Table 1. Selected aspirin RCT performed for CRC prevention in the past

Study name (Drug)	Length of treatment	Subject (no. of enrollment; Mean ± SD age)	Primary objects/Results	Trial site (Ref.)
APACC (Aspirin: 160 or 300 mg/day)	4 Years	Recent history of sporadic colorectal adenomas ($n = 272$; 160 mg/day aspirin group, 59 ± 9)	Reduction in adenoma recurrence/ RR = 0.95 (95% CI: 0.75–1.21)	France (9)
AFPPS (Aspirin: 81 or 325 mg/day)	3 Years	Recent history of sporadic colorectal adenomas ($n = 1121$; 81 mg/day aspirin group, 58 ± 9)	Reduction in adenoma recurrence/ RR = 0.88 (95% CI: 0.77–1.02)	USA and Canada (10)
ukCAP (Aspirin: 300 mg/ day ± folic acid: 0.5 mg/day)	3 Years	Recent history of sporadic colorectal adenomas ($n = 939$; aspirin group, 58 ± 10)	Reduction in adenoma recurrence/ RR = 0.79 (95% CI: 0.63–0.99)	UK and Denmark (12)
CAPP1 (Aspirin: 600 mg/ day ± resistant starch: 30 g/day)	1–12 Years	FAP ($n = 227$; 300 mg/day aspirin group, 18 ± 8)	Prevention of disease progression/the size of the largest polyp was reduced	UK (15)
CAPP2 (Aspirin: 600 mg/day)	1–4 Years	Lynch syndrome gene carriers ($n = 861$)	Intention-to-treat analysis of time to first CRC/HR = 0.63 (95% CI: 0.35–1.13)	UK (16)
J-FAPP2 (Aspirin: 100 mg/day)	6–10 Months	FAP ($n = 17$; aspirin group, 40 ± 13)	Number of subjects with reduced polyps/ Response ratio = 2.33 (95% CI: 0.72–7.55)	Japan (17)

Response ratio = aspirin response rate (no. of subjects with reduced polyps/total)/placebo response rate (no. of subjects with reduced polyps/total).
AFPPS, Aspirin/Folate Polyp Prevention Study; APACC, Association pour la Prevention par l'Aspirine du Cancer Colorectal; CALGB, Cancer and Leukemia Group B; ukCAP, United Kingdom Colorectal Adenoma Prevention; CRC, colorectal cancer; FAP, familial adenomatous polyposis; HR, hazard ratio; RCT, randomized control trials; Ref., reference; RR, relative risk.

placebo ($n = 31$, 10–21 years old), resistant starch (RS) for 30 g/day plus matched placebo ($n = 30$), aspirin plus RS ($n = 31$) and placebo plus placebo ($n = 41$) (15). No significant trend of a reduced number of polyps in the colorectum was observed in the aspirin group compared with the non-aspirin group at the end of intervention. However, the size of the largest polyp was reduced in the overall aspirin group compared with the non-aspirin group. Furthermore, after more than 1 year of intervention, the diameter of the largest polyp recorded in the aspirin group (3 mm) was only half of that in the placebo group (6 mm; $P = 0.02$).

CAPP2

The CAPP2 was a 2X2 factorial randomized trial in 861 Lynch syndrome gene carriers. The subjects were divided into intervention groups of an aspirin enteric-coated tablet (600 mg/day for a minimum 2 years) and a matched placebo (427 participants) for between 1 and 4 years, with a pre-planned design for a 10-year follow-up (16). This trial is the first double-blind randomized trial of aspirin chemoprevention with cancer as a primary endpoint. After a mean observation period of 29 months of intervention, there was no evidence showing that aspirin influenced development of colorectal neoplasia. After a mean of 55.7 months, the hazard ratio for new CRC development for aspirin was 0.63 (CI 0.35–1.13 $P = 0.02$). Adverse events in the aspirin and placebo group were almost the same. This finding suggests that a follow-up for several years after a randomized trial is necessary for evaluating the effects of aspirin, and this may be true with other CRC chemopreventive agents.

J-FAPP2

In Japan, a double-blind randomized trial was performed, using a low-dose aspirin enteric-coated tablet (100 mg/day for 6–10 months) in 34 subjects with FAP (17 each in the aspirin and placebo groups) (17). This trial is the first double-blind randomized trial of aspirin in Japanese subjects. The J-FAPP2 trial resulted in a tendency of reduction in the size of colorectal polyps in FAP with aspirin administration, when compared with placebo administration. Furthermore, subgroup analysis indicated that the number of subjects with a small polyp with a mean baseline polyp diameter ≤ 2 mm was significantly reduced in the aspirin group. Adverse effects of aspirin, such as astomotic ulcer, aphtha in the colorectum and progression of anemia, occurred in three subjects. All of these subjects were non-smoking women, with an age lower than 40 years with high β-catenin staining of their polyps. Moreover, none of the subjects developed CRC.

Insights into the Mechanism of Aspirin Chemoprevention

In the CAPP2 study, aspirin reduced development of CRC long after cessation of exposure to aspirin. It is assumed that the primary action of aspirin on COX in colonic tumors is not likely to be the important mechanism, but that other mechanisms could exist. Several pieces of evidence have shown that aspirin can inhibit proliferation and induce apoptosis of colon cancer cells independently from its inhibitory effects on prostanoid biosynthesis (18). Reported COX-independent molecular mechanisms are: (i) the interruption of nuclear factor kappa B (NF-κB) (19, 20); (ii) the

interruption of extracellular signal-regulated kinases (21); (iii) the induction of caspase 8 and 9 (22,23); (iv) the inhibition of β-catenin signaling (24) and (v) the activation of 5′ adenosine monophosphate-activated protein kinase (AMPK) (25) (Table 2).

Ongoing Trials Using Aspirin

CAPP3

To determine ideal doses of aspirin for all Lynch syndrome gene carriers, a CAPP3 study is recruiting 3000 gene carriers to test the relative benefits of 100, 300 or 600 mg/day.

J-CAPP Study

The aim of the study was to present the evidence that aspirin is useful as a chemopreventive agent in general Asian populations. The J-CAPP study aimed to investigate the effects of low-dose aspirin for 2 years in Japanese in a double-blind, randomized, placebo-controlled clinical study in patients whose colorectal tumors (one or more) were all excised by colon endoscopy. The research protocol of the J-CAPP study is described elsewhere (26).

Trials Using Other NSAIDs and Selective COX-2 Inhibitors

Related to PG biosynthesis, many human studies using various NSAIDs have been conducted. There are small, randomized clinical trials using sulindac as a chemopreventive agent. Forty-five FAP patients were enrolled in these studies, and sulindac showed a statistically significant decrease in the number of colorectal tumors (27,28). On the other hand, 77 FAP patients were enrolled in a double-blind placebo-controlled study using a selective COX-2 inhibitor, celecoxib, at a dose of 100 mg twice a day, and 400 mg twice a day for 6 months (29). The dose of 100 mg resulted in a 12% decrease in the number of colorectal tumors. Celecoxib at 400 mg reduced the number of colorectal tumors by 28% from the baseline, evaluated by endoscopy at the beginning of the trial.

Table 2. Summary of cyclooxygenase-independent targets of aspirin

Targets	Target-reactive molecules	Target-related bioactivity
NF-κB	COX-2, iNOS, IL-6, TNFα, etc.	Inflammation, cell survival, etc.
ERK	Elk1, AP-1	Cell growth, cell differentiation
Caspase-8 and-9	Caspase-3, -6 and -7	Apoptosis
β-catenin	c-Myc, Cyclin D1, etc.	Cell growth, cell survival, cell differentiation, etc.
AMPK	GLUT4, PGC-1α, PPAR, etc.	Cellular energy homeostasis, modulation of insulin secretion, etc.

AMPK, 5′-adenosine monophosphate-activated protein kinase; COX-2, cyclooxygenase-2; ERK, extracellular signal-regulated kinases; GLUT4, glucose transporter 4; iNOS, induced nitric oxide synthase; IL-6, interleukin-6; NF-κB, nuclear factor kappa B; PGC-1α, PPAR-γ-coactivator 1α; PPAR, peroxisome proliferater-activated receptor; TNF-α, tumor necrosis factor-α.

However, the promising use of coxibs in chemoprevention was halted abruptly due to the enhancement of cardiovascular risks. This could be explained partly by the inhibition of COX-2-dependent PGI_2 production, which plays an important role in vasoprotective and anti-thrombotic pathways. In addition, other major problems of some NSAIDs and COX-2 inhibitors are that the suppressive effects on tumorigenesis are transient and disappear soon after drug withdrawal.

To use such NSAIDs and selective COX-2 inhibitors for a long time, we need to give careful consideration by comparing the benefits of use and the risks of adverse effects, such as gastrointestinal bleeding and cardiovascular events. To prevent such adverse effects, several approaches can be considered: (i) reduction of doses, (ii) co-prescription of a proton-pump inhibitor and (iii) treatment to eradicate *Helicobacter pylori* infection possibly to overcome bleeding complications. Moreover, mPGES-1 inhibitors cause a selective inhibition of PGE_2 by affecting a PGE_2 synthase downstream of COX-2 and, thus, they may not affect the production of PGI_2 (30). Other ongoing studies are additionally listed in Table 3.

