Review

Anti-tumor Promotion with Food Phytochemicals: A Strategy for Cancer Chemoprevention

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Cancer chemoprevention is currently regarded as a promising avenue for cancer control. In particular, the inhibition of tumor promotion (anti-tumor promotion) in multistage carcinogenesis is expected to be an efficient strategy, because tumor promotion is experimentally accomplished through the long-term, repetitive exposures of rodents to a tumor promoter, and premalignant lesions caused by a tumor promoter regress, at least in their earlier stages. In this review, we first describe the background of cancer chemoprevention studies as well as recent results of clinical trials. Subsequently, some hypothetical biological and cellular pathways in tumor promotion are explored. In addition, the anti-tumor promoting properties of vegetables, fruits, and edible marine algae, together with their active constituents and action mechanisms thus far known, are also described. Anti-tumor promotion with food phytochemicals may be characterized as an efficient and reliable strategy for cancer chemoprevention.

Key words: cancer chemoprevention; anti-tumor promotion; food phytochemicals; multistage carcinogenesis; vegetables and fruits.

In 1915, Yamagiwa and Ichikawa reported that repetitive topical applications of coal tar on rabbit ears for many months resulted in the formation of epithelial carcinomas. This epoch-making achievement has prompted many researchers to search for and identify carcinogens as well as anti-carcinogens, especially those in our environment. In addition to the experimental animal models, in vitro short-term tests (e.g., the Ames test) have greatly facilitated carcinogenesis studies. Such tests undoubtedly contributed to the understanding of: multistage carcinogenesis; dietary carcinogens (e.g., N-nitrosamines, aflatoxins) and their inhibitors (e.g., ascorbic acid, polyphenols); metabolic activation and detoxification pathways of carcinogens; and so forth.

Accumulated data at that time implied that cancer in humans may be preventable by the administration of natural or synthetic compounds, the suppressive effects of which on carcinogenesis were confirmed in experimental animal models. However, almost all cancer prevention studies remained in the domain of laboratory research before the end of the 1970s. Then, this vague and passive situation was broken by the establishment of the Chemoprevention Branch of the National Cancer Institute in the U.S.A. in 1982, which systematically dose various studies from primary screening tests to clinical trials. Today, cancer prevention research has taken on a new aspect on the basis of massive experimental data from epidemiological and animals studies, launching out into a new phase of cancer control.

In this review, we wish to first describe the background and current aspects of cancer chemoprevention studies, and recent topics of clinical trials. It is evident that an understanding of the mechanisms of the carcinogenesis process is essential for cancer chemoprevention. Most cancer prevention research is based on the concept of multistage carcinogenesis: initiation → promotion → progression. Among these stages, in contrast to both initiation and progression stages, animal studies indicate that the promotion stage takes a long time to occur and may be reversible, at least in its earlier stages. Therefore, the inhibition of tumor promotion in the multiple stages is expected to be an efficient approach to cancer control.

Though a critical biological step in the initiation of carcinogenesis has been discovered (i.e., point mutation of the Ha-ras gene), those of promotion are more complicated and remain to be identified in detail. Discovery of one common denominator in the biological pathways of tumor promotion would be indispensable for a strategy of anti-tumor promotion. To explore this, some current topics in hypothetical biological and cellular pathways of tumor promotion are discussed. In addition, the anti-tumor promoting properties of food items, their active constituents, and action mechanisms are highlighted.

Trends of Cancer in Japan

Cancer has been the leading cause of death in Japan since 1981. In recent years, while gastric cancer mortality in Japan has declined along with that in the U.S.A., cancers
of the lungs, pancreas, and colon are increasing steadily. The former tendency is probably caused by a results of diminished salt intake due to the improvement of food storage. The latter tendency may be attributable, at least in part, to the change of diet style among Japanese people from a traditional to more western style (i.e., high fat, high energy intake). The fact that aging is well-correlated with the onset of cancer makes cancer control, in countries having advanced medical treatment, a crucial social issue to be solved.

Cancer Chemoprevention

Vigorous efforts for the development of chemotherapy or radiotherapy to cure and eradicate cancer have been made by a wide variety of scientific fields over the past several decades. However, as mentioned above, increasing tendencies for cancer-related mortality in Japan and the U.S.A. have not declined. Most invasive cancers, especially solid tumors, are still difficult to cure. Such grim circumstances encouraged the concept of cancer chemoprevention to mature in a number of scientific fields.

