Review Article

Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish

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Research leading to the discovery of a series of mutagenic and carcinogenic heterocyclic amines (HCAs) was inspired by the idea that smoke produced during cooking of food, especially meat or fish, might be carcinogenic. More than ten kinds of HCAs, actually produced by cooking or heating of meat or fish, have now been isolated and their structures determined, most being previously unregistered compounds. They are highly mutagenic towards *Salmonella typhimurium* in the presence of S9 mix and are also mutagenic *in vitro* and *in vivo* toward mammalian cells. HCAs have now been chemically synthesized in quantity and subjected to long-term animal testing. When HCAs were fed in the diet, rodents developed cancers in many organs, including the colon, breast and prostate, and one HCA produced hepatomas in monkeys. The lesions exhibited alteration in genes including *Apc*, *β-catenin* and Ha-*ras*, and these changes provide clues to the induction mechanisms. The HCAs are oxidized to hydroxyamino derivatives by cytochrome P450s, and further converted to ester forms by acetyltransferase and sulfotransferase. Eventually, they produce DNA adducts through the formation of N-C bonds at guanine bases. There are HCA-sensitive and resistant strains of rodents and a search for the responsible genes is now under way. While the content of HCAs in dishes consumed in ordinary life is low and not sufficient in itself to explain human cancer, the coexistence of many other mutagens/carcinogens of either autobiotic or xenobiotic type and the possibility that HCAs induce genomic instability and heightened sensitivity to tumor promoters suggest that avoidance of exposure to HCAs or reduction of HCAs' biological effects as far as possible are to be highly recommended. Usage of microwave ovens for cooking and supplementation of the diet, for example with soy-isoflavones, which have been found to suppress the occurrence of HCA-induced breast cancers, should be encouraged. Advice to the general public about how to reduce the carcinogenic load imposed by HCAs would be an important contribution to cancer prevention. (Cancer Sci 2004; 95: 290–299)

Importance of analysis of causative factors for human carcinogenesis

Much is now known concerning the mechanisms of carcinogenesis, and genetic alterations and epigenetic changes of crucial genes related to cancer phenotypes have been well elucidated. Knowledge on qualitative and quantitative changes of gene products during carcinogenesis has also been accumulated, and the dissection and integration of information on changes in signal transduction pathways should eventually reveal the details of the molecular mechanisms underlying malignant cancer phenotypes, namely escape from cell cycle regulation, cellular and structural atypia, and cellular behaviors such as infiltration and metastasis. Scientific research has focused on DNA modification and its repair processes relevant to genetic alterations, and much progress has been made over the past decades. For prevention of cancer, however, further information and analysis of causative factors of both genetic and epigenetic changes are required.

Early ideas on cancer causation

Soon after the discovery of radium, it was noted by Furth and Lorenz that ionizing radiation was a carcinogenic hazard.[1] Prior to this, Sir Percival Pott had described chimney sweeps in London developing scrotal skin tumors,[2] and urinary bladder cancers in workers in the aniline dye industry were reported in 1895,[3] pointing to environmental influences. Lung cancers frequently occur among miners[4] and liver tumors with special histological features, hemangioendothelial sarcomas, develop in workers occupationally exposed to vinyl chloride monomer,[5] removing any doubt that human cancers can be caused by heavy exposure to environmental xenobiotic carcinogens, mostly related to occupational or iatrogenic events or industrial accidents. Currently, autobiotic oxidative carcinogens are attracting much attention.

Meanwhile, scientists working on the genetic background of cancer development have provided us with important evidence about the roles of specific genes from studies of hereditary cancers, such as childhood retinoblastoma (*Rb*),[6] adenomatous polyposis associated malignancies of the colon (*APC*),[7] familial breast cancers (*BRCA-1* and *-2*)[8, 9] and familial gastric cancers (*E-cadherin*).[10] However, heavy exposure to environmental xenobiotic carcinogens and clearly definable genetic alterations can explain only a relatively small proportion of the total cancer burden.

Most neoplasms occurring in the general population, our relatives and friends, are due to both environmental factors and genetic influences. Exposure to individual xenobiotics may be minute, so that the impact of one agent may be small, but the presence of many kinds of environmental factors that can act in concert must be considered. Among the common cancers, the contribution and penetrance of single genetic factors may similarly be low, but multiple genetic factors can interact to play major roles. Of particular interest, studies using identical twins have provided very reasonable guesses as to the relative contributions of environmental and genetic factors. For instance, Lichtenstein *et al*. indicated that genetic factors account for 42%, 35% and 27% of the risk for prostate, colon and breast cancers, respectively.[11] The remaining contributions come from the environment.