Metformin

As denoted above, NSAIDs and selective COX-2 inhibitors are the first candidates for CRC chemopreventive agents. In addition to those powerful and well-noted drugs, the anti-diabetic drug metformin has been thrown into the limelight recently (31).

Metformin (dimethylbiguanide) was first discovered as a derivative of mono-substituted guanidine, which showed less lipophilic interaction and considerably safer disposition than the original European anti-diabetic agent Galegine, in 1922. Now, almost a century has passed from then, and metformin has become the most widely prescribed anti-hyperglycemic agent (32,33). Metformin has a powerful metabolic effect, especially for lowering blood triglyceride levels in diabetic patients, targeting phosphorylation/activation of AMPK. AMPK is one of the possible candidates for a carcinogenesis-associated molecule, as written in the aspirin section. A tumor-suppressor gene product, LKB1 kinase, has been proved to be the upstream regulator of AMPK, and therefore restraining AMPK signal pathway activation would affect carcinogenesis. Based on this logic, metformin was presumed to have anti-tumor activity (34).

Observational Studies

To test this hypothesis, many observational case–control studies have been performed. The first large cohort study was Diabetes Audit and Research in Tayside Scotland/

Table 3. Selected ongoing RCT whose primary purpose is prevention of CRC

Drug	Length of treatment	Subject (estimated enrollment; ages)	Phase	Primary objects	Protocol ID (trial site)
Erlotinib (75 mg/day) + sulindac (150 mg/day)	6 months	FAP ($n = 100$; 18–69)	II	Regression of adenoma	NCT01187901 (USA)
Celecoxib (16 mg/kg/day)	5 years	FAP ($n = 200$; 10–17)	III	Time reduction from randomization to treatment failure	NCT00585312 (USA, UK, Belgium and others)
Aspirin + DFMO	Treatment repeated every 28 days for 1 year	High risk of CRC ($n = 104$; 40–120)	II	Reduction of adenoma recurrence rate	NCT00983580 (USA)
DFMO (500 mg/day) + sulindac (150 mg/day)	2 years	FAP ($n = 150$; >19)	III	Delay time to the first occurrence of any FAP-related event.	NCT01483144 (USA)
EPA (465 mg/day) + DHA (375 mg/day)	6 months	History of >1 polyps + known genotype for rs174535 in *FADS1* ($n = 150$; 49–79)	II	Decrease in rectal epithelial cell proliferation indexes and markers of rectal crypt apoptosis	NCT01661764 (USA)

Information obtained December 2012 from the websites (www.clinicaltrials.gov). DFMO, difluoromethylornithine/eflornithine hydrochloride; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Medicines Monitoring Unit conducted in Scotland (35). This study demonstrated that diabetic patients taking metformin showed reduced all-cancer risk, compared with those taking other diabetes therapies (adjusted odds ratio, OR = 0.86, 95% CI 0.73–1.02). This study triggered scientific interest in this field, and more than dozens of studies have confirmed its effectiveness in cancer chemoprevention.

In the field of gastroenterological cancer, multiple studies indicated the anti-neoplastic capability of metformin. For example, Lee et al. (36) performed the first prospective cohort study in Asia, recruiting 800 000 Taiwanese diabetic patients treated with or without metformin. This study revealed that metformin was able to decrease incidence rates of CRC and hepatocellular carcinoma (HCC) close to the levels of non-diabetic individuals. Of note, there was a significant gender difference with metformin interaction, i.e. in CRC it favored women (HR = 0.36, 95% CI 0.13–0.98), and in HCC it favored men (HR = 0.06, 95% CI 0.02–0.16).

Some meta-analyses also certified that metformin could reduce cancer risk. Zhang et al. (37) reported that metformin treatment was associated with a significantly lower risk of colorectal neoplasm, analyzing five studies, with 108 161 patients (RR = 0.63, 95% CI 0.50–0.79, $P < 0.001$). In another meta-analysis, it was shown that metformin treatment was able to reduce risks of cancer mortality and incidence (38). In the observational cohort studies, the pooled RR for all-cancer mortality among metformin users was 0.62 (95% CI 0.46–0.82). Especially, the incidence of cancer risks was also significantly decreased for CRC, HCC and lung cancer. In contrast, prostate, breast, pancreatic and gastric cancer were not significant. Of note, some bias could be involved in the data of the meta-analysis. Thus, it is still worthwhile to describe that metoformin use was associated with a reduced risk of pancreatic cancer in a hospital-based case–control study (973 case vs 863 controls) (39). Almost all of these observational studies indicated that metformin treatment is associated with a reduced cancer risk and/or improved prognosis; however, these data are mostly from retrospective and non-randomized studies.

Randomized Controlled Trials

A pilot study was performed in Japan to evaluate the chemopreventive effect of metformin on rectal aberrant crypt foci (ACF), an endoscopic surrogate marker of CRC. Non-diabetic patients with ACF ($n = 26$) were prospectively randomized into a metformin group (250 mg/day, $n = 12$) or a non-treatment group (control, $n = 14$) for 1 month in a blinded manner. The metformin group had a significant decrease in the mean number of ACF, whereas the mean ACF number did not change significantly in the non-treatment group (40). Furthermore, Japanese researchers recently reported the trial protocol of an ongoing double-blind, randomized controlled trial of metformin against colorectal polyp formation (41).

If metformin were clearly proved to be effective for the prevention of CRC, and any other cancers, the impact would be extremely large, in the context of drug repositioning. Needless to say, more information is needed for making the design of clinical trials, i.e. evaluation of appropriate doses for metformin against CRC. It would be very effective to use metformin at conventional doses as an anti-diabetic agent, because attenuation of high levels of insulin may contribute to anti-neoplastic activity. In participants under 18 years old, no dosing or adverse event data are currently

available with regard to the use of metformin, which results in the exclusion of children in trials, but it will be eligible for future pediatric trials. Other desired information is the effects of metformin on the developing human fetus at recommended therapeutic doses. Therefore, a serum pregnancy test must be performed and be negative in all women of childbearing potential prior to starting the trials. The verification of these points may also explore more aggressive dosing of metformin.

NATURAL PRODUCTS

ω3 Polyunsaturated Fatty Acids

High-fat diets are generally associated with a high risk of colon cancer (42). However, there are several types of fat and the effects on carcinogenesis are different. Animal fat, rich in saturated fatty acids and cholesterol, and corn and safflower oils, rich in ω6 polyunsaturated fatty acids (PUFAs) such as linoleic acid, have been shown to promote colon carcinogenesis in animal studies. On the other hand, olive oil (rich in ω9 monounsaturated fatty acids) and fish oil (rich in ω3 PUFA) have been demonstrated to have no promotive effects on carcinogenesis; rather, fish oil suppresses colon carcinogenesis in animal models (43,44).

Docosahexaenoic acid (DHA, C22:6, ω3) and eicosapentaenoic acid (EPA, C20:4, ω3) are major components of fish oil. DHA and EPA have lowering effects on serum lipids (45). Thus, EPA has been approved as a therapeutic agent for the treatment of dyslipidemia, and suppressive effects have been demonstrated in the Japan EPA Lipid Intervention Study on the incidence of coronary events in hypercholesterolemia with impaired glucose metabolism (46). Besides, DHA has important physiological activities in the brain and retina (45), and thus capsules are sold as health supplements at drug stores.

Observational Studies

Epidemiologically, there is only limited, but suggestive evidence for the beneficial effects of fish intake or ω3 PUFA consumption on the risk of CRC. In a systematic review published in 2006 summarizing prospective cohort studies of estimated consumption of ω3 PUFA and risk of several cancers, nine studies of the risk of CRC from seven different cohorts were identified (47), but only one study, the New York University Women's Health Study, demonstrated a statistically significant reduction in the risk of CRC in the highest ω3 PUFA intake category compared with the lowest (RR = 0.49, 95% CI 0.27–0.89) (48). In addition, the Physicians' Health Study demonstrated that intake of fish and ω3 PUFAs was inversely associated with risk of CRC in men; multivariate RR for highest vs lowest category for fish intake was 0.60 (95% CI 0.40–0.91) and that for ω3 PUFAs was 0.74 (95% CI 0.57–0.95) (49). In a population-based case–control study in Caucasians and African Americans, increased consumption of long-chain ω3 PUFAs was associated with a reduced risk of distal large bowel cancer in Caucasians, but not in African Americans; multivariable odds ratio for the highest vs lowest category in Whites was 0.49 (95% CI 0.34–0.71) (50). Recently, a Japan Public Health Center-based prospective study has demonstrated that intake of ω3 PUFAs was inversely associated with cancer risk in the colon in women (RR for the highest vs lowest category = 0.60, 95% CI 0.31–1.14), and in the proximal colon in men (RR for highest vs lowest category = 0.35, 95% CI 0.14–0.88) (51).

The available observational evidence on the effect of ω3 PUFA exposure on risk of CRC has been summarized in detail in the Second Expert Report of the World Cancer Research Fund and American Institute for Cancer Research in 2007 (52), which has been updated as part of the Continuous Update Project of these organizations in 2011 (53). The results show heterogeneity of the effects of ω3 PUFA on the risk of CRC and remain inconsistent.

