Review

Cancer Prevention by Natural Compounds

Hiroyuki Tsuda1,2*, Yutaka Ohshima1, Hiroshi Nomoto2, Ken-ichi Fujita3, Eiji Matsuda3, Masaaki Iigo2, Nobuo Takasuka2,3 and Malcolm A. Moore1,2

1Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
2Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute, Tokyo, Japan

Summary: Increasing attention is being paid to the possibility of applying cancer chemopreventive agents for individuals at high risk of neoplastic development. For this purpose by natural compounds have practical advantages with regard to availability, suitability for oral application, regulatory approval and mechanisms of action. Candidate substances such as phytochemicals present in foods and their derivatives have been identified by a combination of epidemiological and experimental studies. Plant constituents include vitamin derivatives, phenolic and flavonoid agents, organic sulfur compounds, isothiocyanates, curcumin, fatty acids and d-limonene. Examples of compounds from animals are unsaturated fatty acids and lactoferrin. Recent studies have indicated that mechanisms underlying chemopreventive potential may be combinations of anti-oxidant, anti-inflammatory, immune-enhancing, and anti-hormone effects, with modification of drug-metabolizing enzymes, influence on the cell cycle and cell differentiation, induction of apoptosis and suppression of proliferation and angiogenesis playing roles in the initiation and secondary modification stages of neoplastic development. Accordingly, natural agents are advantageous for application to humans because of their combined mild mechanism. Here we review naturally occurring compounds useful for cancer chemoprevention based on in vivo studies with reference to their structures, sources and mechanisms of action.

Key words: cancer chemoprevention; natural agents; combined mechanisms

Introduction

The purpose of cancer prevention is to cause delay in onset of cancer, progression from precancerous lesion or recurrence after treatment, as an alternative to treatment of cancer cases after clinical symptoms have appeared. Therefore, ultimate goal of cancer prevention is preferably to live without cancer or with cancer without suffering from symptoms until the natural termination of life (Fig. 1).1,2

Cancer can be prevented by either avoiding life style-related risk factors such as the smoking habit, a Western diet, physical inactivity and carcinogen containing foods, or alternatively by increasing exposure to beneficial influences, including intake of chemopreventive agents.3) The latter may be particularly practical because they can be taken as supplements or by modulation of the current diet status. One can envisage subjects for cancer chemoprevention falling into two groups, one at high risk of cancer because of the presence of precancerous lesions or predisposing conditions, and the other being the apparently healthy general population (Fig. 2).

Given difficulties in ensuring that any compound will not have any toxicity besides proven efficacy and convenience for use in the long term, the practical aim of chemoprevention, for the present, should best be focused on high risk-groups. Furthermore, there are advantages with application of natural compounds so...
Fig. 1. "Natural-End Cancer" indicates either living without cancer or living with cancer but without suffering from cancer related symptoms until the natural termination of life comes.

Fig. 2. The population are divided into two groups, one at high risk with predisposing conditions, and the other being the apparently healthy general population. Use of chemopreventive measures by asymptomatic persons requires highly reliable toxicology data.

Fig. 3. Stages of cancer chemoprevention. In the 'initiation stage', a cell is 'hit' by carcinogens with resultant DNA injury. The subsequent promotion and progression (post-initiation) phase is characterized by clonal cell proliferation. Chemopreventive compounds act to work in initiation stage by blocking carcinogens to "hit" DNA; those which act on post-initiation stage suppress the growth of preneoplastic cells.

Goal of Cancer Chemoprevention

Clearly, if we are to be successful in cancer chemoprevention we need to target specific processes which are known to be involved in the multistage development of cancers. In the prevailing paradigm, three stages can be recognized, 'initiation', 'modification' (promotion or inhibition) and 'progression', as illustrated graphically in Fig. 3. In 'initiation', either of single cell or multiple individual cells within a tissue, giving a field effect, occurs through exposure to a carcinogen. This is enhanced by proliferation, because of fixation of DNA damage so that it becomes replicable as a mutation. The subsequent modulation phase is characterized by clonal expansion of initiated cell populations, these eventually becoming foci or nodules from which malignancies are thought most likely to arise.

