Carcinogenicity of Food Mutagens

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Cancer cells are produced by the accumulation of genetic alterations in somatic cells. Those genetic alterations are produced by xenobiotics, which enter the human body from the environment, and by autobiotics, which are produced in the human body. Food contains many different types of xenobiocut mutagens/carcinogens and tumor promoters. Food can influence the formation of autobiotic mutagens/carcinogens and give rise to tumor-promoting conditions. In spite of this, it can also contain many antimutagenic, anticarcinogenic, and antitumor-promoting substances. Carcinogenic risk and anticarcinogenic efficacy are hard to express quantitatively; however, holistic approaches that are designed to improve lifestyle are realistic for cancer prevention. — Environ Health Perspect 104(Suppl 3):429-433 (1996)

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Cancer as a Genetic Disease

Cancer is now generally regarded as a disease caused by accumulated alterations of genes in somatic cells. The involved genes are related to the classical cancer phenotype in that they are responsible for control of the cell cycle, growth, differentiation, cellular and structural atypisms, and ability of cells to infiltrate into surrounding tissues or metastasize to distant sites. Genes that regulate scheduled cell death have also been implicated.

Those gene alterations are produced by genotoxic substances, either environmental xenobiotics entering the body or autobiotics generated inside the body, or even through an enhanced frequency of cell proliferation that increases the chance of incorrect replication of genetic materials.

Cells acquire a growth advantage because of initial genetic alterations that accelerate passage through the cell cycle; in the course of carcinogenesis, during the multistep conversion of a normal cell to a malignant one, mutations accumulate successively. The thus formed final malignant cell eventually dominates. In other words, cancer masses are due to successive monoclonal growths, in each case originally derived from a single cell.

Exogenous Food Mutagens/Carcinogens

Food mutagens are major xenobiotic genotoxic substances. They include, as typical examples, aflatoxin B1 from Aspergillus flavus, nitrosamines in fermented foods, polycyclic aromatic hydrocarbons in heated foods, heterocyclic amines in heated meat and fish, and pyrrolizidine alkaloids from plants (1-3). Most of them are metabolically oxidized by cytochrome P450s and then esterified to ultimately reactive forms to produce DNA adducts through electrophile and nucleophile reactions as documented by Miller and Miller (4).

Autobiotic Mutagens/Carcinogens

Endogenous genotoxic substances have also attracted much attention. They are not directly food related but can act in collaboration with exogenous food mutagens to cause accumulation of genetic alterations in cells.

Typical examples are active oxygen molecular species which can inflict various types of oxidative damage to DNA, a typical example being the 8-hydroxyguanine that was first discovered by Kasai and Nishimura (5). Nitrates are considered as a precursor for the formation of nitroso compounds under acidic conditions, and food additives and vegetables are known to be sources of nitrite/nitrate (6). It is well documented that N-nitrosamines are formed from the reaction of nitrite with secondary amines under acidic conditions. Many phenol and indole derivatives present in our environment have also been demonstrated to give rise to mutagenic and carcinogenic nitrosated compounds (7). Products from phenolic compounds and nitrite are diazo derivatives, and those from indole compounds and nitrite are N-1 and/or C-3 nitrosated products. However, recent findings indicate that nitric oxide synthase, both constitutively expressed in organs such as the liver and inducible in macrophages, produces nitric oxide from L-arginine. This nitric oxide is involved in endogenous formation of nitroso compounds (8,9). Nitric oxide is also a potent deaminating agent for 5-methylcytosine residues of DNA, converting them to thymine residues (10). Thus, CG pairs at CpG sites change to TA pairs.

Inflammation most likely contributes to the induction of nitric oxide synthase, providing one reason for the fact that chronic gastritis, ulcerative colitis, chronic hepatitis, and chronic pancreatitis are all carcinogenesis-prone conditions. Furthermore, any chronic mechanical stimuli causing chronic tissue damage, such as inappropriate tooth protheses and calculi in the gallbladder, urinary pelvis, and bladder, enhance epithelial cell proliferation through regenerative processes.

Mutagens/Carcinogens in Food

Ordinary Constituents in Food Acting as Conditional Carcinogenic Factors

Areas where people ingest more sodium chloride show an elevated incidence of gastric cancers (11). Such salt intake causes mucous membrane damage to the stomach, as evidenced by the existence of malondialdehyde in extracts of gastric mucous membranes under sodium chloride administration (12). Malondialdehyde, a product of lipid peroxidation that could be generated by damage of cell membranes, has previously been reported to be mutagenic to bacteria and mammalian cells and carcinogenic to rats (13-15). Recently, malondialdehyde–deoxyguanosine adducts were detected in human liver (16). It is therefore likely that sodium chloride–lipid peroxidation–malondialdehyde–mutation
scenarios play a role in carcinogenesis in the stomach.

_Helicobacter pylori_ infection in the mucosa of the stomach results in gastritis, which may increase formation of nitric oxide (17) as well other proliferative stimuli. It is therefore a factor that might facilitate accumulation of gene alterations.