Animal Model Studies

There are many pre-clinical studies evaluating preventive effects of fish oil or ω3 PUFAs on colon carcinogenesis using rodent models, and they have recently been reviewed in detail by Cockbain et al. (54). EPA has been shown to decrease tumor incidence and multiplicity in a rat colon carcinogenesis model induced by azoxymethane (AOM) (55), and intestinal tumor number and size in $Apc^{Min/+}$ mice (56–58). DHA has also been demonstrated to decrease numbers of ACF, putative pre-neoplastic lesions and tumors in a rat colon carcinogenesis model induced by 1,2-dimethylhydrazine or AOM (59,60), and intestinal polyp number and size in female Apc^{D716} mice (61).

Clinical Studies

In seven of nine clinical studies of ω3 PUFA treatment on colorectal mucosa biomarkers, a reduction in the cell proliferation index was observed (54). There have been two reports of clinical studies of ω3 PUFA treatment with FAP patients using colorectal polyps as the primary endpoint for the risk of CRC (62,63). A small, open-label study in three patients with FAP, and two patients with multiple (more than 30) colorectal polyps demonstrated no significant change in the number of colorectal polyps by treatment with 2.2 g DHA + 0.6 g EPA daily for 1–2 years (62). A recent phase III randomized, double-blind, placebo-controlled trial of EPA-FFA 2g daily for 6 months in 55 FAP patients undergoing sigmoidoscopic surveillance of a rectal stump after total colectomy (EPA-FFA 28, placebo 27) demonstrated a 22.4% reduction in the number of polyps ($P = 0.012$), and a 29.8% decrease in the sum of polyp diameters ($P = 0.027$) in the EPA-FFA group, while the global polyp burden worsened over 6 months in the placebo group (63) (Table 4). The chemopreventive efficacy of EPA-FFA in FAP patients was similar to that previously observed with

Table 4. Selected RCT with natural products for CRC prevention

Natural products	Length of treatment	Subject (no. of enrollment; age)	Primary objects/Results	Trial site (Ref.)
EPA-FFA (2 g/day)	6 months	FAP ($n = 55$; 18–74)	Reduction in number and size of polyps/Polyp number and size were reduced 22.4% ($P = 0.012$) and 29.8% ($P = 0.027$), respectively.	UK (63)
bLF (1.5 or 3 g/day)	12 months	Patients with colorectal polyps ($\leq$5 mm in diameter) ($n = 104$; 40–75)	Inhibition of the growth of colorectal polyps /3 g bLF inhibited growth of the polyps in patients less than 64 years old ($P = 0.006$).	Japan (84)

bLF, bovine lactoferrin; FFA, free fatty acid.

selective COX-2 inhibitors, and EPA-FFA was safe and well tolerated (63).

Insights into the Mechanism of Chemoprevention by ω3 Polyunsaturated Fatty Acids

There are several putative mechanisms underlying the anti-inflammatory and anti-neoplastic activity of ω3 PUFAs (54,64–68). (i) Inhibition of COX activity: COX-2 overexpressed in colon tumors stimulates cell proliferation and angiogenesis via PGE_2 production (69). Reduction of PGE_2 synthesis via inhibition of COX activity (54,64) is considered to be the main mechanism of the anti-neoplastic activity of ω3 PUFAs. (ii) Activation of PPARs and transrepression of NF-κB: PPARα and γ activation has the ability to inhibit expression of pro-inflammatory genes by inhibiting NF-κB activation. ω3 PUFAs have been implicated as PPAR-α/γ-agonists and inhibit NF-κB binding activity (54,65). (iii) Production of novel anti-inflammatory lipid mediators: ω3 PUFA-derived lipid mediators, resolvins and protectins, bind to G-protein-coupled receptors (GPCRs) and show anti-inflammatory and inflammation resolution activity. EPA and DHA can also act as direct ligands for GPCRs (54,64,65). (iv) Increase in membrane fluidity and alteration of lipid rafts and cell surface receptor function: lipid rafts are involved in modulating intracellular signaling cascades, including EGF receptor, insulin receptor, T cell receptor and B cell receptor. ω3 PUFAs are capable of suppressing CD4 + T cell proliferation and function via altering lipid rafts (66). (v) Increased oxidative stress: PUFAs are highly peroxidizable, and generated reactive oxygen species may induce apoptosis (54,67). (vi) Improvement of dyslipidemia: hyperlipidemia is a putative risk factor of colon cancer (70,71). ω3 PUFAs lower serum lipid levels via activation of PPARα (increase in FA oxidation) and suppression of SREBP-1c expression (decrease in triglyceride synthesis) (68). (vii) Activation of AMPK (72).

Lactoferrin

Lactoferrin is a component of whey/milk serum, which remains after milk has curdled and has been strained, i.e. a by-product of cheese or casein. The whey fraction also contains a large number of ingredients: α- and β-lactoalbumin, immunoglobulin, lactoferrin, etc. In humans, lactoferrin exists at relatively high concentrations in various secretions, i.e. tears, saliva and seminal fluid, with colostrum having particularly high levels (10 mg/ml) (73). We ingest bovine lactoferrin (bLF) as a component of cow's milk. Most ingested bLF is easily digested to lactoferricin (bLFcin) and its related peptides by acid pepsin hydrolysis (bLFH). bLFcin is detected in epithelial cells of the small intestine by immunohistochemical methods (74).

Animal Model Studies

Whey protein concentrate was found to exert a protective effect in a colon cancer models in rats (75), and the administration of whey protein to mice in the post-initiation stage resulted in a decrease in the colon tumor burden and prolongation of survival (76). These protective effects are thought to be due to a boost of the immune cells (77). Besides, α-lactoalbumin has been shown to be a calcium-elevating and apoptosis-inducing agent (78).

Bezault et al. (79) have shown protective effects of lactoferrin on the growth of solid tumors and the development of experimental metastases in mice. Moreover, we previously reported that bLF is a promising chemopreventor of colon carcinogenesis in rats (80,81). In rats administered AOM for initiation of colon carcinogenesis, the incidence of adenocarcinoma in the colorectum was markedly decreased (26%: $P < 0.01$ and 43%: $P < 0.05$ of the control in 2% and 0.2% bLF group, respectively) in rats fed bLF. The multiplicity (number of tumors per animal) was also significantly reduced in the bLF-fed groups. Cell proliferation in the carcinoma lesions, as assessed by 5-bromo-2′-deoxyuridine labeling indices, was significantly decreased in the 2 and 0.2% bLF-fed rats, compared with those in the control group. In addition to bLF, both bLFH and bLFcin also inhibited AOM-initiated colorectal carcinogenesis (82).

Randomized Controlled Trials

In 2002, a randomized, double-blind, placebo-controlled trial was conducted by the National Cancer Center Hospital, Tokyo to determine whether oral intake of bLF would inhibit the growth of adenomatous colorectal polyps in patients

(Table 4). Prior to the course of the 3-year trial, colorectal polyps were evaluated by colonoscopy. Target polyps were less than 5 mm in diameter with a pit pattern III (83). During the initial colonoscopic examination, the location of target polyps was marked, and the size of polyps was measured on the final day of one year of treatment.

Trial participants ingested 0, 1.5 or 3 g of bLF, and the results of the trial were published in 2009 (84). Participants aged 63 years or younger ingesting 3 g bLF had a significant reduction in target polyp size compared with the age-matched placebo subjects, and this group also had a significant increase in their levels of serum lactoferrin (hLF), but in participants 64 years or older, ingestion of bLF did not have a significant effect on the polyp size or serum hLF. Of note, serum bLF was undetectable in all the participants. The study also found that the participants ingesting 3 g bLF showed decreased induction of serum hLF with age.

Overall, participants with higher levels of NK cell activity had smaller polyps, but the effect of bLF ingestion on serum NK cell activity was inconclusive. A significant increase in NK cell activity was seen in the participants in the 1.5 g bLF group, but not in the 3 g bLF group. A larger study is needed to explore this point more conclusively.

No serious adverse effects associated with bLF ingestion occurred during the trial period, verifying the safety of bLF ingestion. Moreover, no malignant lesions were observed during the course of the trial.

bLF and bLFcin inhibit endothelial cell growth together with activation of immune cells that contribute to the anti-carcinogenesis and anti-metastatic activity. (i) bLF and bLFcin inhibit angiogenesis (85). In animal studies, bLF and bLFcin exhibited dose-dependent anti-angiogenesis effects on chick embryo chorioallantoic membrane. Human lactoferrin also exhibited strong anti-angiogenic effects. Moreover, bLF inhibited formation of tube-like structures by bovine pulmonary arterial endothelial cells in 1% FCS DMEM supplemented with VEGF in *in vitro* studies. (ii) During the examination aimed to inhibit tumor development and metastases by B16 melanoma and colon 26 tumor cells by bLF (86), marked increases in the number of cytotoxic T and NK cells in the mucosal layer of the small intestine and in the peripheral blood were found. This is possibly due to enhanced levels of interleukin-18 (87,88). Notably, in colon 26 tumor-cell-bearing SCID mice (origin BALB/c), which are deficient in T and B cells, bLF still showed significant inhibition of lung metastatic colony formation. On the other hand, anti-asialo GM1 antibody treatment results in markedly increased lung metastatic colonies in SCID mice with weakened NK cell activity. Those results suggest that inhibition of metastases by bLF is mediated through NK cells. (iii) In addition to the data in human trials, which show an increase in NK cell activity, we found that induction of serum hLF was associated with lower infiltration of polymorphonuclear leukocytes (PMNs) into target polyps. Moreover, lower infiltration of PMNs into polyp tissue was associated with growth suppression of polyps. Infiltration of PMNs into a polyp has been known to enhance tumor growth (89,90). All of these results suggest that bLF treatment can reduce the risk of colon carcinogenesis and have anti-tumor activity in humans. (iv) Lactoferrin is also reported to increase AMPK phosphorylation (91).