Exposure to chemical carcinogens—whether by topical, subcutaneous, intragastric, intraperitoneal or any other route—results in absorption, transport, entry into cells and metabolism by the phase I and phase II enzymes. This results in activation and binding to macromolecules, or inactivation and excretion of detoxified products, whether in man or experimental animals. Thus the products of phase I P450 metabolism may be more electrophilic than the parent compounds and therefore will interact with all constituents of cells, both within the cytoplasm and within the nuclei. However, with few exceptions, the vast majority of attention has been paid to interactions with the heritable material locked up in the DNA. Adduct formation has been described, not only for typical direct or indirect-acting chemical carcinogens, but also hormones, irradiation, UV light and physical agents or procedures such as in situ freezing, known to be capable of initiating carcinogenesis. Phase II enzymes, like sulfotransferase, quinine reductase and the glutathione S-transferases, protect cells from such insults by generating water-soluble conjugated intermediates which are more readily
It is generally accepted that, from oxidative processes alone, production of adducts by endogenous sources is exceedingly high and evolution has demanded an ability of cells to remove or repair damage to their DNA. Indeed, it has been shown that a number of repair systems have been generated and this protective influence clearly needs to be taken into consideration. Experimentally it has been shown with transgenic animals that increased repair activity correlates with decreased sensitivity to carcinogens.\textsuperscript{11}

However, if cell division occurs before repair can take place then any transmitted changes in one DNA strand will be replicated and no longer recognizable as an error. This is the basis for postulated proliferation requirement for initiation to occur. The observed positive relationship between increased cell division and elevated sensitivity to initiation is perhaps best exemplified by performance of partial hepatectomy prior to application of a carcinogen. Thus the initiation phase is dependent on carcinogen activation, adduct formation and cell proliferation.\textsuperscript{12}

For the second modulatory phase, growth is essential, and this is dependent on the balance between cell division and apoptosis. For the final progression to malignancy, further genetic changes are considered to be involved, due to a combination of exogenous and endogenous factors. For assessment of cancer chemopreventive agents we thus need to look at effects of different processes and therefore types of influence. Thus for the assessment of chemopreventive effects, modulation of carcinogenesis is conceptually divided into 3 stages, modification of initiation by chemical and physical carcinogens, suppression of cell growth and delay in progression (Fig. 3).

**Processes and Potential for Preventive Action**

Chemoprevention aims to utilize a part of physiological activities of compounds from plants/animals in their natural environment (Fig. 4). Representative activities relevant to prevention of carcinogenesis are illustrated in Fig. 5. The following are factors with relevance to prevention of carcinogenesis in initiation-promotion-progression stages.

a) **Oxidative stress and anti-oxidant action**: Reactive oxygen and/or nitrogen oxide species-induced stress (RONOSS) and its downstream events are clearly important for carcinogenesis. RONOSS can be induced by exposure of animals and humans to a variety of carcinogenic xenobiotics and microorganisms\textsuperscript{13,14} and the various cellular alterations induced by RONOSS play crucial roles in carcinogenesis.\textsuperscript{15-17} DNA adduct products, such as 8-hydroxydeoxyguanine (8-OHdG), 2-hydroxyadenine, 8-hydroxyadenine, 5-hydroxycytosine, and 8-nitroadenine, are pre-mutagenic and induce specific types of gene mutations. Furthermore, the presence of DNA damage can affect gene expression and cause aberrant gene regulation and abnormal signaling. Thus RONOSS can contribute to carcinogenesis as “preneoplastic” lesions by virtue of both genetic and epigenetic mechanisms and antioxidants would therefore be expected to inhibit, both because of alteration in relevant enzyme profiles and quenching.\textsuperscript{15,18}

b) **Inflammation and anti-inflammatory action**: Proliferation due to infectious agents such as hepatitis C virus and Helicobacter pylori is a major factor in development of cancer in humans.\textsuperscript{19,20} Indeed, inflammatory states and associated chronically elevated levels of proliferation appear to predispose to cancer development in any site of the body and the metabolic pathways that are switched on under such conditions are natural targets for chemoprevention.\textsuperscript{21,22} Therefore a great
deal of interest has been concentrated on non-steroidal anti-inflammatory drugs (NSAID'S) which act by inhibition of prostaglandin endoperoxidase/cyclooxygenase (COX) and other factors which may have an impact.\textsuperscript{21–23} It should be remembered that under certain conditions, the proliferation stimulus associated with inflammation may preferentially act on tumor precursors, as stressed earlier.\textsuperscript{21–23}