Definition and concept

Prevention is the most reliable strategy for remedying all diseases including cancer, and the prevention of cancer has widely been anticipated as a foremost paradigm for cancer control in recent years. Cancer chemoprevention, coined by Sporn et al. in 1976, is defined as a strategy for cancer control by the administration of synthetic or natural compounds capable of halting or inhibiting the onset of cancer. Cancer chemoprevention is based on a rationale of epidemiological surveys, laboratory animal models, and the concept of field and multistage carcinogenesis.

A great number of epidemiological studies have demonstrated the relationship between carcinogenesis and dietary habits or their components. For instance, it is generally accepted that cigarette smoking is a cause of lung cancer, and ingestion of green-yellow vegetables is inversely related to several cancers. Studies on animal models have also demonstrated that experimentally induced cancer is preventable with some synthetic or naturally occurring compounds. Field carcinization, named by Slaughter et al. in 1953, is well illustrated by recent studies showing a high rate of incidence of second primary tumors in patients with previous primary carcinomas in the head and neck, and lungs. Current advances in DNA analysis, such as in situ hybridization and polymerase chain reaction, have enabled researchers to detect some multiple genetic abnormalities in normal and premalignant epithelial lesions induced after operation on primary tumors. Chemopreventive treatments should promptly be applied to such cases.

Clinical trials

In clinical trials, experimental design is very important for the validity of research and its fruitful outcome, i.e., selection of chemopreventive agents and target populations, dose determination from pharmacokinetic studies, and determination of the trial period and target intermediate endpoints. Chemoprevention clinical trials must be concerned with the relationship between benefit and toxicity, which are variable among trial subjects. In this regard, even mild toxicity is not acceptable to healthy populations. Thereby, to date, the target populations for cancer chemoprevention are generally recognized as high-risk groups such as: (1) individuals in occupational contact with carcinogens (e.g., asbestos workers); (2) survivors of primary cancer or patients with a disease possibly proceeding to cancer (e.g., hepatic cholangitis); (3) individuals with a genetic background with a high frequency of cancer incidence (e.g., familial colonic polyposis); and (4) individuals with predicted premalignancy by diagnosis with biomarkers (e.g., aneuploidy, p53 mutations). At present, over 30 randomized clinical trials have been done. For example, a fruitful result was reported by Blot et al., who observed that the combined administration of β-carotene, α-tocopherol, and selenium could reduce stomach cancer mortality by 21% in Linxian county in China, where one of the world's highest rates of esophageal/gastric cardia cancer and a persistently low intake of micronutrients are known. In Japan, Muto et al. recently did preliminary randomized placebo-controlled phase II double blind trials with 89 post-operative patients with hepatoma. ES166, a synthetic acetic retinoid, at 600 mg·day was orally administered for 48 weeks. Out of the 71 analytically significant cases, the recurrence rate of hepatoma in the ES166 group was 15% (5/33), which was markedly lower than that (39%; 15/38) in the control group.

Multistage Carcinogenesis

When carcinogenesis is accomplished through a multiple and discernible process, one may be able to delay the onset of cancer or to halt it at a preinvasive stage (i.e., at initiation or promotion). The classical "two-stage carcinogenesis theory," advocated by Brenblau in 1941 with a mouse skin model, is still accepted with some modifications. Currently, the chemical carcinogenesis process is substantially divided into three stages of initiation, promotion, and progression (Fig. 1). The promotion stage was further divided into conversion and propagation by Slaga et al. Based on experimental animal models, the multistage carcinogenesis theory has recently been accepted as valid in diverse types of cancer such as kidney, breast, lungs, stomach, and liver. In reality, daily exposure to initiators, promoters, and progressors is in disorder, and the carcinogenic process in humans is distinctly more complicated than that in experimental animal models. Nevertheless, the multistage carcinogenesis theory is evaluated as a useful and indispensable model to explore the detailed biological and cellular mechanisms of chemical carcinogenesis.

