds in digestive organs and cancer cytology.