—Review—

Cancer chemoprevention by natural compounds

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Abstract: There is growing interest in the use of natural compounds for the treatment and prevention of a wide variety of diseases, including cancer. Several herb-derived components are currently evaluated in preclinical studies as potential cancer chemopreventive agents. We have recently found that several herbal plants in the Ryukyu Islands, or any other natural compound, have a potential chemopreventive effect on biomarkers of colon carcinogenesis and a growth inhibitory effect on human cancer cells. These studies together with related *in vivo* and *in vitro* investigations are summarized and discussed in this review.

Key phrases: chemoprevention, colonic preneoplastic lesions, natural compounds

Introduction

Cancer chemoprevention is defined as the prevention of cancer by intake of one or several compounds [1, 2]. There has been increasing interest in the use of traditional herbal medicines for the treatment and chemoprevention of a variety of diseases [3]. For instance, investigators have focused on the medical properties of natural compounds such as traditional Japanese or Chinese herbal medicines called Kampo that may have a broad range of biological activities, including anticancer effects [4, 5]. It is well accepted that one-third of human malignancies might be associated with dietary habits and lifestyle [4-7]. The Ryukyu Islands, which belong to Okinawa Japan, are located in the most south part of Japan. Especially in females, Okinawa is known globally as one of longevity areas in Japan [8]. In fact, the mortality of solid-type cancer in Okinawa is much lower than that in mainland Japanese population [9]. Although many studies on the longevity have been done in relation to food culture, environment, lifestyle, and genetics, the precise reason why Okinawans have much longevity has not yet been elucidated. There are many kinds of herbal plants in the Ryukyu Islands. Of these, *Peucedanum japonicum* (PJ) and *Terminalia catappa* (TC) are widely distributed in Southern and Eastern Asia including Okinawa. In ancient times people used their leaves or roots as antipyretic or haemostatic agents and to treat patients with hepatitis [10]. Their leaves were also commonly consumed in ordinary life as a dietary supplement. Pericarps of *Garcinia mangostana* (GM), called mangosteen, are growing in the Southern and Eastern Asian countries and have been in use for hundreds of years as a traditional herbal medicine for a variety of purposes, including treatment of skin infection [11] and wounds, or malaria infection [12]. *Azadirachta indica* (AI), called Neem, is a tropical evergreen tree in South and Middle Asian countries. In recent studies, we examined a possible short-term chemopreventive effect of PJ, TC, AI, and crude α-mangostin (CαM) on carcinogen-induced rat colon carcinogenesis [13-15].

Sphingolipids are a group of structural and functional derivatives that have a long chain (sphingoid) base backbone [16-18]. Milk, eggs, and soybeans are rich sources [19]. Ceramide and glucosylceramide (cerebroside) are included in the bran and endoderm of rice grains [20]. Of these sphingoids, we examined the possible chemopreventive effect of enriched dietary monoglucosylceramide, (4E,8E)-1-*O*-β-glucosyl-*N*-2'-hydroxyarachidoyl-4,8-sphingadienine (G_1CM),on reneoplastic biomarker lesions involved in rat colon carcinogenesis [21]. Phytochemicals are non-nutritive components in the plant-based diet that have substantial anticarcinogenic and antimutagenic properties [22]. Indole-3-carbinol (I3C) is a naturally occurring phytochemical and found in cruciferous vegetables including cabbage, broccoli, Brussels sprouts, and cauliflower [23]. We also examined the effects of I3C on colon carcinogenesis, cell proliferation, cell cycle progression, apoptosis, and on the levels of expression of several cell cycle control molecules [24].