Initiation

Initiation is considered to occur in one or a few cells of tissue, and to be provoked by the metastatic activation of procarcinogens by phase I enzymes such as cytochrome P-450-dependent monooxygenases, which catalyze oxidation, hydroxylation, reduction, and hydrolysis. These enzymes can convert procarcinogens into ultimate carcinogens, most of which are reactive electrophiles. The multistep activation process of procarcinogens by phase I enzymes is, however, inhibited by phase II enzymes such as glucuronosyltransferases, sulfotransferases, and glutathione S-transferases, which primarily catalyze conjugating reactions. The activated electrophiles, that are not
detoxified by phase II enzymes can interact with and bind to cellular DNA to trigger gene mutation(s). With treatment using an appropriate dose of 7,12-dimethylbenz[a]-anthracene (DMBA), a potent polycyclic aromatic hydrocarbon tumor initiator, in mouse skin, more than 90% of the papillomas are observed to possess an A to T transversion at the second position of code 61 in the Ha-ras gene. After mutation fixation is accomplished by cellular replicative DNA synthesis, the initiating process is virtually irreversible. Another important aspect of initiation is the absence of a threshold for initiators. Such characteristics of initiators imply that continuous exposure to initiators in daily life would result in suffering higher risks of cancer development in humans. The data showing the relationship between aging and cancer may demonstrate such etiological characteristics of initiation. However, by contrast, there also exists a controversial report that daily exposure to initiators is assessed as trivial for illustrating actual human cancer incidence. In this regard, it is reasonable to assume that tumor promoting factors in our environment play a critical role in human carcinogenesis.

**Promotion**

Promotion involves clonal proliferation of the initiated cells, and converts them into premalignant tumor cells. During this event, a variety of intracellular signaling pathways are activated. Tumor promotion research was earnestly started with the identification of a tumor-promoting constituent in croton oil. 12-O-tetradecanoylphorbol-13-acetate (TPA), by Hecker et al. in 1967. TPA-type tumor promoters such as phorbol esters, teleocidins, or aplysias-toxin can activate both phospholipid and Ca$^{2+}$-dependent protein kinase C (PKC), an enzyme activated by endogenous diacylglycerol released by an activation of phospholipase C. PKC is widely accepted as one of the major intracellular targets of TPA-type tumor promoters. Activated PKC undergoes phosphorylation of proteins regulating cellular differentiation and or proliferation. Recently nine different PKC isozymes have been identified by cDNA coding. They are divided into three groups: conventional PKC (PKC-α, βI, βII, and γ); novel PKC (PKC-δ, ε, η, and θ); and apical PKC (PKC-ζ). Expression of PKC-βI, γ, and ε can cause a transformed phenotype in NIH 3T3 cells. Most PKC isozymes are translocated from the cytosol to the cellular membrane, possibly to bind and activate target membrane proteins. In this regard, it is interesting that the two isozymes, PKC-β and ε, are selectively translocated to the nucleus.

Okadaic acid, a toxic polyether compound, is a representative non-TPA-type tumor promoter isolated from a black sponge, *Halicidonia okaidai*. Okadaic acid possesses potent tumor-promoting activity in mouse skin, rat glandular stomach, and rat liver. An intrinsic action mechanism of okadaic acid is the inhibition of protein phosphatases 1A and 2A, which results in an increase of phosphorylated proteins. This hyperphosphorylation is observed in such cytoskeletal proteins as vimentin and cytokeratins, and nuclear proteins such as RB and p53, both of which are proposed to be tumor suppressor proteins. Recently Wang et al. reported that the continuous treatment of murine NIH 3T3 fibroblast cell cultures with okadaic acid resulted in a 50-fold amplification of multidrug-resistance genes, mdr-1a and mdr-1b. As okadaic acid-type promoters, dinophysistoxin-1, calyculin A, microcystin-LR, nodularin, and tutaomycin have been identified.