c) Hormone-dependent growth and anti-hormone action: Enhanced cell proliferation by the hormonal milieu, with elevated cell turnover result from receptor-based signaling, is risk factor of cancer development.\textsuperscript{5,24–27} For example, estrogen is a growth factor in the absence of progesterone\textsuperscript{28} and is a major factor for breast and ovarian cancer.\textsuperscript{29} High levels of testosterone and low levels of sex-hormone binding globulin (SHBG) are similarly considered to positively linked to prostate cancer development.\textsuperscript{30} Promotion of prostate carcinogenesis by testosterone has been shown in genetically susceptible rats.\textsuperscript{31} Since natural compounds with obvious anti-hormone effects are not well known except isoflavone and related compounds, cancer prevention by these compounds require further experimentation.

d) Immune activity and modulation: Since the immune system can influence on growth either via effects on the inflammation status or by causing apoptosis through the function of various cytokine species, it may naturally be of importance for early stages in neoplasia, in addition to its effects on frank malignancies.\textsuperscript{32–34}

e) Xenobiotic metabolism and enzyme induction: Most carcinogens are metabolized into DNA-attacking moieties by phase I cytochrome P450 (CYP) enzymes. Phase II enzymes, NAD(P)H (quinone acceptor):oxidoreductase (NQO1), glutathione S-transferases (GST) and UDP-glucuronosyltransferases (UGT), in contrast, are responsible for detoxification and excretion of activated products. Thus the balance between these plays important roles in preventing initiation of neoplasia because of its role in formation of adducts and mutations and agents inducing drug-metabolizing enzymes are therefore obvious candidates for cancer chemoprevention. Metabolism may lead to an increased rate of chemical detoxification, but in other cases it causes chemical activation to toxic products.\textsuperscript{35} As indicated in a recent study, methoxsalen from the \textit{Ammi majus} plant, a potent inhibitor of CYP 2A6, which is an activating enzyme of tobacco specific nitrosamines, clearly reduced mouse lung tumor development.\textsuperscript{36} A great deal is known about individual CYP influence\textsuperscript{29,30} and specific phase II species.\textsuperscript{37}

f) Molecular association with carcinogen: Chlorophyllin was known to cause molecular association with heterocyclic amines to form larger molecule to block passage the cell membrane, thus causing reduction of heterocyclic amines-DNA adducts formation. These compounds are called an "interceptor molecule".\textsuperscript{40–42} These compounds are summarized in Table 1. Chemopreventive Agents by their Structure Group

a) Carotenoids: Carotenoid group include beta-carotene, alpha-carotene, lycopene, lutein, astaxanthin, cryptoxanthin and zeaxanthin. The majority of carotenoids are derived from a 40-carbon polyene chain, which could be considered the backbone of the molecule (Fig. 6). Carotenoids are responsible for colors of plants, fruits, flowers and fish such as the orange of carrots and citrus fruits, the reds of peppers and tomatoes, and the pink of salmon. Carotenoids also have effects on immune functions.\textsuperscript{43} Carotenoids were shown to be effective in reducing tumor development in organs such as lung, liver, colon and skin in experimental animals.\textsuperscript{44} The hydrocarban carotenoids are known as carotenes, while oxygenated derivatives of these hydrocarbons are known as xanthophylls. They possess anti-oxidant action as one of the mechanism for their cancer preventive effects.\textsuperscript{45,46} Vitamin A (Ascorbic acid); Fruit sources are citrus fruits, fresh strawberries, cantaloupe, pineapples and guava. Vegetable sources are broccoli, Brussels sprouts, tomatoes, spinach, kale, green peppers, cabbage and turnips. Vitamin C acts as a scavenger of oxygen radicals and also as competitive inhibitor of nitrosamine formation from nitrate and amines \textit{in vivo}.\textsuperscript{47,48} Many epidemiological studies indicate vitamin C intake is clearly related with cancer
Table 1. Summary of natural chemopreventive agents in different category of function

a. Anti-oxidant effects
   Carotenoids, Vitamin C, E, Sulfur compounds, Anthocyanin, Catechins, Ellagic acid, Flavonoids, Phenolic compounds and Polyphenols, Conjugated fatty acids, Curcumin