Preneoplastic biomarker lesions in the colon tissue

Aberrant crypt foci (ACF) are putative precursor lesions of colon carcinogenesis in rodents [25, 26] and humans [27]. These lesions are induced in the colonic mucosa of rats or mice treated with chemical carcinogens such as azoxymethane (AOM), methylazoxymethanol (MAM) acetate, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), and 1,2-dimethylhydrazine (DMH) [13, 25, 28-31]. ACF are classified into two distinct fractions such as histological ACF and dysplastic foci (DF). DF are characterized by nuclear

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stratification, loss of nuclear polarity, structural abnormality of the crypts, Paneth cell metaplasia, decrease or loss of goblet cells, and presence of mitosis [31]. In a recent study, we found that focal lesions that display the accumulation of the β-catenin protein predispose to carcinogen-induced colon carcinogenesis [32]. Mucin-depleted foci (MDF) have also been demonstrated to be preneoplastic lesions in the colon tissue [33, 34]. These lesions have been used as useful biomarkers for short-term colon carcinogenesis bioassays and are also useful to examine chemopreventive effects of a wide variety of candidate compounds [35].

Cancer preventive activities of natural plants

1. *Peucedanum japonicum* (Botanbofu) and *Terminalia catappa* (Momotamana)

Two different categories of colon preneoplastic lesions such as ACF and BCAC were used as biomarkers to evaluate possible chemopreventive activity of PJ and TC. These lesions were induced with subcutaneous (sc) injections of a carcinogen AOM (20 mg/kg body weight, once a week for 2 weeks) in F344 rats on the colonic mucosa. Powdered PJ leaves and hot water-soluble extracts of TC were provided by the Okinawa Industrial Technology Center (OITC, Okinawa, Japan). One week before the first injection of AOM, the rats were fed a diet containing 0.2 and 1% PJ and 0.02 and 0.1% TC for 5 weeks. Effects of these samples were evaluated by calculating the average number of ACF or BCAC per rat. Dietary administration of rats with PJ and TC at both dose levels significantly decreased the average number of both ACF ($P<0.05$ for PJ and $P<0.02$ for TC) and BCAC ($P<0.0001$ for PJ and $P<0.02$ for TC), when compared to the control untreated rats [13, 14]. Also, PJ and TC caused a significant decrease ($P<0.001$ for PJ and $P<0.005$ for TC) in the proliferating cell nuclear antigen (PCNA) labeling index in the colonic epithelial cells [13, 14]. The liver homogenate of the rats treated with PJ displayed relatively weak antioxidative activity compared to that of vitamin C [13]. These findings suggest that PJ and TC have a potent short-term chemopreventive effect on two different biomarkers of colon carcinogenesis and this effect may be associated with the inhibition of the development of ACF and BCACs.

2. *Garcinia mangostana* (Mangosteen)

Dried mangosteen pericarps were extracted with ethyl acetate and crude preparation was obtained by simple crystallization. The crude crystals were composed of 77.8% α-mangostin, 15.9% γ-mangostin, and some other unknown constituents. Of these, CαM was used for our study. Four-week-old male F344 rats were given sc injections of a carcinogen DMH (40 mg/kg body weight, once a week for 2 weeks) to induce preneoplastic lesions on the colonic mucosa. One week before the first injection of DMH, the rats were fed a diet containing 0.02 and 0.1% CαM for 5 weeks. We evaluated the effect of CαM by calculating the average number of ACF or BCAC per rat. Dietary treatment of rats with CαM at both doses significantly inhibited the induction of ACF ($P<0.05$) and BCAC ($P<0.05$), when compared to the control untreated rats [15]. Treatment of rats with CαM also decreased the PCNA labeling index in the colonic epithelial cells and in the cells consisting of the crypts of ACF and BCAC [15]. These results indicate possible chemopreventive effect of CαM in a short-term colon carcinogenesis bioassay system.

3. *Azadirachta indica* (Neem)

Neem leaf was provided by the Ryu-Celo Co., Ltd. (Okinawa, Japan). Powdered leaf was boiled with distilled water at 95°C for 10 min. and filtered with a filter paper. The extract was used for the experiment. ACF were induced by sc injections of AOM (20 mg/kg body weight, once a week for 2 weeks) in the colon of F344 rats. We found that dietary feeding of the Neem extract inhibited the development of ACF and PCNA labeling index, when compared to the control [36]. Also, the Neem extract showed antioxidative activity. The finding that dietary Neem has possible chemopreventive effects in colon carcinogenesis suggests that longer-term exposure may cause suppression of tumor development.