FUTURE ASPECTS

Other selected ongoing randomized control trials whose primary purpose is prevention of CRC are additionally listed in Table 1. Recent advanced technologies allow us to investigate further detailed mechanisms associated with the adenoma–carcinoma sequence and, thus, to obtain improved strategies to identify patients for CRC high-risk groups. Recently, genome-wide association studies identified four single nucleotide polymorphisms, such as *THADA*, *JAZF1*, *KCNJ11* and *TSPAN8*, as susceptibility loci for type II diabetes mellitus that affect the risk of CRC (92). Although the tools for an accurate estimation of cancer risk are increasing, problems still remain, such as lack of biomarkers for early detection and safe and effective chemopreventive agents. Taking a look at CRC management, the challenge of the next decade will be to explore paths for a double approach based on the development of innovative preventive strategies and anticancer therapies.

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Conflict of interest statement

None declared.

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Review Article

Cancer Chemoprevention by Citrus Pulp and Juices Containing High Amounts of β-Cryptoxanthin and Hesperidin

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β-Cryptoxanthin, a carotenoid, and hesperidin, a flavonoid, possess inhibitory effects on carcinogenesis in several tissues. We recently have prepared a pulp (CHRP) and citrus juices (MJ2 and MJ5) from a satsuma mandarin (*Citrus unshiu* Mar.) juice (MJ). They contain high amounts of β-cryptoxanthin and hesperidin. We have demonstrated that CHRP and/or MJs inhibit chemically induced rat colon, rat tongue, and mouse lung tumorigenesis. Gavage with CHRP resulted in an increase of activities of detoxifying enzymes in the liver, colon, and tongue rats'. CHRP and MJs were also able to suppress the expression of proinflammatory cytokines and inflammatory enzymes in the target tissues. This paper describes the findings of our in vivo preclinical experiments to develop a strategy for cancer chemoprevention of colon, tongue, and lung neoplasms by use of CHRP and MJs.

1. Introduction

Epithelial malignant neoplasms remain a major health challenge in the world. Despite improvements in staging and the application and integration of therapies, including surgery, radiotherapy, and chemotherapy, the 5-year survival rate for individuals with malignancies is still low. Even if strategies for early detection are successful and malignancies are detected at a stage where local tumor resection and treatment is curative, the patients will still have significant risk for developing second primary malignancy associated with the problem, including "field cancerization" [1–6]. For this reason, it is important to focus on chemopreventive strategies to prevent the development of epithelial malignancies [5, 6].

Cancer prevention is a rapidly expanding discipline that focuses on the discovery and identification of dietary agents and drugs that prevent or inhibit epithelial malignant tumor development [5–8]. Since approximately one-third of the overall risk of cancer is attributable to diet, a large number of dietary compounds have been tested to determine their chemopreventive ability using animal carcinogenesis models [4, 9–12]. It is known that certain carotenoids and flavonoids can inhibit cancer development in animal carcinogenesis models [4, 10–12]. β-Cryptoxanthin, (Figure 1(a)), a carotenoid, and hesperidin (Figure 1(b)), a flavonoid, are such compounds. β-Cryptoxanthin with nonsubstituted β-ionone cycles and provitamin A property possesses several biological activities including scavenging of free radicals, enhancement of gap junctions, immunomodulation, and regulation of enzyme activity involved in carcinogenesis [13, 14]. β-Cryptoxanthin is reported to inhibit mouse skin tumorigenesis [15] and rat colon carcinogenesis [16]. Hesperidin, present in several vegetables and fruits, has antioxidant property, anti-inflammatory effect, and inhibiting effect on prostaglandin biosynthesis. This flavonoid inhibits chemically induced carcinogenesis in several organs [4, 10–12]. β-Cryptoxanthin and hesperidin are thus considered to be potential cancer chemopreventive compounds. However, edible plants contain only small amounts of these chemicals. To obtain higher contents of these compounds in foods, therefore, we prepared a pulp (CHRP) containing high amounts of β-cryptoxanthin and hesperidin during the process of making satsuma

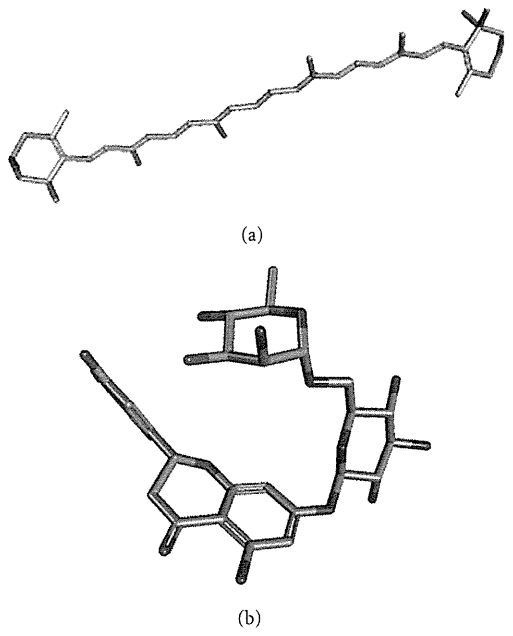

FIGURE 1: 3D chemical structures of (a) β-cryptoxanthin and (b) hesperidin.

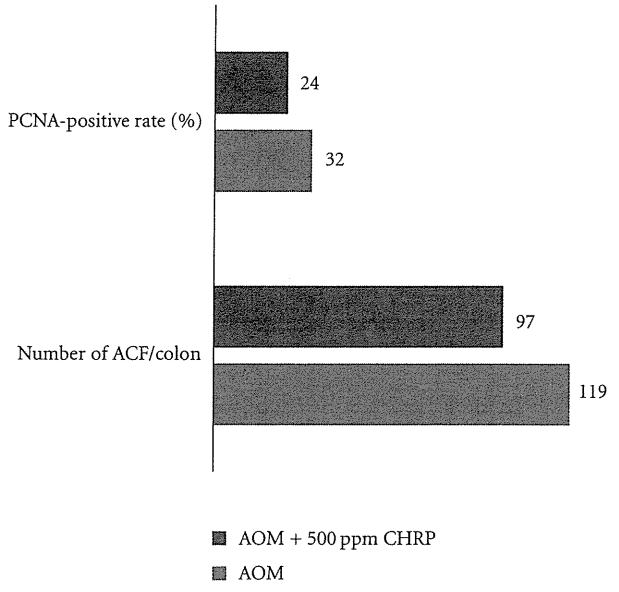

FIGURE 2: Dietary CHRP suppresses AOM-induced colonic aberrant crypt foci (ACF) in rats.

mandarin (*Citrus unshiu* Marc.) juice (MJ). CHRP (100 g) contained 0.67 g β-cryptoxanthin and 3.58 g hesperidin: the contents of β-cryptoxanthin and hesperidin are 583 times and 38 times greater than those in edible parts of satsuma mandarin (*Citrus unshiu* Marc.), respectively. In addition, we prepared Satsuma mandarin (*Citrus unshiu* Marc.) juices, called MJ2 (1.7 mg β-cryptoxanthin and 84 mg hesperidin/100 g) and MJ5 (84 mg β-cryptoxanthin and 100 mg hesperidin/100 g), by adding CHRP to usual mandarin orange juice (MJ: 0.8 mg β-cryptoxanthin and 79 mg hesperidin/100 g).

We describe the chemopreventive effects of CHRP and MJs on chemically induced oncogenesis in the colon and tongue of rats and mouse lung [17–19].

2. Dietary CHRP Inhibits Chemically Induced Rat Colon Carcinogenesis

To predict possible inhibitory action of CHRP in colon carcinogenesis, the effects of CHRP on the development of aberrant crypt foci (ACF), which are putative precursor lesions of colonic adenocarcinoma (ADC) of rodents and human [20–22], were examined in rats initiated with a colon carcinogen, azoxymethane (AOM) [17]. A total of 32 male F344 rats were used in the study. Animals were divided into the AOM alone, AOM and 500 ppm HCRP, 500 ppm HCRP, and untreated groups. Rats were initiated with AOM by two weekly subcutaneous injections (20 mg/kg bw) and were fed the diets containing CHRP at 500 ppm for 4 weeks (initiation stage), starting one week before the first dose of AOM. At week 11 of the experimental week, the frequency of ACF was determined. As illustrated in Figure 2, all rats in the AOM-alone group developed ACF with a frequency of 119 $\pm$ 5 ACF per rat, while rats in the AOM + 500 ppm CHRP group had a frequency of 97 $\pm$ 11 ($P < 0.05$). The CHRP-alone and untreated groups did not develop ACF. Immunohistochemical analysis of proliferation activity, proliferating-cell-nuclear-antigen- (PCNA-) positive index, was done, since promising chemopreventive compounds act through modulation of cell proliferative activity in the target organs [11, 23]. As presented in Figure 2, the PCNA-labeling index in ACF was significantly decreased by feeding with the CHIRP diet ($P < 0.05$).