**Antimutagenic and Anticarcinogenic Factors in Food**

There are many substances in food that can counteract mutagenic and carcinogenic processes. Testing of vegetables and fruits for mutagenic activity with _Salmonella typhimurium_ strains have indicated that flavonoids account for a large part of the activity (18,19). The most commonly found flavonoids are quercetin and its glycoside, rutin; however, quercetin exerts no carcinogenicity in mice, rats, and hamsters in long-term animal feeding experiments (20–23). The experimental results of the National Toxicological Program in F344 rats only demonstrated formation of renal tubular cell adenomas in males (24), and in this case α_{2}-globulin nephropathy may have played some role. Thus, the data are not regarded as unequivocal evidence of any carcinogenicity for quercetin.

On the contrary, many papers have suggested that flavonoid and isoflavonoid compounds should be regarded as anticarcinogens in nature (25–27). One related mechanism could be inhibition of the arachidonic acid cascade, especially at the lipoxygenase step (25). Some flavonoids may also function as active oxygen scavengers. Furthermore, there are many other polyphenolic compounds showing anticarcinogenic activity in plants, including epigallocatechin gallate from tea leaves and curcumin from the turmeric plant, _Curcuma longa_, of the ginger family (28–31).

β-Carotene in vegetables and fruits is an effective cancer preventive agent, especially for carcinomas arising from squamous epithelium and squamous metaplastic lesions (32). However, an adverse effect of β-carotene was reported in an intervention study on lung cancer development among smokers (33). β-Carotene is understood to be converted to vitamin A. α-Carotene is also convertible to vitamin A, but its pro-vitamin A potency is only half that of β-carotene; therefore, it was not expected to be so effective for cancer prevention. However, recently it was demonstrated that α-carotene is in fact more efficient than β-carotene in a mouse liver carcinogenesis experiment and in two-step carcinogenesis experiments focusing on mouse lung and skin (34).

Fish oils are rich in polyunsaturated ω3 fatty acids, including eicosapentaenoic acid and docosahexaenoic acid, which can suppress dimethylhydrazine and azoxymethane-induced aberrant crypt foci and cancer in the colon of rats (35,36). Docosahexaenoic acid is an inhibitor of prostaglandin synthesis. Nonsteroidal anti-inflammatory agents such as indomethacin, sulindac, and aspirin, which also inhibit prostaglandin synthesis, have similarly been reported to reduce development of colon cancer in animals (37–39). Thus, the mechanism underlying the inhibitory effects of ω3 fatty acids on colon cancer development may be the same as that of nonsteroidal anti-inflammatory agents.

Another factor in food relevant to cancer prevention is fibers from vegetables, fruits, and grains. They dilute concentration of mutagens and carcinogens in the intestinal tract due to the increased mass and make the passage of intestinal material much faster so that uptake of included mutagens and carcinogens may be reduced. These characteristics are consistent with data indicating that a high risk of colon cancer is associated with a low intake of dietary fiber (40).

**Heterocyclic Amines: A New Series of Mutagens/Carcinogens in Heated Foods**

It has been well documented that cigarette smoke condensate contains a variety of mutagens and carcinogens. Our group therefore expected that smoke derived from burning foodstuffs might similarly contain mutagens and carcinogens. In fact, smoke condensate obtained by broiling fish showed mutagenicity to _Salmonella typhimurium_ TA98 with a metabolic activation system (41). It was also proved that charred parts of meat and fish, cooked under ordinary conditions, exert much higher mutagenic activity to TA98 after metabolic activation (41). By monitoring the mutagenic activity, the actual mutagenic substances could be purified from cooked food as well as from pyrolyzed amino acids and proteins. Up to the present, a total of 23 heterocyclic amines have been isolated as mutagens, and structures of 19 of them were determined, as shown in Figure 1 (2,42). All the compounds except one were newly registered chemicals. Among them, most heterocyclic amines were found by our group, while α-carboline compounds were isolated by Yoshida et al. (43) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQx) were isolated by Felton et al. (44) and Becher et al. (45). Moreover, 10 of these compounds have subsequently been chemically synthesized on a large scale to allow long-term carcinogenesis experiments to be performed. All of the 10 compounds were proven to be carcinogenic in rats or mice (2,42). They are metabolically activated mainly by cytochrome P450IA2 with conversion of an amino group to a hydroxyamino group and further esterified to give the ultimate forms that produce DNA adducts. The series of heterocyclic amines can be divided into two groups: the 2-amino-3-methylimidazo[4,5-f]quinoxilne (IQ)-type heterocyclic amines and non-IQ-type heterocyclic amines. IQ-type-heterocyclic amines have a 2-aminoimidazole moiety as a common structure and are formed by heating mixtures of creatine, amino acids, and sugars. Non-IQ-type heterocyclic amines contain a 2-aminoopyridine moiety as a common structure and are produced by heating amino acids such as l-tryptophan and l-glutamic acid. In routinely cooked meat and fish, the former type of heterocyclic amines, amino imidazoquinolines, amino imidazoquinoxalinines, and amino imidazopyridines account for the major portion of the mutagenicity.