4. Crude extracts obtained from herbal plants in the Ryukyu Islands

We screened the chloroform extracts obtained from 44 herbal plants in the Ryukyu Islands for the growth-inhibitory activity in several human colon carcinoma cell lines [37]. These plants were obtained in a public market in mainland Okinawa. Of these 44 herbal plants, extracts of *Hemerocallis fulva*, *Ipomoea batatas*, *Curcuma longa*, and *Nasturium officinale* displayed a dose-dependent growth inhibition in these cell lines [37]. The IC_{50} values of these extracts were in the range of 10-80 μg/ml. Extracts of *Hemerocallis fulva* and *Ipomoea batatas* arrested HCT116 cells in the G1 phase of the cell cycle, and extracts of *Curcuma longa* and *Nasturium officinale* induced apoptosis in the same cell line. These findings demonstrate that several plants in the Ryukyu Islands contain a component(s) that may have anticancer activity.

5. Monoglucosylceramide (G_1CM)

We evaluated the preventive effect of dietary G_1CM on DMH-induced ACF and BCAC formation in F344 rats during initiation stage [21]. Pure G_1CM obtained from rice bran was provided by the Oryza Oil and Fat Chemical Co. (Aichi, Japan). Purity was determined by high-performance liquid chromatography (HPLC) analysis, and structural information

was obtained with mass spectrometric analysis. After the exposure of AOM, the rats were fed a diet containing 200 and 1,000 ppm G_1CM for 5 weeks. Dietary G_1CM at both doses significantly inhibited the occurrence of ACF and BCAC ($P<0.001$) compared to the control [21]. The PCNA labeling index of GiCM-treated group was also lower than that of the control. These findings, that dietary G_1CM has possible short-term chemopreventive effect in the colon, suggest that longer exposure of G_1CM may cause suppression of tumor formation.

6. Indole-3-carbinol (I3C)

In a recent study, we found the I3C inhibits growth and induced G1-phase cell cycle arrest and apoptosis in human colon carcinoma cell lines [24]. I3C also caused an increase in the expression levels of $p27^{KIP1}$ and $p21^{CIP1}$ mRNA. These results suggest that I3C inhibits the growth of human colon carcinoma cells, at least in part, by inducing expression of these molecules. In parallel studies, we also investigated cancer-preventive effect of I3C in carcinogen-induced rat colon carcinogenesis. F344 rats were given sc injections of AOM (20 mg/kg body weight) once a week for 2 weeks to induce colon tumors. One week after the last injection of AOM, the rats were fed a diet containing 0.01 or 0.05% I3C (>97% pure, Sigma Chemical Co., St. Louis, USA) for 35 weeks. In contrast to the results of the cell culture, I3C caused a significant increase in the tumor multiplicity and volume of adenocarcinoma in the colon [24]. Further, I3C increased the PCNA labeling index and decreased the apoptotic index of the colonic adenocarcinoma. These findings suggest that I3C promotes carcinogen-induced rat colon carcinogenesis by increasing cell proliferation and inhibiting apoptosis of colon tumors. Most *in vivo* and *in vitro* studies demonstrate I3C's inhibitory or preventive activities for colon carcinogenesis [38-43] but some provide evidence for promotion or enhancement of colon carcinogenesis [44, 45]. Therefore, our study may provide evidence for the ambivalent modulatory effect of I3C and this information may be useful when including this compound in cancer chemoprevention or extensive clinical trials.