b. Anti-inflammatory effects
   Cox1/2 inhibition
   Carotenoids, Vitamin C, E, Sulfur compounds, Anthocyanin, Catechins Ellagic acid, Flavonoids, Phenolic compounds, Polyphenols, Conjugated fatty acids, Curcumin
   Arachidonic acid cascade modification
   n-3 PUFAs, Curcumin

c. Immune enhancing effects
   NK, T cell activity
   Carotenoids, Flavonoids, Lactoferrin

d. Hormone activity modulation
   Estrogenic: Genistein

e. Xenobiotic metabolism and enzyme induction
   Phase I—CYP suppression
   Diallyl sulfide, Catechins, CLA, Curcumin, Lactoferrin, Methoxsalen
   Phase II—GST, Quinone reductase induction
   Azapetene, Phenolic compounds, Curcumin, d-Limonene
   Carotenoids, Sulfur compounds, Catechins, Flavonoids, Indole-3-carbinol

f. Apoptosis induction
   d-Limonene, Sulfur compounds, EGCG, CLA, lactoferrin

g. Anti-angiogenesis
   CLA, Lactoferrin

h. Cell differentiation induction
   Vitamin A, Retinoids

i. Interaction to cell cycle
   Genistein (G0-G1 arrest)

j. Modulation of oncogene signal transduction
   d-Limonene, Dehydroepiandrosterone (Ras cascade inhibition)

k. Inhibition of intestinal absorption (Molecular association with HCA)
   Chlorophyll, Chlorophyllin

l. Alteration in bacterial microflora in the intestine
   Curcumin

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**Fig. 6.** The structure of beta-carotene, alpha-carotene and lycopene.
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Fig. 7. The structure of vitamins A, C and E.

Fig. 8. The structure of polyunsaturated fatty acids, docosahexaenoic acid (DHA), α-linolenic acid, and trans-9, cis-11 conjugated linoleic acid. Conjugated linoleic acid has many isoforms depending on the positions of the double bond and whether they are cis- or trans-forms.

incidence.57,58) 2) Vitamin E (alpha-tocopherol); Vitamin E is a fat-soluble vitamin that exists in different forms. Vegetable oils, nuts, and green vegetables are the main dietary sources of vitamin E. Alpha-tocopherol is the most active form of vitamin E in humans, and is a powerful anti-oxidant with multiple roles.79) As with vitamin C, vitamin E also blocks the formation of nitrosamines in the stomach from nitrates ingested with the diet.80) In addition to anti-oxidant properties, it shows other functions such as anti-inflammatory action.10) 3) Vitamin A; The source of vitamin A is from carotenoids (precursors of vitamin A) in vegetables, marine foods and from retinyl esters, mostly retinyl palmitate, and from foods of animal origin. Carotenoids are metabolized to retinol or retinoic acid to act as vitamin A. Although not clearly shown in human studies, some data indicate that vitamin A inhibited mammary carcinogenesis in experimental animals. Possible mechanism includes modulation of cell proliferation, differentiation, communication and adhesion.81,82)

c) Polyunsaturated fatty acids (PUFAs) (Fig. 8): Unsaturated fatty acids are usually found in the form of vegetable-derived liquid oils, while saturated fatty acids are usually found in solid animal fat (such as butter). Polyunsaturated fatty acids (PUFAs) are long-chain fatty acids containing two or more double bonds. Docosahexaenoic acid (DHA), an omega-3 (n-3) fatty acid with six cis double bonds and 22 carbons (22:6n-3) found in fish oil. Alpha-linolenic acid (ALA) is an essential fatty acid highly concentrated in certain plant oils such as flaxseed oil and to a lesser extent, canola, soy, perilla, and walnut oils. ALA is converted to eicosapentaenoic (EPA) and DHA after ingestion. Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers showed a good correlation.84,85) DHA is well known to inhibit colon and other organ carcinogenesis in rats.86–88) Perilla oil inhibited 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumor induction in rats.89) Possible mechanism includes anti-inflammatory action by inhibiting prostaglandin biosynthesis from linoleic acid.90) 4) Conjugated linoleic acid (CLA) (Fig. 8): CLA is found naturally in beef, cheese and whole milk. Two CLA isomers (cis-9, trans-11 CLA and trans-10, cis-12 CLA) have been shown to inhibit mammary and colon carcinogenesis in animals. The mechanisms are anti-angiogenesis, apoptosis induction to cancer cells and alteration of fat metabolism.90–92) CLA might inhibit
angiogenesis in vivo, a hypothesis that was subsequently confirmed. The anti-angiogenic effect is mediated, in part, through a CLA-induced decrease in serum and mammary gland VEGF (vascular endothelial growth factor) and in inflammatory cytokines. The data suggest that CLA may be an excellent candidate for prevention of breast cancer. Recent studies showed CLA also have similar inhibitory effect on development of colon preneoplastic aberrant crypt foci.