Based on the findings from this preliminary experiment, a long-term animal experiment was conducted to determine the suppressing effects of CHRP on the development of colonic ADC induced by AOM. A total of 69 rats were randomly divided into 5 groups, namely, the AOM alone, AOM + 500 ppm CHRP for 4 weeks of the initiation stage, AOM $\rightarrow$ 500 ppm CHRP, for 28 weeks of the promotion stage, 500 ppm CHRP for the entire experimental period (32 weeks), and untreated groups. Animals were initiated with three weekly subcutaneous injections of AOM (15 mg/kg bw) to induce colonic neoplasms. As given in Figure 3, the frequencies of colonic ADC in the AOM + 500 ppm CHRP group (47%) and the AOM $\rightarrow$ 500 ppm CHRP group (21%, $P < 0.05$) were smaller than in group 1 (60%). In Figure 3, the PCNA-labeling indices of large bowel ADC developed in the the AOM + 500 ppm CHRP group and the AOM $\rightarrow$ 500 ppm CHRP group were significantly smaller than the AOM alone group ($P < 0.05$).

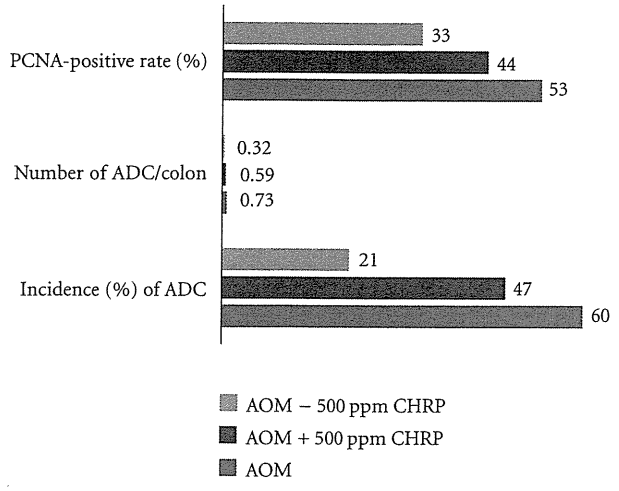

FIGURE 3: Dietary CHRP inhibits AOM-induced colonic adenocarcinoma (ADC) in rats.

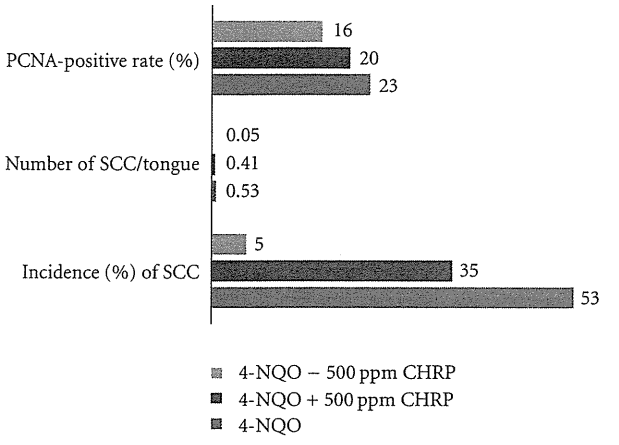

FIGURE 4: CHRP in diet suppresses 4-NQO-induced rat tongue squamous cell carcinoma (SCC).

3. Supplementation with CHRP in Diet Inhibits 4-Nitroquinoline-1-Oxide- (4-NQO-) Induced Rat Tongue Carcinogenesis

Oral cancer is an important public health issue because its occurrence is strongly associated with cigarette smoking and alcohol drinking [2, 3, 24]. Oral cancer is one of the ten most frequent cancers worldwide, with three-quarter of all cases occurring in the developing countries [2, 3]. It varies in the frequency greatly among different countries and geographic regions [2, 3]. The incidence and mortality of oral cancer have increased over the past decades in Europe [25] and in the United States [26]. In particular, the incidence and mortality rate of tongue cancer, as compared to other types of oral cancer, have increased in younger adults [27]. Chemoprevention against oral/tongue cancer development is thus important. To determine whether CHRP can inhibit chemically induced rat tongue carcinogenesis, a total of 67 male F344 rats were divided into 5 groups, including the 4-NQO alone group (8-week treatment with 20 ppm 4-NQO in drinking water), 4-NQO + 500 ppm CHRP group (10-week treatment during the initiation stage), 4-NQO → 500 ppm CHRP group (22-week treatment during the promotion stage), CHRP alone group (32-week of the entire experiment), and untreated group. At week 32, tongue squamous cell tumors (papilloma and carcinoma) and dysplasia developed in the posterior tongue (dorsal region) of rats that received 4-NQO. As shown in Figure 4 the incidences of tongue squamous cell carcinoma (SCC) were 53% in the 4-NQO alone group, 35% in the 4-NQO + 500 ppm CHRP group, and 5% in the 4-NQO → 500 ppm CHRP group. The incidence of tongue carcinoma in the 4-NQO → 500 ppm CHRP group was significantly smaller than the 4-NQOO alone group ($P < 0.05$). The multiplicity of tongue carcinoma of this group was also lower than the 4-NQO alone group ($P < 0.05$). The PCNA-labeling index of tongue SCC in the 4-NQO → 500 ppm CHRP group was significantly smaller than the 4-NQO alone group ($P < 0.05$).

4. MJs, Citrus Juices Rich in β-Cryptoxanthin and Hesperidin, Inhibit AOM-Induced Rat Colon Carcinogenesis

Colorectal cancer (CRC) is the fourth most common malignant neoplasm in the world [21]. Since an inverse relationship between the intake of fruits/vegetables and human CRC has been suggested [5, 6, 28–32], primary prevention, including chemoprevention utilizing the active compounds in edible plants, is important for reducing this malignancy [5, 6]. This experiment was designed to determine the modulatory effects of MJ, MJ2, and MJ5 on the occurrence of colonic neoplasms induced by AOM in rats [19]. Also, PCNA-labeling index in colonic neoplasms was analyzed immunohistochemically. A total of 113 male F344 rats were divided into 6 groups: the AOM alone group, the AOM → MJ group, the AOM → MJ2 group, the AOM → MJ5 group, the MJ5 alone group, and the untreated group. AOM was given to rat by twice weekly subcutaneous injections at a dose level of 20 mg/kg bw. MJ, MJ2, and MJ5 in black bottles were given to rats for 12 h (from 8:00 p.m. to 8:00 a.m.). Figure 5 shows the incidence and multiplicity of colonic ADC at week 38. AOM administration induced large intestinal ADC with an incidence of 69% and a multiplicity of 0.76 ± 0.57. The incidences and multiplicities of the AOM → MJ group, the AOM → MJ2 group, and the AOM → MJ5 group were significantly smaller than the AOM alone group ($P < 0.05$). The mean PCNA-labeling index of adenocarcinoma in rats of group 1 was 55 ± 7% ($n = 20$). The mean PCNA-labeling indices of adenocarcinomas present in the AOM → MJ2 and the AOM → MJ5 groups ($P < 0.05$) were significantly lower than the AOM alone group (55 ± 7%, Figure 5).

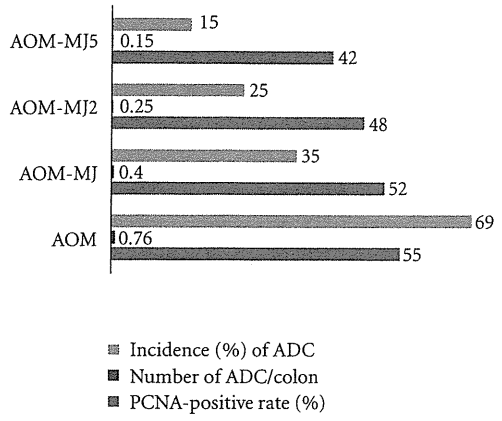

FIGURE 5: MJs suppress AOM-induced colonic adenocarcinoma (ADCs) in rats.

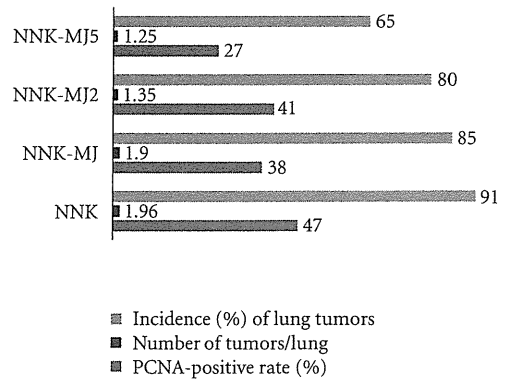

FIGURE 6: MJs inhibit NNK-induced lung tumors in mice.