7. Resveratrol and epigallocatechin-3-gallate (EGCG)

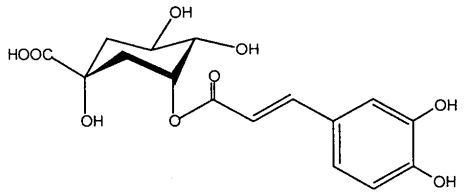
The polyphenolic compound resveratrol (3, 5, 4'-trihydroxy-*trans*-stibene) is a naturally occurring phytochemical. Red wine and grapes are probably its main sources in Western diets. One of its richest sources is the herb *Polygonum cuspidatum*, which has been used in Asian folk medicine. We found that resveratrol induces growth inhibition, S-phase arrest of the cell cycle distribution, and changes in expression of cyclin D1, cyclin B1, and β-catenin in human colon carcinoma, esophageal adenocarcinoma, esophageal squamous cell carcinoma, breast carcinoma, and leukemia cell lines [46]. These results provide evidence that this compound may be useful in chemoprevention and cancer therapy trials. Epigallocatechin-3-gallate (EGCG) is a major active component of green tea. EGCG inhibited the growth of human head and neck squamous cell and breast carcinoma cell lines [47-49] In these cell lines, EGCG decreased VEGF production by inhibiting EGFR-related pathways of signal transduction [47-49]. Therefore, EGCG may be useful in treating head and neck and breast carcinoma because it can exert both antiproliferative and antiangiogenic activities.

Potential active components of herbal plants

Neochlorogenic acid (4(*S*)-3-*O*-caffeoylquinic acid, 3-COA) (Figure 1A) is one of the isomers of chlorogenic acid (4(*R*)-3-*O*-caffeoylquinic acid) (Figure 1B) [50]. Oxidative stress is closely associated with the carcinogenic process [51] and both of these compounds have been shown to have antioxidative activity measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays [13]. In addition, 3-COA is reported to have an inhibitory effect for human low-density lipoprotein (LDL) oxidation [52]. 3-COA was found in the fruit of plum [45]. PJ (Botanbofu) also contains approximately 4% 3-COA and this plant displays antioxidative activity [13], suggesting that 3-COA may be a main active component of the hot water extract of PJ. Corilagin (β-1-*O*-galloyl-3,6-[(*R*)-4,5,6,4',5'6'-hexahydroxybiphenyl-2,2'-dicarbonyl]-*D*-glucose) (Figure 1C) is the member of the tannin family of some kind of fruits and this compound is present in Japanese and Chinese traditional herbs, *Geranium thunbergii* (Genno-shoko) and *Phyllanthus embolica* (Yu-Gan-Zi), respectively [53, 54]. One possible active component of TC (Momotamana) is corilagin because this compound is found to be present in TC [14] and also exerts antioxidative effect [14, 50, 55]. GM usually contains xanthone derivatives, such as α, β, and γ-mangostin [56]. Of these, α-mangostin (Figure 1D) has been demonstrated to have biological activities including growth-inhibitory and apoptosis-inducing effects on human leukemia [56] and colon carcinoma cell lines. γ-Mangostin (Figure 1E) also inhibits the cyclooxygenase-2 (COX-2) activity in C6 rat glioma cell line [57]. Mangostins are found to be one of the constituents of GM [15] and CαM exerts biological activities [15], indicating that the possible active components of GM are mangostins. Of 44 herbal plants, we found that the extracts from *Hemerocallis fulva*, *Ipomoea batatas*, *Curcuma longa*, and *Nasturium officinale* caused a significant growth inhibition of human colon carcinoma cells [37]. The possible active compounds are anthraquinone derivatives, anthocyanine compounds, curcumin, and isothiocyanates, respectively. These compounds are demonstrated to have a broad range of biological effects including the growth inhibition of cancer cells, anti-oxidation, anti-inflammation, apoptosis induction, and the prevention of rat colon carcinogenesis [58-67]. Isothiocyanate of *Nasturium officinale* inhibited growth of human cancer cell lines, and