Based on quantitative data on amounts of heterocyclic amines in cooked food, cigarette smoke, and urine samples from healthy volunteers, daily intakes of heterocyclic amines have been estimated. For example, the daily levels of exposure to 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) were calculated to be less than 1/800 of their tumorigenic dose rate 50 (TD_{50}) values, obtained from studies of their carcinogenicity in mice and rats.

**Risk of Mutagens and Carcinogens in Food and Cancer Prevention**

Diet is known to be an important factor determining cancer risk together with other lifestyle parameters including cigarette smoking, alcohol intake, and physical exercise, as well as infection and disease. As described in the previous section, diet may be directly involved in carcinogenicity through damage to DNA. Human cells are continuously being exposed to mutagenic substances and conditions yielding DNA damage; it is clear that mutations resulting in genomic instability, including DNA...
Figure 1. Structures of mutagenic and carcinogenic heterocyclic amines. Abbreviations: Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole; Trp-P-2, 3-amino-1-methyl-5H-pyrido[4,3-b]indole; Glu-P-1, 2-amino-6-methylpyridin(1,2-a3',2'-d)imidazole; Glu-P-2, 2-amino(1,2-a3',2'-d)imidazole; Phe-P-1, 2-amino-5-phenylpyridine; Orn-P-1, 4-amino-6-methyl-1H-2,5,10,16-tetraazafurazan; AcC, 2-amino-9H-pyrido[2,3-b]indole, MeAcC, 2-amino-3-methyl-9H-pyrido[2,3-b]indole; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoline; 7,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; 4'-OH-PhIP, 2-amino-1-methyl-6-[4-hydroxyphenylimidazo[4,5-b]pyridine; Cre-P-1, 4-amino-1,6-dimethyl-2-methylamino-1H/6H-pyrrolo[3,4-f]benzimidazole-5,7-dione; 4-CH2OH-8-MelQx, 2-amino-4-hydroxy-7,8-dimethylimidazo[4,5-f]quinoline; 7,9-DiMelQx, 2-amino-1,7,9-trimethylimidazo[4,5-g]quinoxaline. The following ten compounds have been proven to be carcinogenic: Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AcC, MeAcC, IQ, MeIQ, MelQx, PhIP.

Figure 2. Scheme for genomic instability in carcinogenesis. Arrows indicate the gene alteration related to genomic instability. The light gray represents non-malignancy and the dark gray represents full malignancy.

repair deficiency, may have a pronounced effect on accumulation of genetic alterations as shown in Figure 2 (46,47). On the other hand, the diet also contains numerous antimutagenic and anticarcinogenic factors. Therefore, the risk of cancer development in man from food-derived mutagens and carcinogens is hard to estimate only from the basis of data of daily human exposure and of long-term animal experiments conducted with maximum tolerated doses.

Furthermore, it is becoming increasingly evident that the human cancers are not produced by a single carcinogenic agent but rather are the result of many carcinogenic compounds, each acting at very low exposure levels. Certain types of malignancies have a known etiology such as adult T-cell leukemia, which is initiated by infection with the adult T-cell leukemia virus through breast-feeding of newborns. Even in this case, however, the onset of disease only occurs when the affected individuals reach 30 to 60 years of age. Mathematical analysis shows that several additional events must occur during the long latent period (48). Viral infection is thus in itself not sufficient for the complete disease process. This is also true for the case of hepatitis B or hepatitis C virus infection and development of hepatocellular carcinomas during the course of which genetic alterations accumulate. Susceptibility to food-derived mutagens and carcinogens might be increased when cell turnover is accelerated. Therefore, the mathematical approach is less easily applicable to the general population among whom there are different backgrounds on the presence of cells having genetic alterations.

Nevertheless, we can say that human beings are exposed synchronously and heterochronously to various mutagenic and carcinogenic factors and that these could be responsible for the genetic alterations found in human cancer cells. The pitfall that scientists working on certain factors tend to consider is that they think their compounds are more important than others for production of cancers. The real situation probably is that exposure to the agents on which scientists themselves are working might correspond to the tip of an iceberg floating on the sea. The submerged unseen portion clearly is the major problem, namely exposures to other agents. We can therefore conclude that any exposure
to food mutagens and carcinogens should be decreased as far as possible. In some cases, from the viewpoints of culture, tradition, and economics, it may be better to accept the presence of tiny amounts of mutagens and carcinogens in food. The same principle in reverse, stands for antimutagenic/anticarcinogenic factors. Various factors that belong to entirely different categories may be expected to collaborate in cancer prevention. Again it is recommended that appropriate measures be taken to optimize our exposure to anticarcinogenic/antimutagenic factors. This approach to cancer prevention has the twin advantages of being both pragmatic and achievable.

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