5. MJs, Citrus Juices Rich in β-Cryptoxanthin and Hesperidin, Inhibit Chemically Induced Mouse Lung Tumorigenesis

Lung cancer is the largest cause of cancer deaths in industrial countries, and cigarette smoking is regarded as the overwhelming cause of lung cancer. Chemoprevention using naturally occurring or synthetic compounds to arrest or reverse the carcinogenic process is extremely important as a lung cancer prevention strategy. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is the most important carcinogenic nitrosamine, because of its strong potential in inducing lung neoplasms in rodents and its exposure to humans through smoking [33, 34]. Using the animal model for lung tumorigenesis with NNK, several promising agents for prevention of lung cancers have been reported [33–38]. This experiment was aimed to determine possible modulatory effects of MJs on the development of lung tumors induced by a pulmonary carcinogen, NNK, in mice [18]. The PCNA-labeling index in lung tumors was also determined. A total of 103 male A/J mice were divided into 6 groups: the NNK alone group, the NNK → MJ group, the NNK → MJ2 group, the NNK → MJ5 group, the MJ5 alone group, and the untreated group. NNK was given to mice by a single intraperitoneal injection (10 μmol in saline/mouse). MJ, MJ2, and MJ5 were administered to mice as a drinking water for 21 weeks, starting one week after the NNK injection. MJs in the black bottles were given to mice for 12 hr (from 8:00 p.m. to 8:00 a.m.) At week 22, lung proliferative lesions were diagnosed as hyperplasia and tumors, and we did not subclassify the tumors into adenoma and adenocarcinoma, because of the difficulty in evaluating malignancy [39, 40]. Pulmonary tumors (adenoma or adenocarcinoma) were developed in all mice treated with NNK. As illustrated in Figure 6, the incidences and the multiplicities of lung tumor of the NNK → MJ group, the NNK → MJ2 group, and the NNK → MJ5 group were smaller than NNK alone group. Statistically, the incidence of lung tumors in mice that received NNK and MJ5 was significantly lower than the NNK alone group ($P < 0.05$). The multiplicities of lung tumors of mice in the NNK → MJ group, the NNK → MJ2 group, and the NNK → MJ5 group were also low, but the differences were insignificant. The mean PCNA-labeling indices of pulmonary tumors of the NNK → MJ group and the NNK → MJ5 group were significantly lower than the NNK alone group ($P < 0.05$, Figure 6).

6. CHRP Increases Detoxifying Enzymes in the Liver, Tongue, and Colon

Induction of enzymes that enhance the detoxification of chemical carcinogens has been a broadly effective strategy for chemoprevention of experimental carcinogenesis in rodent models. Several inducing agents are now in clinical trials to evaluate utility for prevention of cancers associated with unavoidable high exposures to environmental carcinogens. Thus, certain phase II detoxifying enzyme inducers, including phenolic antioxidants, dithiolethiones, isothiocyanates, and triterpenoids, are considered to be promising chemopreventive in preclinical and clinical interventions [41–46].

To determine whether CHRP modifies glutathione S-tansferase (GST) and quinone reductase (QR) activities in the liver, colon, and tongue, male F344 rats were gavaged with CHRP at four dose levels (0, 40, 200 or 400 mg/kg body wt in 0.5 mL of 5% gum Arabic) of CHRP for 5 consecutive days [17]. Thirty min after the last gavage, the liver, colon, and tongue were excised immediately to measure GST and QR activities. Dosing of 40, 200, and 400 mg/kg bw of CHRP significantly elevated liver GST (1.27-fold, 1.25-fold, and 1.50-fold increases, resp., $P < 0.05$) and QR activities (1.24-fold, 1.22-fold, and 1.33-fold increases, resp., $P < 0.05$) when compared to rats that received 0 mg/kg bw. Similarly, gavage with CHRP significantly increased GST activity in the colonic (1.11-fold, 1.12-fold, and 1.15-fold increases, resp., $P < 0.05$) and tongue mucosa (1.33-fold, 1.29-fold, and 1.23-fold increases, resp., $P < 0.05$). CHRP treatment significantly increased the colonic QR activity at a dose of 400 mg/kg bw (1.13-fold increase, $P < 0.05$) and the tongue QR activity at doses of 200 and 400 mg/kg bw (1.25-fold and 1.21-fold increases, resp., $P < 0.05$).

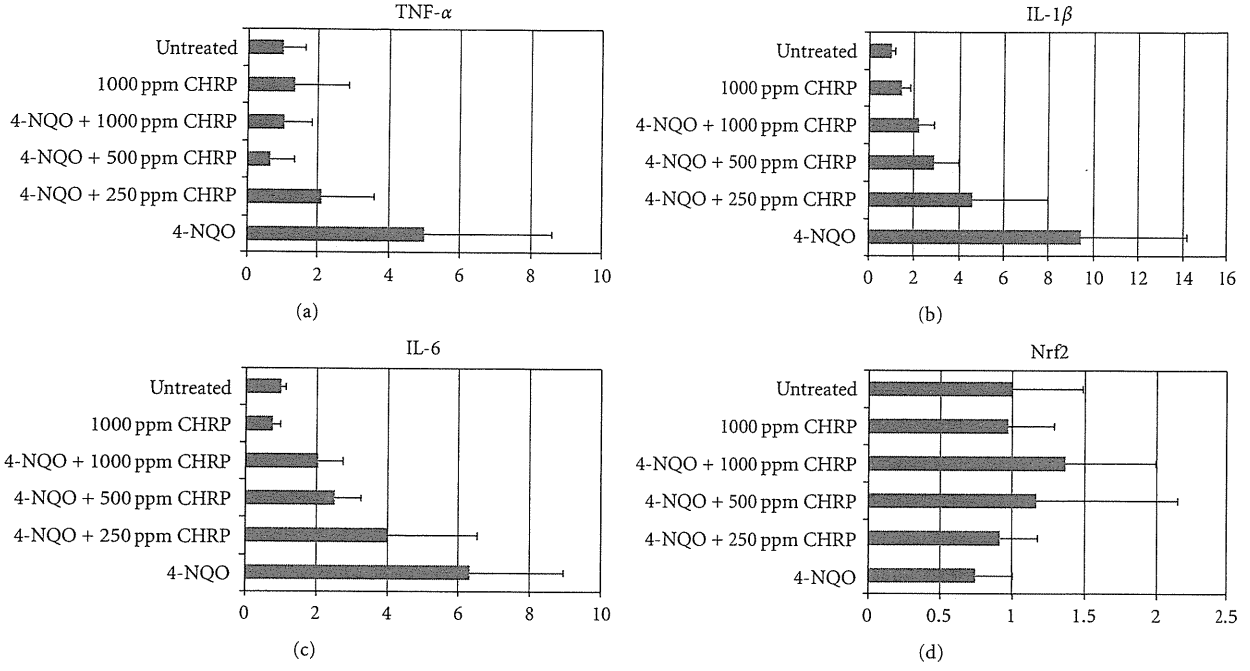

FIGURE 7: Dietary CHRP lowers mRNA expression of proinflammatory cytokines in the tongue of rats that received 4-NQO at week 8 of the study.

7. Modulation of Cytokine Expression by CHRP and MJs in the Tongue and Colon of Rats Initiated with Carcinogens

Chronic inflammation is closely associated with cancer development [47]. Cytokines are an important group of proteins that regulate and mediate inflammation and angiogenesis. Deregulation in their production results in tumor growth, invasion, and metastasis being facilitated. In addition to proinflammatory cytokines, inflammatory enzymes, such as cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS), are involved in carcinogenesis [48–50], and they are good targets for cancer chemoprevention [51–54]. The NF-E2-related factor 2 (Nrf2) is a key regulator of the inducible expression of enzymes such as GST and QR in catalyzing the detoxification of reactive electrophiles and oxidants that contribute to the formation of mutations and ultimately cancers. Nrf2 is now recognized to regulate a broad cytoprotective, transcriptional response leading to prevention of damage to DNA. Nrf2 is also a good target for cancer chemoprevention in certain tissues [55–59].

Based on these reports, we assayed mRNA expression of tumor necrosis factor (TNF)-α, interleukin (IL)1-β, IL-6, COX-2, iNOS, and Nrf2 in the tongue and colonic mucosa of rats to determine whether CHRP and MJs can affect this mRNA expression. Male F344 rats were initiated with a tongue carcinogen 4-NQO (20 ppm) by giving drinking water for 8 weeks, and they were also fed diets containing CHRP at doses of 250, 500, and 1000 ppm for 8 weeks, starting 4-NQO administration. At week 8, tongue mucosa was scraped. In a different experiment, male F344 rats were initiated with a colonic carcinogen AOM by twice weekly subcutaneous injections (20 mg/kg bw), and they also received MJ, MJ2, and MJ5 for 4 weeks from starting the AOM injection. Colonic mucosa was scraped at week 4 of the experimental period. Total RNA was extracted from tongue and colonic mucosa using the RNeasy Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. The cDNA was then synthesized from total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Japan Ltd, Tokyo, Japan). Quantitative real-time PCR analysis of individual cDNA was performed with ABI Prism 7500 (Applied Biosystems Japan Ltd, Tokyo, Japan) with rat gene specific primers for TNF-α, IL-1β, IL-6, COX-2, and iNOS (Applied Biosystems) on a GeneAmp 5700 Sequence Detection System (Applied Biosystems). Fold change was calculated using the $\Delta\Delta$Ct method relative to untreated with β-actin as the endogenous control. In addition, the specific primer (forward primer 5′-tgcccctggaagtgtcaaa-3′, reverse 5′-ggctgtactgtatccccagaaga-3′) was used to measure the expression of Nrf2. Data are illustrated in Figures 7, 8, 9, and 10. Administration with CHRP and MJs decreased mRNA expression of TNF-α, IL1-β, IL-6, COX-2, and iNOS in the tongue and colonic mucosa, while the treatments increased mRNA expression of Nrf2 in both mucosal tissues.