Studies on TPA resulted in the establishment of assays using their biological and physiological activities. There-after, tumor promoters more closely associated with our daily lives have been discovered as the following: glioxal in stomach (coffee), 2.2,3,7,8-tetrachlorodibenzo-p-dioxin in liver (a trace contaminant in paper bleaching and synthesis of a herbicide), polybrominated and polychlorinated biphenyls in liver (accidentally mixed in livestock feed), Roussin red methyl ester in stomach (pickled vegetables), lithocholic and bile acids in colon (endogenous factor), sodium chloride in stomach, and a high-fat diet in breast and colon. Dietary high fat intake is known
to be significantly related to tumor promotion. Birt et al. reported that, in a two-stage carcinogenesis experiment, increases in both the number and incidence of papillomas, and the earlier appearance of carcinomas were observed in mice fed on a high-fat diet during the promotion phase, and PKC activity in epidermal cells from those mice were higher than that in mice fed on a control diet.30

Tumor promoters are not genotoxic but can induce the expression of several genes. The activation of PKC by TPA-type tumor promoters is followed by the induction and/or activation of several proliferation-relating genes such as c-myc, c-fos, and c-jun.31 Okadaic acid can also enhance the expression of c-fos or c-jun. Activator protein (AP)-1, a heterodimer of the nuclear oncoproteins Fos and Jun, is a transcription factor capable of activating some genes containing the TPA-responsive element (TRE, TGACTCA).32

Interleukin-2 (IL-2) is also genetically regulated by TPA. This transcription is caused by binding of the nuclear factor κB (NF-κB) to the NF-κB recognition site of DNA. NF-κB forms a complex with an inhibitory protein IκB in cytoplasm where this complex is inactive in binding to DNA. With TPA treatment, the IκB NF-κB complex dissociates by the phosphorylation of IκB by PKC, then NF-κB migrates into the nucleus and can bind to DNA.33 The role of IL-2 in tumor promotion has not yet been discovered.

A hypomethylated gene has more potential for expression than a hypermethylated gene. Counts et al. proposed the hypothesis that hypomethylation of DNA could be one of the mechanisms in tumor promotion.34 DNA methylation is observed in cytosine as 5-methylcytosine (5MeC), and is frequently decreased along with carcinogenesis. The genomic level of 5MeC is lower in rat or mouse liver tumors than normal liver tissue. When 5-azacytidine (5AzC), an analog to 5MeC, is noncompetitively incorporated into DNA, it can inhibit methyltransferase, resulting in the hypomethylation of DNA. 5AzC actually has tumor-promoting activity in the rat liver and causes cell transformation in vitro.34 Moreover, while choline and methionine are essential factors for the synthesis of S-adenosylmethionine, a cofactor of DNA methylation, the potentiated expression of Ha-ras, myc, and fos by hypomethylation were observed in rats fed on a choline and methionine-deficient diet.34

Tumor necrosis factor (TNF), a cytokine with a molecular weight of 17,000, has been accepted to act as a serum factor inducing necrosis of transplanted solid tumors in the mouse. TNF is also known to be important in inflammation, immunoregulation, and mitogenesis. Recently Fujiki et al. reported that okadaic acid induced mTNF-α transcription in Bhas 42 and BALB 3T3 cells, resulting in stimulated growth of the initiated cells via an autocrine or paracrine mechanism.35 TNF-α markedly stimulated transformation of BALB 3T3 cells initiated with 3-methylcholanthrene. Surprisingly, the transformation potential of TNF-α in the initiated cells was about 1000 times higher than that of TP or okadaic acid. These findings raise the possibility that TNF-α, originally discovered and proposed as a tumor necrosis factor, may be an endogenous tumor promoter in humans.

Apoptosis is a homeostatic control mechanism of cell death (for review, see ref 36). Recent accumulating reports imply that the inhibition of apoptosis may be a common denominator in the action mechanism of tumor promotion. Unlike necrosis, apoptotic cells undergo DNA fragmentation in multiples of 180 base pairs released by cellular endonucleases. Because an essential function of apoptosis is to eliminate damaged or undesirable cells, blocking apoptosis, possibly by tumor promoters, may be beneficial for premalignant cells to be maintained or developed. Recently Wright et al. found that a total of 10 tumor promoters tested significantly inhibited DNA fragmentation in a variety of cultured cells such as fibroblasts, myeloid, and monocytic leukemias, while 4z-phorbol-12,13-diaceate, an inactive analog of TPA, could not inhibit apoptosis.37 Interestingly, tumor promoter-induced resistance to apoptosis was reversible after removing promoters, reminding us of the reversibility of the tumor promotion process in vivo. Furthermore, Schulte-Hermann et al. found a decrease in the appearance of apoptotic bodies in phenobarbital-promoted liver carcinogenesis.38