It is generally believed that about one-fourth to one-third of all cancers are produced by smoking, dietary factors and inflammation/infection.[12, 13] Dietary factors for carcinogenesis include two categories, namely cancer-producing and cancer-

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preventing agents. Since most occupational human and experimental carcinogens have been found to show mutagenic activity toward prokaryotes,[14–17] it is rational to expect mutagens in the diet to be carcinogenic. As proven mutagenic substances in the diet, we can point to mycotoxins, including aflatoxin B_1, which is carcinogenic to humans, rodents and fish.[18] However, the presence of aflatoxin B_1 up to certain levels in foodstuffs such as peanut butter is accepted in many developed nations, since it is almost impossible to completely eliminate it.[19] Various plant alkaloids are also illustrative. For example, cycasin, a β-D-glucoside of methylazoxymethanol, exerts carcinogenic activity in the intestines of rodents and is neurotoxic in man.[20] Bracken fern containing mutagenic ptaquiloside/aquilide A induces tumors in rodents, and also causes urinary bladder-bleeding in cows.[21, 22] Bracken fern is nevertheless accepted as an edible plant by most Asian nations.

Based on the colorful history of environmental carcinogenesis, we initiated studies on genotoxic substances in food in 1970 and by coincidence, AF-2 [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide], which had been used as a food preservative for several years in Japan, was revealed to be mutagenic toward *Escherichia coli*.[23–25] Based on this, intensive tests were carried out on rodents, demonstrating positive carcinogenicity[26, 27] and the use of AF-2 was banned by the Ministry of Health and Welfare, Japan in 1974.

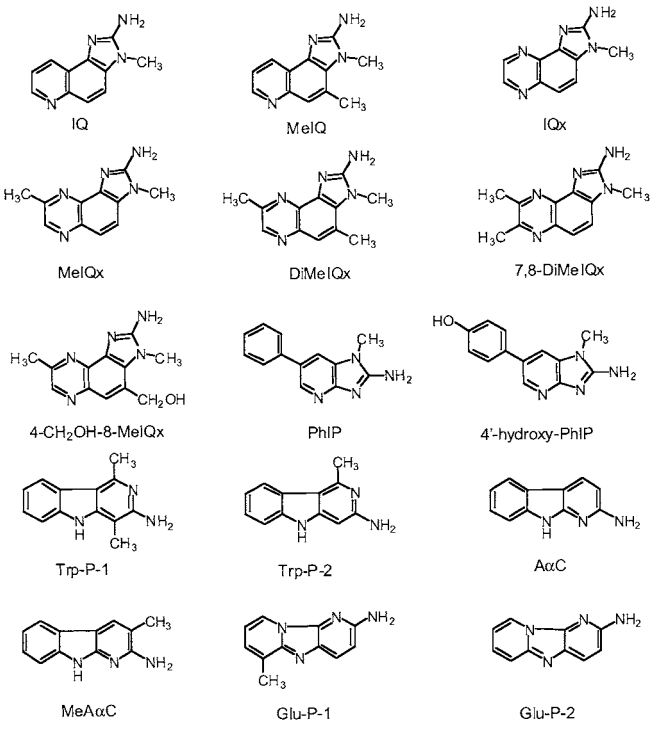

Fig. 1. Structures of HCAs.

A serendipitous scenario for the discovery of heterocyclic amines (HCAs) in cooked meat

One of the authors (T.S.) was inspired by an experience on holiday that has now lead to a major new research area in environmental carcinogenesis. His wife was broiling fish in the kitchen, and the smoke caught his attention. If cigarette smoke contains many mutagens, why not also smoke produced by broiling fish? On the basis of this speculation, it was soon confirmed in the laboratory that smoke produced by broiling fish, collected on glass-fiber filters and dissolved in dimethyl sulfoxide, showed strong mutagenicity to *Salmonella typhimurium* TA98.[28, 29] This was a memorable day, initiating three-decade worldwide investigations of mutagens derived from broiled proteinaceous foods. These data were first introduced in a milestone symposium entitled "The Origins of Human Cancer" held in Cold Spring Harbor in 1976, and the proceedings of the meeting were published in 1977.[28] Commoner *et al*. also reported in 1978 that cooking of meat results in the formation of mutagens.[30]