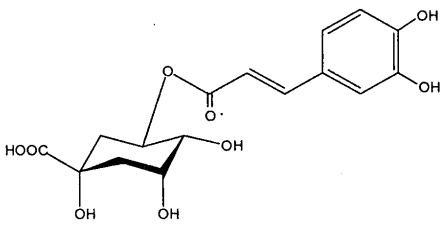
phenyisothiocyanate enhanced c-Jun *N*-terminal kinase (JNK) activity [68, 69]. The Neem leaf has been demonstrated to include potent chemopreventive phytochemicals such as limonoids and flavonoid querucetin [70]. It is reported that these compounds have potent cancer preventive, growth inhibitory, and antioxidative activities [71-77]. Chemical structure of G_1CM is shown in Figure 1(F). About 88% of dietary sphingomyelin is degraded to ceramine or sphingoid bases in the small intestine, and the remaining 12% of dietary sphingomyelin is present in the colon [78]. Thus, colonic epithelial cells can be exposed to ceramide and sphingoid bases. These bioactive metabolites are taken up by the colonic epithelial cells and affect various cellular target molecules [16, 17, 79, 80].

A



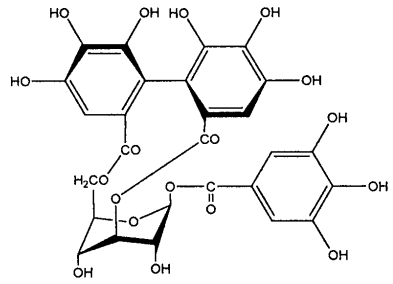
Neochlorogenic acid (4(*S*)-3-*O*-caffeoylquinic acid)

B



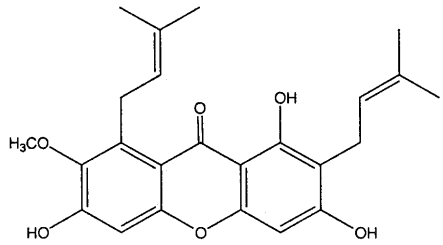
Chlorogenic acid (4(*R*)-3-*O*-caffeoylquinic acid)

C



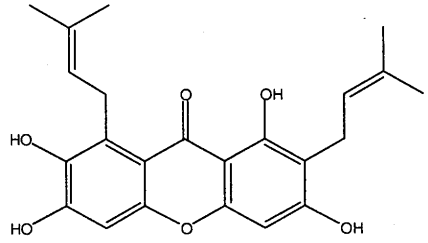
Corilagin
(β-1-*O*-galloyl-3,6-[(*R*)-4,5,6,4',5'6'-hexahydroxybiphenyl-2,2'-dicarbonyl]-*D*-glucose)

D



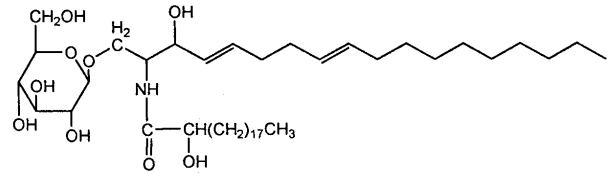
α-Mangostin

E



γ-Mangostin

F



(4E,8E)-1-*O*-β-glucosyl-*N*-2'-hydroxyarachidoyl-4,8-sphingadienine (G_1CM)

Figure 1. Chemical structures of the natural compounds

Medical application of herbal products

Our recent studies provide evidence that several plants in the Ryukyu Islands prevent short-term rat colon carcinogenesis and induce growth inhibition and apoptosis, strongly indicating that these plants contain a component(s) that may have anticancer activity. Several herb-derived natural compounds introduced in this article are currently being evaluated in preclinical studies as potential cancer chemopreventive agents. It is of interest whether phytochemicals derived from the plants are useful in the prevention and therapy of various human malignancies. To address these aspects, more detailed studies are necessary to identify the active component of the plants. It would also be important to examine the precise molecular mechanisms of action of these compounds and their specific cellular targets to predict adverse side effects, and to also use this information to design new drugs for more effective prevention or treatment of human cancer and other diseases.

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