During apoptosis, many intracellular signaling pathways are activated, e.g., protein phosphorylation, activation of an endonuclease and ADP-ribose polymerase, and calcium mobilization. Epstein et al. reported that the ADP-ribose polymerase inhibitor, 3-aminobenzamide, has tumor-promoting activity in mouse skin.39 The level of AP-1 DNA-binding activity, induced by the expression of c-fos by TPA, was closely associated with repression of the apoptotic pathway in T lymphocytes.40 In addition, Trosko et al. recently submitted a hypothesis that apoptosis may be facilitated by gap-junction-mediated intercellular communication.41

Progression

The characteristics of the progression stage are found in irreversibility, somatic aneuploidy, and karyotypic instability.11 Acquisition of such genetic and biological alterations by papillomas results in the conversion of them to carcinomas, with invasiveness and metastatic capability. In a two-stage carcinogenesis experiment, 5% of papillomas were converted to carcinomas spontaneously at approximately 30 weeks.42 Some tumor progresor agents, most of which are genotoxic, were recently identified in mouse skin carcinogenesis experiments. Those progressors are characterized as carcinogens (e.g., urethane, cisplatin, ethyl nitrosourea), free radical generators (e.g., benzoyl peroxide, hydrogen peroxide), and others (e.g., acetic acid, diethyl maleate).43 Accordingly, oxidative stress is suggested to be a biologically important factor for the progression stage. However, Warren et al. reported that seven radical scavengers and antioxidants tested, reduced glutathione and disulfiram showed moderate inhibitory activity of tumor progression, and four were inactive.43 Furthermore, surprisingly, tert-butyl-4-hydroxyanisol (BHA), known as an anti-tumor initiator or anti-tumor promoter, significantly enhanced the conversion of papillomas to carcinomas. These data demonstrate that chemical inhibition of the progression stage is not confirmed even in the animal models to date, and useful screening systems for anti-progressing activity are desired to find effective inhibitors of progression.
Suppressive Potentials of Food Items for Tumor Promotion
Vegetables and fruits

Inhibitory potentials against tumor promoter-induced Epstein-Barr virus (EBV) activation are well correlated with those in some animal models. In 1986, Koshimizu et al. conducted screening tests of the methanol extracts from 122 plant species of Japanese vegetables and fruits for inhibitory activity toward EBV activation induced by a TPA analog, 12-O-hexadecanoylphorbol-13-acetate (HPA). As a result, 26% of the total showed significant inhibitory activity (> 30% at 200 μg/ml). On solvent partition of the randomly selected inactive methanol extracts (26 species) with ethyl acetate (EtOAc) and water, 13 and 2 species had the inhibitory activity in the EtOAc and water soluble part, respectively. Maeda et al. recently reported a high correlation (r = 0.82) of the suppressing activities of water extracts from Japanese vegetables and fruits (66 species) between EBV activation and alkyl peroxide radical formation, measured by the luminol-enhanced chemiluminescence method. The hot-water extracts of green leaves of carrot, cruciferous plants, and beans had both the highest inhibitory activities of EBV activation and alkyl peroxide radicals. By contrast, cold-water extracts of vegetables generally had only about 10% or less of the activity of the hot-water extracts. They speculated that these activity differences might come from the thermal destruction of vegetable cell walls, liberating more components, and/or the thermal reaction producing more potent active compounds.

Recently we regard edible Thai plants as promising sources to search for highly effective anti-tumor promoters. Thailand is widely known to have a rich flora, and people frequently use diverse vegetables and fruits for flavors, spices or condiments in their traditional cuisine. Most of the items are concurrently used as traditional folk medicines. Thailand still has many species of vegetables almost never subjected to plant breeding, suggesting that they may contain some biologically active compounds not occurring in long-term bred plants, or that they may surpass them in quantity of bioactive phytochemicals.

We collected a total of 112 species of edible Thai plants (122 parts), and their methanol-extracts were screened for EBV activation inhibitory activity. We found that 60% of the total significantly inhibited EBV activation, and the ratio is markedly higher than that (26%) in the screening tests of edible plants commonly obtained in Japan, suggesting higher cancer preventive potentials of edible Thai plants. In particular, the plant families Rutaceae, Zingiberaeae, Labiatae, and Piperaceae were indicated to contain potent anti-tumor promoters.