From pyrolysates of amino acids and proteins, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC and MeAαC were isolated and identified,[31–33] some being also contained in cooked meat. In collaboration with us, Spingarn *et al*. succeeded in isolating mutagenic compounds from beef extract.[34] We subsequently isolated large quantities of mutagens and identified IQ, MeIQ and MeIQx as mutagenic principles in cooked (broiled) meat and fish.[35–38] PhIP was added by Felton *et al*. in the USA to this series of compounds,[39] all belonging to the heterocyclic amine (HCA) class of chemicals. The structures, chemical names and common abbreviations for these newly identified mutagens are listed in Fig. 1 and Table 1. HCAs are divided in two groups. On 2 m*M* nitrite treatment, Group I HCAs, such as Trp-P-1, Trp-P-2, Aαc, MeAαC, Glu-P-1 and Glu-P-2, lose their mutagenicity through conversion of amino to hydroxyl groups, while the amino group of Group II HCAs, such as IQ, MeIQ, MeIQx, DiMeIQx and 7,8-DiMeIQx, is not changed.[40] For the formation of Group II HCAs, creatine or creatinine in muscles serves as a precursor of imidazo moieties, as reported by Jägerstad.[41] The concentrations of Group II HCAs are generally much higher than those of Group I HCAs in cooked

Table 1. Full names and common abbreviations of HCAs

Common abbreviation	Full name
IQ	2-amino-3-methylimidazo[4,5-*f*]quinoline
MeIQ	2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline
IQx	2-amino-3-methylimidazo[4,5-*f*]quinoxaline
MeIQx	2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
DiMeIQx	2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline
7,8-DiMeIQx	2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline
4-CH_2OH-8-MeIQx	2-amino-4-hydroxymethyl-3,8-dimethylimidazo[4,5-*f*]quinoxaline
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
4′-hydroxy-PhIP	2-amino-6-(4-hydroxyphenyl)-1-methylimidazo[4,5-*b*]pyridine
Trp-P-1	3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole
Trp-P-2	3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole
AαC	2-amino-9*H*-pyrido[2,3-*b*]indole
MeAαC	2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole
Glu-P-1	2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole
Glu-P-2	2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole

meat, as described below. Various ways to prevent formation of heterocyclic amines (HCAs) are available.[42]

Mutagenicity of HCAs

All HCAs listed in Table 1 and shown in Fig. 1 are mutagenic toward *Salmonella typhimurium*, with a range of mutagenic potential of the order of 10^3, as shown in Table 2.[43] *Salmonella typhimurium* TA98, detecting frameshift-type mutagens, shows more susceptibility to HCAs than TA100, which detects base-pair change-type mutagens. The specific mutagenicities of HCAs toward mammalian cell lines using the *Hprt* gene or the *Ef-2* gene as a reporter were almost the same range among various HCAs as in *Salmonella typhimurium*.[44, 45] Mutation spectra *in vivo* have been reported for some HCAs, as shown in Table 3,[46–51] but these are not sufficiently specific to identify causative agents of mutations found in tissues.

Carcinogenicity of HCAs

Carcinogenicity studies of HCAs in mice and rats have mainly been carried out in Japan (Fig. 2). The sites of tumors observed in long-term standard animal tests are listed in Table 4 with references. Matsukura, Ohgaki, Takayama, Ito, and Shirai are among the main contributors.[52–69] It is noteworthy that some HCAs can produce tumors in the colon, mammary glands, and prostate, which are common sites of neoplasms in Western countries and for which rates are increasing in Japan with westernization of dietary habits. Macroscopic and histological features of some HCA-induced tumors are shown in Fig. 3 and Fig. 4. In addition to the lungs, liver, ear ducts, skin and clitoral gland are targets of HCAs. Moreover, Weisburger's group reported that intragastric intubation of IQ resulted in development of tumors in the mammary glands, liver and ear ducts of SD rats.[70] Adamson *et al*. also demonstrated that hepatocellular carcinomas were induced in cynomolgus monkeys (*Macaca fascicularis*) after administration of IQ by gavage.[71]