e) Organosulfur compounds (Fig. 9): Organosulfur compounds such as diallyl sulfide, N-acetylcysteine and S-allyl cysteine present in garlic and onion oil have been shown to inhibit colon, forestomach, esophagus, mammary gland, and lung carcinogenesis of experimental animals. These compounds were known to induce phase II enzymes such as glutathione S-transferase (GST), NAD(P)H-dependent quinone reductase, and UDP-glucuronosyl transferase, in the liver and colonic mucosa. However, they also were found to enhance liver carcinogenesis by causing increase in polyamine biosynthesis. Induction of apoptosis may be the major contributing factor for anti-tumorigenic properties of diallyl sulfide. Anti-proliferative activity has been described in several tumor cell lines, which is possibly mediated by induction of apoptosis and alterations of the cell cycle.

f) Phenolic compounds:

Phenolic compounds include compounds with single or two benzene rings (ferulic acid, auraptene), flavonoid structure and polyphenols. Flavonoids are a ubiquitous group of polyphenolic substances present in most plants, concentrated in seeds, fruit skin or peel, bark, and flowers. The structural components common to these molecules include two benzene rings on either side of a 3-carbon ring (Fig. 10). A great number of plant medicines contain flavonoids, which have been reported as having anti-bacterial, anti-inflammatory, anti-mutagenic and anti-carcinogenic effects. Multiple combinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures form the various classes of flavonoids such as flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, anthocyanidin and isoflavones. Flavonoids have been shown to be potent anti-oxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals.

1) Auraptene (Fig. 11): Auraptene, a citrus polyphenol, showed inhibition of colon, oral cavity and esophagus carcinogenesis. Increased activities of Phase II enzymes and suppression of cell proliferation and lipid peroxidation in the colonic mucosa may explain the inhibitory effects.

2) Ferulic acid (Fig. 11): Ferulic acid, known to be contained in rice germ and coffee bean, was shown to inhibit colon and tongue carcinogenesis. In addition to their anti-oxidant activity, induction of GST and quinone reductase (QR) activities in liver and colonic mucosa was suggested as the mechanism of inhibition of carcinogenesis.

3) Silymarin (Fig. 12): Silymarin, flavonoid complex containing silybin, silydianin, and silychristin derived from the milk thistle plant was shown to inhibit colon, tongue and bladder carcinogenesis, mainly by its strong anti-oxidant effects.

4) Anthocyanin (Fig. 12): Anthocyanin
pigments are responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers. Proanthocyanin from grape seed showed inhibition of rat mammary and colon and mouse skin carcinogenesis.\textsuperscript{19,20} It also reduced tumor development by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a postulated human carcinogen, and nitrosamine-induced lung carcinogenesis.\textsuperscript{49,50} Its action may be related to anti-oxidant, anti-inflammatory, anti-peroxidative and immune-enhancing effects.\textsuperscript{96,97}

5) Soy isoflavones (Fig. 12); Genistein and daidzein, isoflavone derivatives related to coumarin, are found in soy products. They have estrogenic and anti-oxidant activities.\textsuperscript{99} Incidences of breast and prostate cancer are relatively low in China and Japan where relatively high amounts of soy products are consumed and this has lead to a suggestion of protective effects.\textsuperscript{96-102} In fact there is now a very large body of evidence, from both epidemiological and experimental animal studies, pointing to inhibitory potential on cancer development in many organ sites.\textsuperscript{103-105} The overwhelming conclusion from both in vivo and in vitro data is that soy products or constituents such as genistein or daidzein can act as cancer preventive agents in the mammary gland,\textsuperscript{103-105} and possibly in endometrium, ovaries, prostate and stomach, by both hormone-related and non-hormone-related mechanisms.\textsuperscript{106,107} There are also data supporting protective effects on the colon, liver, thyroid, skin and lung tumor development, with very few reports of adverse toxic and carcinogenesis promotion effects.\textsuperscript{102,106} Phytoestrogens may act via effects on vitamin D metabolism.\textsuperscript{109} Reduced level of male sex hormone, dehydroepiandrosterone, by soy isoflavones as a result of inhibition of 5 alpha-reductase activity\textsuperscript{110} may act against tumor development.\textsuperscript{102,111} Genistein also causes cell cycle G2-M arrest.\textsuperscript{112}