8. Discussion

We have described cancer chemopreventive ability of CHRP and MJs in experimental animal carcinogenesis models of

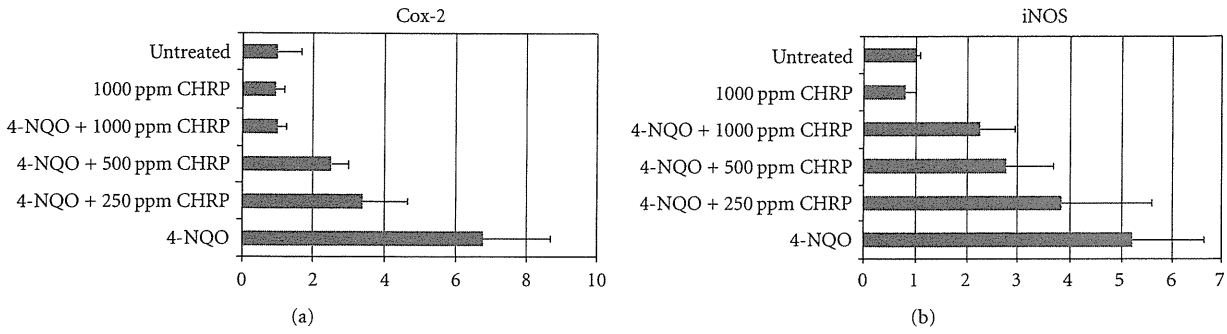

FIGURE 8: Dietary CHRP suppresses mRNA expression of inflammatory enzymes, Cox-2 and iNOS, in the tongue of rats that received 4-NQO at week 8 of the study.

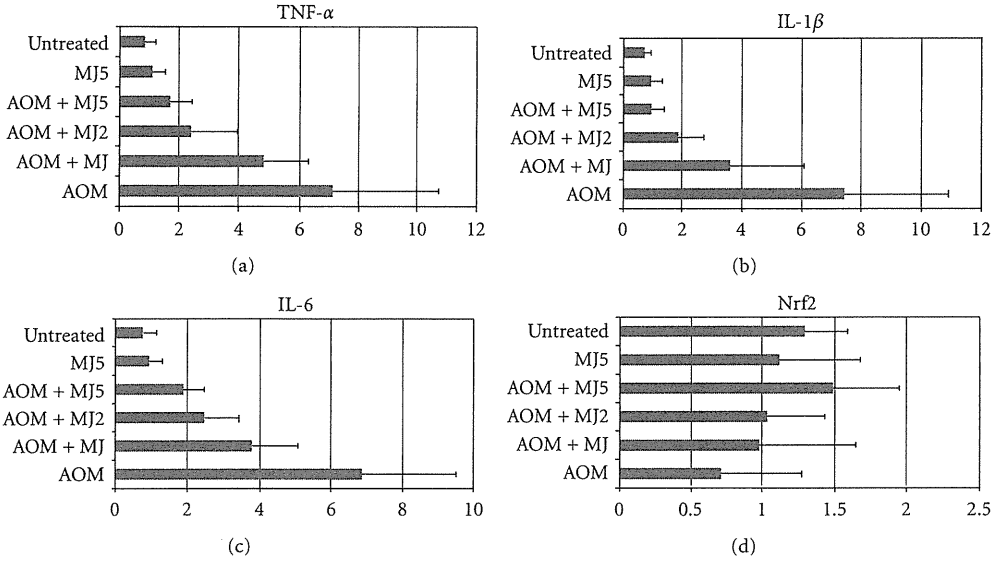

FIGURE 9: MJs lower mRNA expression of proinflammatory cytokines in the colonic mucosa of rats that received AOM at week 4 of the study.

colon, tongue, and lung cancers. Administration of CHRP or MJs after the carcinogen exposure clearly inhibits the development of malignant epithelial neoplasms. Miyagi et al. [60] also reported a protective effect of orange juice on AOM-induced rat colon carcinogenesis. Our data on MJs support their findings. The exact mechanism(s) involved in the suppressing effects of chemically induced tumorigenesis by CHRP and MJs is not fully known. In our studies, gavage with CHRP to rats elevated GST and QR activities in liver, colon, and tongue. Investigations by other researchers also demonstrated that limonin increased GST activities in several organs including liver, colon, and lung [61]. Therefore, it is possible that such elevation in activity of detoxifying enzyme by CHRP treatment may contribute its chemopreventive ability. Also, the modulatory effects of CHRP and MJs on specific species of liver CYPs, which are pertinent to the carcinogen metabolism [38, 62, 63], should be considered, although we did not examine their expression in our studies.

In addition, suppressing effects of CHRP and MJs on hyper-cell-proliferation activity induced by carcinogens in target organs account for their inhibition of carcinogenesis, since control of cell proliferation is one of the important effects of promising chemopreventive agents [23]. In our studies, gavage with CHRP to rats elevated GST and QR activities in liver, colon, and tongue, but strong inhibition in cancer incidence was not found when CHRP was fed to rats during the carcinogen treatment. This indicates that modification of cell proliferation of carcinoma cells rather than modification of activity of detoxifying enzymes may contribute to its chemopreventive ability when the substances were given after carcinogen exposure in long-term experiments.

Interesting findings in our studies are that CHRP and MJs were able to suppress mRNA expression of several cytokines (TNF-α, IL-1β, IL-6) and inflammatory enzymes (COX-2 and iNOS) and enhance mRNA expression of Nrf2 in the tongue and colon of rats that received a carcinogen,

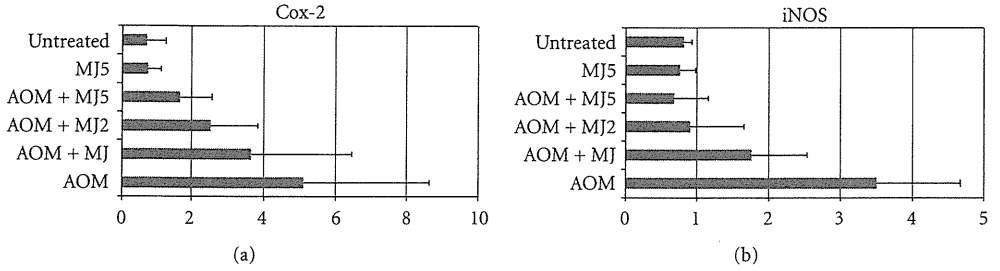

FIGURE 10: MJs suppress mRNA expression of inflammatory enzymes, Cox-2 and iNOS, in the colonic mucosa of rats that received AOM at week 4 of the study.

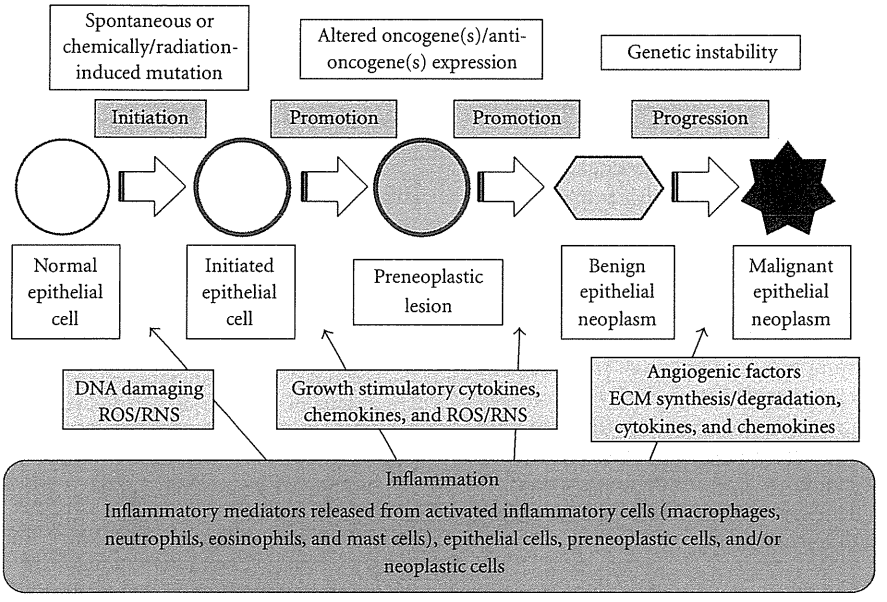

FIGURE 11: Working hypothesis of the involvement of inflammation in multistage carcinogenesis.