Metabolism of HCAs

HCAs are mainly metabolized first by cytochrome P450 (CYP) 1A2 in rodents as well as humans. Other P450 molecular species including CYP1A1, 1B1 and 3A4 are also responsible, to some extent, for oxidation of the exocyclic primary amino group to a hydroxyamino group.[72–74] Recombinant human CYP1A2 shows a much lower K_m for PhIP and a higher activity for MeIQx than rat CYP1A2, and has a low activity for detoxification by hydroxylation at the 4′ position of PhIP.[75] The hydroxyamino group is further metabolized by *N(O)*-acetyltransferase (NAT), of which there are two isozymes, NAT1 and NAT2, in rats and humans. NAT1 is expressed mainly in extrahepatic tissues and NAT2 in the liver and intestinal epithe-

Table 2. Mutagenicity of HCAs in *S. typhimurium* TA98 and TA100 with S9 mix

HCA	Revertants/μg	
	TA98	TA100
MeIQ	661,000	30,000
IQ	433,000	7000
DiMeIQx	183,000	8000
7,8-DiMeIQx	163,000	9900
MeIQx	145,000	14,000
Trp-P-2	104,200	1800
4-CH_2OH-8-MeIQx	99,000	3000
IQx	75,400	1500
Glu-P-1	49,000	3200
Trp-P-1	39,000	1700
Glu-P-2	1900	1200
PhIP	1800	120
AαC	300	20
MeAαC	200	120
4′-hydroxy-PhIP	2	no data available

Fig. 2. Cover of Cancer Res, July 15, 1991, introducing the researchers working HCAs.

Table 3. Mutational spectra of MeIQ and PhIP in mammalian cells *in vitro* and *in vivo*[46–51]

HCA		Target gene	Tissue	No. of total mutations detected/analyzed	Characteristic mutation type[1] (%)	Characteristic mutation and its frequency[1] (%)
MeIQ	Rat	H-*ras*	Zymbal gland tumor	11/14	G:C to T:A, 10 (91)	G to T at 5′-GC-3′, 9 (82)
	Mouse	H-*ras*	Forestomach tumor	22/64	G:C to T:A, 22 (100)	G to T at 5′-GC-3′, 22 (100)
	Mouse	*lacI*	Colon mucosa	92/92	G:C to T:A, 50 (54)	G to T at 5′-GC-3′, 38 (41)
PhIP	Rat	*Apc*	Colon tumor	5/8	G:C deletion, 5 (100)	G deletion from 5′-GGGA-3′, 5 (100)
	Rat	*lacI*	Colon mucosa	227/227	G:C deletion, 82 (36)	G deletion from 5′-GGGA-3′, 23 (10)
	Rat	*lacI*	Mammary gland	149/149	G:C deletion, 31 (21)	G deletion from 5′-GGGA-3′, 9 (6)
	Mouse	*lacI*	Colon mucosa	115/115	G:C deletion, 30 (26)	G deletion from 5′-GGGA-3′, 8 (7)
	Mouse	*lacZ*	Colon mucosa	40/40	G:C deletion, 8 (20)	G deletion from 5′-GGGA-3′, 2 (5)
	Human fibroblast	*supF*		172/172	G:C deletion, 7 (4)	G deletion from 5′-GGGA-3′, 5 (3)
	Chinese hamster fibroblast	*Hprt*		40/40	G:C deletion, 5 (12.5)	G deletion from 5′-GGGA-3′, 4 (10)

1) No. of the same type of mutations among total mutations detected.