6) Green tea polyphenols (Fig. 13); Green tea polyphenols, classified as members of the flavonoid group, including epigallocatechin, epicatechin gallate (ECG) and epigallocatechin-3-gallate (EGCG) have been shown to inhibit carcinogenesis in many organs.\textsuperscript{41,108,113-116} Anti-oxidant activity, apoptotic potentials to carcinogen-initiated and interaction to cell cycle are the mechanism for the inhibitory effects. EGCG inhibits AP-1 activity in the epidermis of a transgenic mouse model.\textsuperscript{113,114}
g) Turmeric and curcumin (Fig. 14): Turmeric is one of the key ingredients in many curries, giving them color and flavor. Curcumin derived from Turmeric (Curcuma longa) gives curry its yellow color and having both strong anti-oxidant and anti-inflammatory properties. Curcumin inhibited chemically induced skin, forestomach, and colon carcinogenesis in post-initiation stage in animals. Curcumin inhibited chemically induced skin, forestomach, and colon carcinogenesis in post-initiation stage in animals.

h) d-Limonene (Fig. 14): d-Limonene is a major constituent of several citrus oils (orange, lemon, mandarin, lime, and grapefruit) and many other plant species. It is used as a component of flavorings and fragrances, as a chemical intermediate. d-Limonene inhibited rat mammary and other tumor development. One of the explanation is inhibition of farnesyl protein transferase and p21ras association to the cell membrane.

i) Chlorophyll/Chlorophyllin (Fig. 15): Chlorophyll is the green pigment found in higher plants, as well as algae. Chlorophyllin is a semi-synthetic sodium/copper derivative of chlorophyll. In contrast to chlorophyll, chlorophyllin is water-soluble. Chlorophyllin is a semi-synthetic form of the natural chlorophyll that makes plants green and is a good blocking agent against free radicals generated through metabolic process of heterocyclic amines and aflatoxin, resulting in modulation of apoptosis, cell proliferation, and beta-catenin/Tcf signaling. They also form molecular association with heterocyclic amines to block absorption from the intestinal epithelium causing reduction of heterocyclic amines-DNA adducts formation. Chlorophyllin administration caused reduced level of aflatoxin-DNA adducts in individuals at high risk of liver cancer. Chlorophyllin also exhibited anti-promotion effect on DMBA-TPA-induced mouse skin carcinogenesis.

j) Chitin and chitosan (Fig. 16): Chitin and chitosan are polysaccharide polymers containing more than 5,000 acetylglucosamine and glucosamine units, respectively, and their molecular weights are over one million Daltons. Chitin is found in fungi, arthropods and marine invertebrates. Commercially, chitin is derived from the exoskeletons of crustaceans (shrimp, crab and other shellfish). Chitosan is obtained from chitin by a
deacetylation process. Chitin, the polysaccharide polymer from which chitosan is derived, is a cellulose-like polymer consisting mainly of unbranched chains of N-acetyl-D-glucosamine. Deacetylated chitin, or chitosan, is comprised of chains of D-glucosamine. When ingested, chitosan can be considered a dietary fiber. Chitin and chitosan inhibit colon carcinogenesis.124,128)

k) Isothiocyanates (Fig. 17): Isothiocyanates present in cruciferous plants such as Brassica vegetables were known to inhibit cytochrome P450 (CYP) isoforms. They induced increased expression of GST, NADPH: quinone oxidoreductase, aldo-keto reductase and gamma-glutamylcysteine synthetase.129) These responses were coordinated at the transcription level by nuclear factor-erythroid 2 p45-related factor-2 acting through the antioxidant/electrophile enhancer response element and stimulated by the mitogen-activated protein kinase extracellular signal-regulated kinase kinase-1 and c-Jun N-terminal kinase-1 (JNK1) pathway. Isothiocyanates also induced apoptosis of pre-cancerous cells and tumor cells activated by caspase-8 and potentiated by JNK1.118,130–132)