In these tests, 20 plant species in Thailand were the same as those in Japan. Though the experiments were done under the same conditions, the activities of 9 out of these 20 species were ranked differently. One of the reasons for such activity variation may come from differences in the cultivar. Supportable causes may be due to the harvest time, preservation, or cultivation conditions such as temperature, humidity, and light. The results suggest that the chemical characteristics of the active constituents or their contents could be changed by environmental factors. Maeda et al. reported that the outer green part and colorless inside part of cabbage leaf had very different inhibitory activity of EBV activation and alkyl peroxide radicals. Plant breeding, taking the content of active constituents into account, is desired to obtain edible plants highly efficient for cancer prevention.

Marine algae

Marine algae are rich in various carotenoids, and recent epidemiological data suggested that the ubiquitous consumption of seaweeds reduces the risk of some types of cancer in Japan. In the experimental model as well, the diet with marine algae powder decreased the incidence of DMBA-induced mammary tumorigenesis in rats. Ohigashi et al. screened 36 dichloromethane extracts from marine algae for EBV activation inhibitory activity. Interestingly, strong activity (> 70% at 4 μg/ml) was observed only in the algae Phaeophyta, which include abundant edible species such as sea tangles (“Kelp” in Japanese) or Wakame seaweed (“Wakame”). Application of a dichloromethane extract of Wakame seaweed (Undaria pinnatifida) (1 mg) on mouse skin almost completely suppressed tumor formation by DMBA and TPA. However, identification of the active constituent of Wakame seaweed was unsuccessful, because the strong inhibitory activity toward EBV activation was not recovered in any separated fraction of it. This suggested that some of the constituents synergistically enhance inhibitory activity.

Recently Okai et al. screened 8 Japanese marine algae for suppressive activity on ornithine decarboxylase (ODC) induced by TPA in BALB/c 3T3 fibroblast cells. Three methanol extracts from U. pinnatifida, Enteromorpha prolifera (“Suisanmori” in Japanese) and Porphyra tenera (“Asakusamori”) showed strong suppressive activity of ODC induction. Though these marine algae contain considerable amounts of β-carotene, comparative studies showed that β-carotene in the marine algae did not contribute to their ODC inhibitory activity. This result suggests that other carotenoids, or other types of components may be responsible for the anti-tumor promoting activity of marine algae.

Promising Anti-tumor Promoters from Food Items
Carotenoids (green-yellow vegetables, marine algae)

Carotenoids are one of the major plant pigments and considered to be responsible for cancer preventive effects of green-yellow vegetables. Among the carotenoids, cancer preventive effects of β-carotene (Fig. 2), a vitamin A precursor, are most intensively studied, and many clinical trials with β-carotene are now undertaken in various populations. However, cancer preventive efficacy of β-carotene in human has not been well defined yet. For example, daily intake of β-carotene (20 mg/day) was given for five to eight years to more than 29,000 male smokers aged 50 to 69 years in Finland. Surprisingly, the lung cancer incidence in β-carotene recipients was higher than that in placebo recipients by 18%. This unexpected outcome was contradictory to the many positive experimental results of β-carotene in animal models. Though further experimental data should be accumulated, this puzzling result led us to doubt the premise that β-carotene plays a critical role in cancer preventive effects of green-yellow vegetables.

In such a context, various carotenoids are currently...
being scrutinized for cancer preventive effects. α-Carotene, fucoxanthine, and halocynthiaxanthin (Fig. 2) were reported to have more potent inhibitory activity of the proliferation of human neuroblastoma GOTO cells than β-carotene.\textsuperscript{53} Murakoshi et al. reported that anti-tumor promoting activity of α-carotene was markedly higher than that of β-carotene in ddY mice promoted with glycerol.\textsuperscript{54} Tsushima et al. recently screened 51 carotenoids for the inhibitory activity of EBV activation induced by TPA in Raji cells.\textsuperscript{55} They found that β-cryptoxanthin, lutein, and lactucaxanthin (Fig. 2) were more potent inhibitors than β-carotene. Moreover, the essential moiety of the carotenoid structure for the inhibitory activity was reported to be the 3-hydroxy-β-ene group, absent in β-carotene. Taken together, it is rationally summarized that cancer preventive effect of green-yellow vegetables is not attributable only to β-carotene, which is merely one component of a diverse number of natural carotenoids.