4-NQO or AOM. Chronic inflammation is known to be closely associated with cancer development in certain tissues [47], including oral cavity, colon, and lung. Cytokines regulate and mediate inflammation and angiogenesis. Tumor growth, invasion, and metastasis are facilitated when there is a deregulation in their production. Functional polymorphisms affecting gene expression of interleukin IL-1β, 4, -6, -8, and -10 as well as TNF-α are strongly associated with increased risk for oral cancer [64]. Recent findings also indicated that interactions between cytokines and sympathetic neurotransmitters and their respective receptors expressed by the nerve, immune, and tumor cells appear to influence tumor growth [65]. In the colon, inflammatory bowel disease (IBD) is an important risk factor for the development of CRC [21, 66, 67]. Inflammation is also likely to be involved with other forms of sporadic as well as heritable CRC [21]. Relationships between lung inflammation/injury and lung cancer in humans are also suggested [39]. Genetic predisposition to chronic obstructive pulmonary disease was associated with increased risk of developing lung cancer [68]. In addition to proinflammatory cytokines, inflammatory enzymes, such as COX-2 and iNOS, are involved in carcinogenesis [48–50], and they are good targets for cancer chemoprevention [51–54]. Nrf2 transcription factor was identified in the mid-1990s as a key regulator of the inducible expression of enzymes such as GST and QR in catalyzing the detoxification of reactive electrophiles and oxidants that contribute to the formation of mutations and ultimately cancers. Nrf2 is now recognized to regulate a broad cytoprotective, transcriptional response leading to prevention of damage to DNA, proteins, and lipids; recognition, repair, and removal of macromolecular damage; and tissue renewal following toxic assaults. The importance of this pathway as a determinant of susceptibility to carcinogenesis was indicated in multiple studies that demonstrated enhanced incidence, multiplicity, and/or tumor burden in Nrf2-deficient mice compared to wild-type ones in models of inflammation and CRC and lung cancer. Nrf2 is thus one of the targets for cancer chemoprevention in certain tissues [55–59]. When considering the relationship between chronic inflammation and cancer development (Figure 11), such effects of CHRP and MJs are attractive for reducing tumor occurrence.

Citrus fruit contains other possible chemopreventive agents. These include *d*-limonene [69], auraptene [70, 71], diosmin [72], limonin [73], and obacunone [73]. Some of these can modify activities and expression of the detoxifying enzymes and CYPs [74]. In addition, CHRP and MJs are able to downregulate mRNA expression of several cytokines and inflammatory enzymes and upregulate Nrf2 mRNA expression. Thus, citrus fruit is one of the rich sources of cancer chemopreventive agents.

9. Conclusion

Our studies demonstrate that oral administration of CHRP and MJs inhibits the development of malignant epithelial neoplasms in colon and tongue and lung tumors of rodents through their multiple biological functions. Further experiments, including preclinical efficacy and mechanistic studies including modification of expression of cancer-related genes in the target tissues, are warranted to fully evaluate these natural compounds for their cancer preventive properties and to understand their mode of action. One of the advantages of CHRP and MJs is that, unlike synthetic chemopreventive agents, they are natural substances present in human foods. On the basis of our observations and knowledge of the carcinogenic process, a strategy for cancer chemoprevention of colon, tongue, and lung can be developed. In conclusion, our findings may indicate that CHRP and MJs that are able to inhibit chemically induced carcinogenesis through modulation of proliferation, detoxifying enzymes, and mRNA expression of several cytokines and inflammatory enzymes are potential cancer chemopreventive agents against tongue, colon, and lung cancer development.

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Review Article

Development of an Inflammation-Associated Colorectal Cancer Model and Its Application for Research on Carcinogenesis and Chemoprevention

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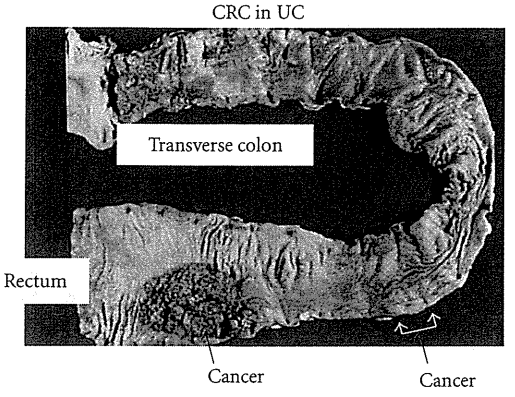
Chronic inflammation is a well-recognized risk factor for development of human cancer in several tissues, including large bowel. Inflammatory bowel disease, including ulcerative colitis and Crohn's disease, is a longstanding inflammatory disease of intestine with increased risk for colorectal cancer development. Several molecular events involved in chronic inflammatory process may contribute to multistep carcinogenesis of human colorectal cancer in the inflamed colon. They include overproduction of reactive oxygen and nitrogen species, overproduction and upregulation of productions and enzymes of arachidonic acid biosynthesis pathway and cytokines, and intestinal immune system dysfunction. In this paper, I will describe several methods to induce colorectal neoplasm in the inflamed colon. First, I will introduce a protocol of a novel inflammation-associated colon carcinogenesis in mice. In addition, powerful tumor-promotion/progression activity of dextran sodium sulfate in the large bowel of $Apc^{Min/+}$ mice will be described. Finally, chemoprevention of inflammation-associated colon carcinogenesis will be mentioned.

1. Introduction

Relationship between inflammation and cancer has been suggested for a long time [1]. Since Marshall and Warren [2], who discovered *Helicobacter pylori* and reported its infection closely associated with gastric cancer development, won the Nobel Prize in Physiology or Medicine in 2005, there have been an increasing number of reports on PubMed as to the relationship between inflammation and carcinogenesis in a variety of tissues (Table 1) and it has been featured in major journals.

In terms of the large bowel, it has been found that the risk of colorectal cancer increases in relation to the degrees of inflammation and the disease duration (duration/risk = 10 years/1.6%, 20 years/8.3%, and 30 years/18.4%) in inflammatory bowl diseases (IBDs) such as ulcerative colitis (UC) and Crohn's disease (CD) (Figure 1) [3]. I have been interested in inflammation-associated colorectal carcinogenesis for a long time, since even younger patients with UC have high risk of colorectal cancer [4].

Patients with UC as well as those with colorectal cancer have been increasing in Asian countries including Japan, similarly to Western countries (Figure 2) [5]. Therefore, it is necessary to investigate the mechanisms of colorectal cancer development with the background of inflammation for establishing the countermeasure strategy such as chemoprevention [6–8]. To this end, a novel animal model is required but there have been few useful animal models. In this paper, I would like to introduce details of my short-term mouse and rat colorectal cancer models with the background of colitis mimicking human UC and our exploration of chemopreventive agents using these models [6–8].



Rate of CRC development in UC patients:

Dis. period (year)	Rate (%)
10	1.6
20	8.3
30	18.4

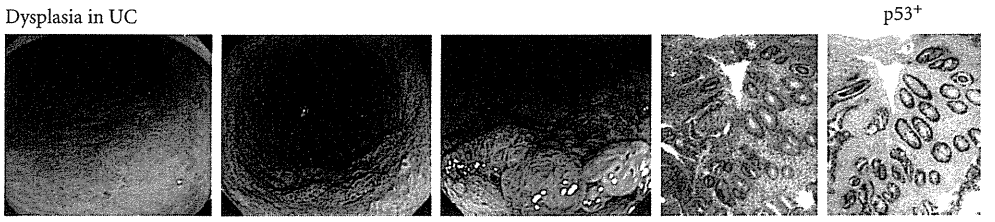


FIGURE 1: UC patients are high-risk groups of colorectal cancer (CRC) development.

TABLE 1: Inflammation and cancer in various tissues.

Chronic inflammation	Site and associated cancer
Chewing tobacco, Oral irritation	Oral squamous cell carcinoma
Smoking, Chronic bronchitis, Chronic obstructive pulmonary disease	Lung cancer
Asbestosis	Mesothelioma
Reflux esophagitis, Barrett's esophagus	Esophageal adenocarcinoma
H. pylori-induced gastritis	Gastric cancer, Mucosa-associated lymphoid tissue lymphoma
Chronic pancreatitis	Pancreatic adenocarcinoma
Viral (Hepatitis B and C virus) hepatitis	Hepatocellular carcinoma
Opisthorchis sinensis infection (liver fluke)	Cholangio carcinoma
Inflammatory bowel disease (IBD)	Colorectal adenocarcinoma
Pelvic inflammatory disease	Ovarian cancer
Human papilloma virus (HPV) infection	Anogenital carcinoma
Schistosomiasis	Bladder cancer
Chronic scar tissue	Scar cancer arising in pre-existing scars in the lung, skin, and other tissues
Human herpes simplex virus type 8	Kaposi sarcoma
Chronic oesteomyelitis	Osteosarcoma

2. Process of Human Colorectal Carcinogenesis

There are at least four types of human colorectal carcinogenesis (adenoma-carcinoma sequence type, hereditary nonpolyposis colorectal cancer (HNPCC) type, *de novo* type, and colitic cancer type) (Figure 3) [9]. Of them, the colitic (colitis-associated) cancer type arises from the background of colitis and DNA injury is induced by production of free radicals by the inducible nitric oxide synthase (iNOS) system in the colonic mucosa with persistent inflammation, followed by *p53* mutation and development of dysplasia, a precancerous lesion. Furthermore, dysplasia is advanced by cyclooxygenase- (COX-) 2, iNOS, and several cytokines produced in the infiltrated inflammatory cells and accumulation of genetic abnormality, such as a loss of the *DCC* gene, leads to invasive colorectal cancer. Unlike common colorectal cancer (adenoma-carcinoma sequence type), it has been thought that the *APC* and K-*ras* genes and microsatellite instability (MSI) are hardly involved in this type, but there remains to be further discussed [9].