l) Dehydroepiandrosterone (Fig. 18): The adrenal hormone dehydroepiandrosterone (DHEA) was shown to inhibit colon, prostate, thyroid and several other organ carcinogenesis in animals. 133,134) It acts as an inhibitor of the mevalonate pathway as well as affecting the phosphate shunt responsible for providing ribose units for DNA synthesis.135,136)

m) Lactoferrin and its digested fragments (Fig. 19): Lactoferrin is a single chain glycoprotein folded into two globular units, each of which can bind one ferric ion (Fe3+) together with a bicarbonate ion. In the “natural state”, bovine lactoferrin (bLF) is only partly saturated with iron (15–20%) and has a salmon pink color. In breast milk, the lactoferrin found is essentially apo-lactoferrin.137) The bLF has a molecular weight of about 80 kDa. The complete amino acid sequence of bovine lactoferrin has been determined to contain 689 amino acids.

bLF and its digested fragments have been found to remarkably inhibit colon, esophagus, lung, and bladder carcinogenesis in rats when administered orally in the post-initiation stage.138–140) It also inhibits colon and liver carcinogenesis when administered concurrently with carcinogenic heterocyclic amines (Table 1), possibly by suppression of phase I enzyme, cytochrome P450 1A2 (CYP1A2) activity.141) Anti-metastatic effects were...
Table 2. Summary of bLF cancer prevention

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Carcinogen</th>
<th>Time of application</th>
<th>Organ</th>
<th>End-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>AOM</td>
<td>○</td>
<td>Colon</td>
<td>Adc</td>
</tr>
<tr>
<td></td>
<td>DHPN</td>
<td>○</td>
<td>Lung</td>
<td>Adc</td>
</tr>
<tr>
<td></td>
<td>4-NOQ</td>
<td>○</td>
<td>Esophagus</td>
<td>Pap + SCC</td>
</tr>
<tr>
<td></td>
<td>BHBN</td>
<td>○ ○○</td>
<td>Tongue</td>
<td>SCC</td>
</tr>
<tr>
<td></td>
<td>PhIP</td>
<td>○</td>
<td>Bladder</td>
<td>Pap + TCC</td>
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<tr>
<td></td>
<td>DEN-MelQx</td>
<td>○ ○○</td>
<td>Prostate</td>
<td>PIN</td>
</tr>
<tr>
<td>Mouse</td>
<td>ApcMin</td>
<td>○ ○○</td>
<td>Colon</td>
<td>ACF</td>
</tr>
</tbody>
</table>

○. Inhibition of carcinogenesis.

a, 2.0% and 0.2% dose = 90 and 9 g/60 kg B.W., respectively

Ad, Adenoma; Adc, Adenocarcinoma.

Pap, Papilloma; SCC, Squamous cell carcinoma; TCC, Transitional cell carcinoma GST-P +, Glutathione S-transferase positive foci; ACF, Aberrant crypt foci.

Fig. 20. Mechanism of lactoferrin mediated by lactoferrin. Lactoferrin satisfies 5 out of the 10 mechanistic categories of chemopreventive action as shown in Fig. 5.

Moreover detected when bLF was given intragastrically to mice bearing metastatic Colon carcinoma 26 cells (Co 26Lu), with apparent enhancing influence on local and systemic immunity. Marked increase in the number of cytotoxic T and NK cells in the mucosal layer of the small intestine and peripheral blood cells was thus found, this in turn enhancing the production of interleukin 18 (IL-18) and caspase-1 in the epithelial cells of the intestine, with possible consequent induction of interferon (IFN) gamma positive cells. bLF also inhibited angiogenesis stimulated by tumor cells in vivo and in vitro. Induction of apoptosis in the colon epithelial and also leukemic cells possibly related with increase in expression of Fas and apoptosis linked caspase was also demonstrated (Table 2). Furthermore, the recombinant bovine and human lactoferrin was shown to inhibit growth of human cell carcinomas transplanted in mice. As summarized in Fig. 20, inhibitory effect of bLF is mediated by mixed activities, which satisfy a half, 5 (see shaded letters) out of 10, of important functions of chemoprevention.