\textit{Hydrolyzable (HTs) and condensed (CTs) tannins and (−)-epigallocatechin gallate (EGCG) (teas, edible plants)}

Hydrolyzable (HTs) and condensed (CTs) tannins are known to inhibit the formation of hydroperoxide (HPx). Perchellet et al. investigated the inhibitory effects of HTs and CTs on the TPA-induced biochemical and biological activities such as ODC activity, HPx production, and DNA synthesis in mouse skin.\textsuperscript{56} Topical application of ellagic acid (EA, 5 μmol) (Fig. 2) inhibited HPx formation and DNA synthesis by 100% and 31%, respectively, in mouse skin promoted with TPA (8.5 nmol).

Anti-tumor promoting activity of green tea extracts or their active constituents, catechins, have been most intensively studied in this decade. Especially (−)-epigallocatechin gallate (EGCG, Fig. 2), 60% of the total catechins in tea, is reported to inhibit tumor promotion by TPA or okadaic acid in mouse skin.\textsuperscript{57} Also EGCG has been shown to suppress carcinogenesis in a variety of organs such as the duodenum, lungs, stomach, colon, or liver.\textsuperscript{58} Catechins are known to have antioxidative effects, which undoubtedly contribute to the anti-tumor promoting activity of EGCG. It is of interest to note that the index of stomach cancer risk in those who take more than 10 cups of green tea per day is reduced by 0.5–0.3.\textsuperscript{59} Drinking green tea may be one of the most practical or safe strategies for cancer prevention at the public level, on account of its low toxicity.

\textit{Curcumín (termeric)}

Food additives are notable sources of anti-tumor promoters with respect to confirmation of their low toxicity. Curcumín (Fig. 2) is used as yellow pigment throughout the world in such foods as curry and mustard. Curcumín possesses potent anti-tumor promoting activity in mouse skin.\textsuperscript{59} Recently Tanaka et al. reported that oral administration of curcumín in the postinitiation (promotion) phase markedly reduced tumor numbers and incidences induced by 4-nitrosoquinoline 1-oxide (4-NQO) in rat tongue. This activity was significantly higher than that of β-carotene.\textsuperscript{60} Inhibitory mechanisms of curcumín in carcinogenesis are diverse and complicated.\textsuperscript{61} Curcumín at 10 μM suppresses TRE binding by c-Jun/AP-1 protein in mouse fibroblast NIH 3T3 cells, and while c-jun mRNA is selectively inhibited by curcumín, c-fos is not affected. Both enzymes, PKC and ODC, are also significantly inhibited by curcumín. Furthermore, the suppression of the arachidonic acid cascade by the inhibition of both cyclooxygenase and
lipoxygenase is an important function of curcumin in its anti-tumor promoting activity. Free radical scavenging effects of curcumin are also notable. Curcumin at 10 \( \mu \text{M} \) can inhibit TPA induced formation of 8-hydroxydeoxyguanosine and lipid peroxidation in NIH 3T3 cells. Osawa et al. recently reported the metabolic pathway of curcumin.\(^{6,21}\) In the colon, curcumin is converted to tetrahydrocurcumin, a more potent antioxidant than curcumin, then to dihydroferulic acid in the liver.

**Henriciacontane (Natto, Japanese soybean fermented food)**

The inhibition of gap junctional intercellular communication is one of the pleiotropic effects induced by a tumor promoter. Recently Takahashi et al. reported that hydroxycarbons were identified in Natto as potent inhibitors of the intercellular communication inhibition by TPA in BALB/3T3 cells.\(^{9,33}\) Natto is one of the representative, traditional fermented foods in Japan. Henriciacontane (C\(_{31}\)H\(_{44}\), Fig. 2) at a very low concentration of 0.65 ng ml was shown to recover the cell-cell communication inhibited by treatment with TPA (20 ng ml), lathocholic acid (tumor promoter in colon, 10 \( \mu \text{g ml} \)), or NaCl (in stomach, 0.72%).\(^{6,34}\) Though in vitro anti-tumor promoting activity of henriciacontane has not yet been confirmed, it may be a new type of anti-tumor promoter.

**\( \alpha \)-Acetoxychavicol acetate (ACA, great galangal)**

The rhizomes of *Languas galanga* (Zingiberaceae) are widely ingested as a ginger substitute in Thailand. Activity-guided separation of the methanol extract resulted in the isolation of (1'S)-\( \alpha \)-acetoxychavicol acetate (ACA, Fig. 2).\(^{6,41}\) This compound was originally identified as an anti-ulcer component of the plant.\(^{62,5}\) ACA showed marked inhibitory activity toward EBV activation (IC\(_{50}\) = 1.3 \( \mu \text{M} \)). In a two-stage carcinogenesis experiment using mouse skin with DMBA (0.19 \( \mu \text{mol} \)) and TPA (1.6 mmol), topical application of ACA even at 1.6 mmol reduced the number of tumors per mouse by 44%.\(^{6,61}\) In a 4-NQO induced rat tongue carcinogenesis experiment as well, rats fed on a diet containing ACA at 100 ppm in both initiation and promotion phases bare no tumors while 58% of the rats in the control group did bear tumors.\(^{6,71}\) In the tests, ACA significantly inhibited polyamine synthesis, BrdUrd-labeling indices, the number of AgNORs per nucleus, as well as incidences of precancerous lesions such as dysplasia and hyperplasia.\(^{6,71}\) The cancer chemopreventive potential of ACA in the rat tongue carcinogenesis experiment was markedly higher than those of \( \alpha \)-difluoromethylornithine (DFMO),\(^{6,80}\) \( \beta \)-carotene,\(^{6,80}\) or curcumin.\(^{6,80}\)

The inhibitory mechanism of ACA in anti-tumor promotion is not known in detail. However, it is worth noting that ACA is a dual inhibitor of two major superoxide (\( O_2^- \))-generating systems in cells. Noro et al. reported that ACA showed inhibitory activity of xanthine oxidase generating \( O_2^- \) through the conversion of xanthine into uric acid.\(^{6,80}\) In addition, we found that ACA at 10 \( \mu \text{M} \) almost completely inhibited \( O_2^- \) generation induced by TPA in differentiated HL-60 cells.\(^{6,80}\) Because ACA has no \( O_2^- \) scavenging effect up to 100 \( \mu \text{M} \) in the xanthine oxidase system, it may inhibit the \( O_2^- \) generating system including NAD(P)H oxidase in differentiated HL-60 cells.

**Conclusions and Perspectives**

Hundreds of synthetic or naturally occurring chemicals have thus far been reported as possible anti-tumor promoters. Their cancer preventive potentials were estimated using various methods ranging from convenient short-term assays to clinical trials. To apply promising candidates to human use, they should first of all be scrutinized for their cancer preventive efficacy, acute and chronic toxicities, the metabolic or degradation pathways, agent availability, cost, action mechanisms, and so forth. With respect to toxicity and availability, anti-tumor promoters from daily food items may be suitable for the general population. As mentioned above, in Japan, changes of diet style in the last few decades are supposed to have resulted in significant increases in lung, pancreatic, and colon cancer mortalities and a decrease in stomach cancer mortality. Some of the developing countries are showing similar trends with Japan in cancer mortality and diet style in recent years. Diet intervention is a convenient way being accompanied with no severe side effects for most disease prevention, not only for cancer. At present, there is a consensus that cancer in humans is, at least in part, controlled by food ingredients or habits in a positive or negative manner. On the other hand, it can not be said often enough that cessation of cigarette smoking is a markedly effective strategy for cancer prevention. People in Asian countries have traditional and diverse types of food. It is important to note that in Asia there is a diverse variety of edible plants whose pharmaceutical effects have traditionally been confirmed. Further identification of new types of active constituents with anti-tumor promoting potentials together with their use for cancer prevention studies including clinical trials may become a breakthrough for the reduction of cancer mortality in the future. To meet this endpoint, prompt establishment of co-operative research systems, consisting of various scientific fields, for cancer prevention are necessary.

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