Current Situation for Chemopreventive Agents

An overview of the findings for a variety of different types of agents, where they inhibit and the mechanisms is given in Table 1. The great interest in developing chemical agents that might have appreciation as chemopreventive agents has driven a large body of research and a great deal has been published. However, in contrast to the large number of compounds for which efficacy has been proven in experimental models, relatively few clinical trials have been performed and none has, so far, proven a net benefit conferred by natural compounds (Tables 6 and 7).

Previous clinical trials have shown the effectiveness of the following: polyprenoic acid (acyclic retinoid) for hepatocellular carcinoma; tamoxifen for breast cancer; retinoic acids for head and neck tumor; and aspirin, a COX-2 inhibitor, for colorectal cancer. Despite the advantageous effects of some of these agents, their toxic...
Table 4. Summary of action, target organ, stages of application, tested animals and results of clinical trial (1)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Action</th>
<th>Organ</th>
<th>stage</th>
<th>Animal</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene</td>
<td>Anti-oxidant</td>
<td>Oral cavity, Liver, Skin</td>
<td>I, P</td>
<td>R, M</td>
<td>×</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Immune enhancing</td>
<td>Stomach, Colon, Mammary gl.</td>
<td>I, P</td>
<td>R</td>
<td>×</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Cell differentiation</td>
<td>Colon, Liver, Prostate, Mammary gl.</td>
<td>I, P</td>
<td>R, M</td>
<td>×</td>
</tr>
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a. I, Initiation stage; P, Post Initiation stage; C, Concurrent administration
b. R, Rat; M, Mouse
c. ×, Limited – Inadequate; –, Lack of evidence of preventive effect
d. gl., gland

Effects must also be of concern at the same time. For example, in a chemoprevention trial of lung cancer, beta-carotene was unexpectedly found to increase the risk of lung cancer among high-risk groups. It is also noted that large-scale clinical trials demand large research grants, which may not be affordable in Japan and other countries of Asia. Chemoprevention is still an emerging field of oncology where researchers in both basic and clinical sciences face great challenges. According to publication from International Agency for Research on Cancer (IARC) and the authors survey, number of compounds shown to be effective in human clinical trial are extremely limited as compared to those shown to be effective in animal studies (Figs. 6 and 7). Based on preclinical results, selected agents have been and are now being evaluated in phase I, II and III clinical interventions for various cancers. Development of valid surrogate endpoints is essential to accelerate progress in cancer prevention clinical and intervention studies.

Micronutrients currently being examined in National Cancer Institute (NCI)-sponsored phase I, II, or III chemoprevention trials for prostate, breast, and colon cancers include isoflavones, lycopene, selenized yeast, selenomethionine, selenium, vitamin E, perillyl alcohol, folic acid, vitamin D, calcium, and curcumin. The response to micronutrients may vary not only in magnitude but also in direction. This variation and response likely depend on individual genetic polymorphisms and/or interactions among dietary components that influence absorption, metabolism, or site of action. Research priorities include investigation of possible molecular targets for micronutrients and whether genetic and epigenetic events dictate direction and magnitude of the response.

It should be borne in mind that health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The response to micronutrients may vary not only in magnitude but also in direction. This variation and response likely depend on individual genetic polymorphisms and/or interactions among dietary components that influence absorption, metabolism, or site of action. Research priorities include investigation of possible molecular targets for micronutrients and whether genetic and epigenetic events dictate direction and magnitude of the response.

Acknowledgements: Studies conducted by the author were supported by a Grant-in Aid for the Second Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare, a Grant-in Aid from the Ministry of Health, Labour and Welfare and a Grant-in Aid from the Ministry of Education, Science, Sports, Culture and Technology, Japan. During the drafting of this paper Malcolm A Moore was the recipient of a Foreign Research Fellowship from the Foundation for Promotion of Cancer.
Table 5. Summary of action, target organ, stages of application, tested animals and results of clinical trial (2)

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a. I, Initiation stage; P, Post initiation stage; C, Concurrent administration
b. R, Rat; M, Mouse
c. ×, Limited – Inadequate; −, Lack of evidence of preventive effect
d. gl., gland

Table 6. Evaluation of chemopreventive agents tested in animals
(from IARC and authors)

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Table 7. Evaluation of chemopreventive agents tested in humans
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Research Program for Invitation of Foreign Researchers. The author would thank Dr. Mitsuaki Maeda of Kitazato University for his kind advice regarding the chemical structure of compounds.

References

Cancer Prevention by Natural Compounds


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