Table 4. Carcinogenicity of HCAs in rats and mice

HCA	Animal	Strain	Concentration in diet (ppm)	Experimental peroid (weeks)	Target organs	Reference
Trp-P-1	Rat	F344	150	52	Liver	59
	Mouse	CDF_1	200	89	Liver	52
Trp-P-2	Rat	F344	100	112	Liver, Urinary bladder	60
	Mouse	CDF_1	200	89	Liver	52
Glu-P-1	Rat	F344	500	64	Liver, Small and large intestine, Zymbal gland, Clitoral gland	61
	Mouse	CDF_1	500	57	Liver, Blood vessels	53
Glu-P-2	Rat	F344	500	104	Liver, Small and large intestine, Zymbal gland, Clitoral gland	61
	Mouse	CDF_1	500	84	Liver, Blood vessels	54
AαC	Rat	F344	800	104	No tumors	69
	Mouse	CDF_1	800	98	Liver, Blood vessels	53
MeAαC	Rat	F344	100	100	Liver	62
	Mouse	CDF_1	800	84	Liver, Blood vessels	53
IQ	Rat	F344	300	55–72	Liver, Small and large intestine, Zymbal gland, Clitoral gland, Skin	63
	Mouse	CDF_1	300	96	Liver, Forestomach, Lung	54
MeIQ	Rat	F344	300	40	Large intestine, Zymbal gland, Skin, Oral cavity, Mammary gland	64
	Mouse	CDF_1	400, 100	91	Liver, Forestomach	55
		C57BL/6	300		Liver, Large intestine	57
MeIQx	Rat	F344	400	61	Liver, Zymbal gland,Clitoral gland, Skin	65
	Mouse	CDF_1	600	84	Liver, Lung, Hematopoietic system	56
PhIP	Rat	F344	400	52	Large intestine, Mammary gland, Prostate, Lymphoid tissue	66, 67
	Mouse	CDF_1	400	82	Lymphoid tissue	58
		C57BL/6N	300	70–95	Small intestine, Lymphoid tissue	68

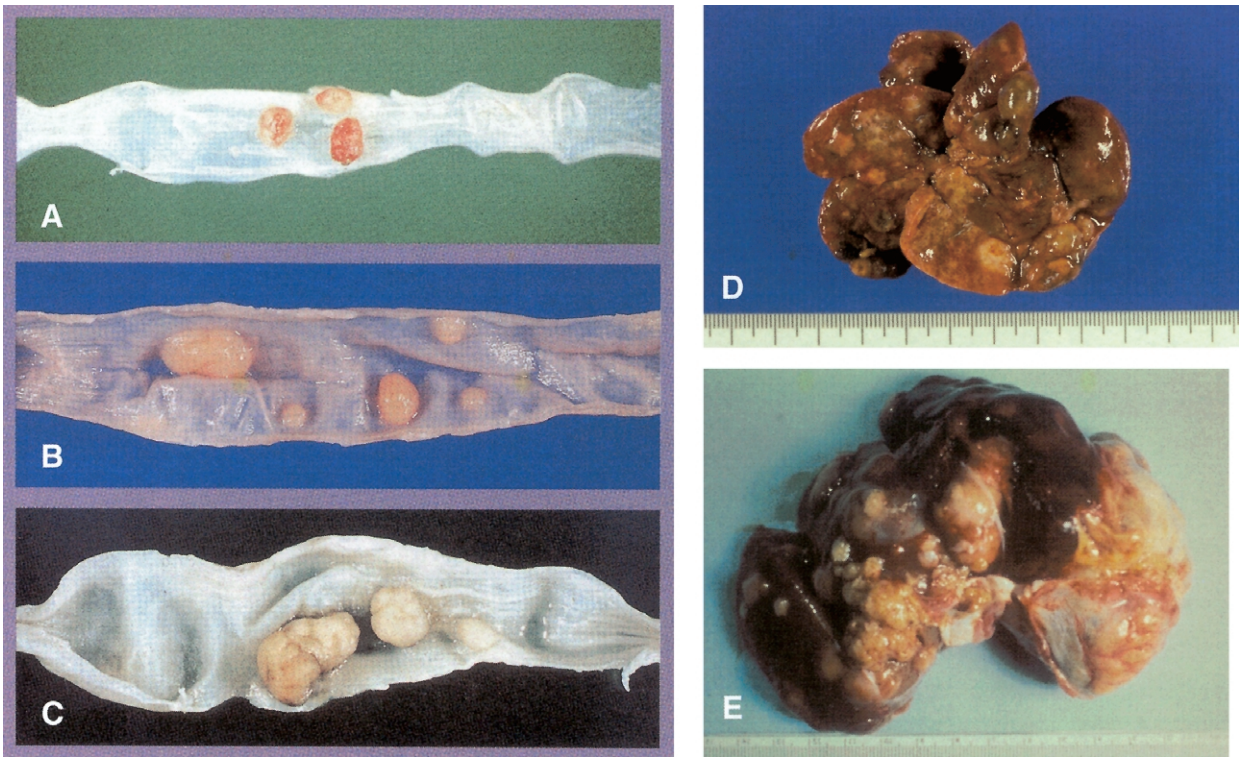

Fig. 3. Macroscopic features of HCA-induced cancers in experimental animals. (A–C) Rat colon cancers induced by IQ (A), PhIP (B) and Glu-P-1 (C), respectively. (D and E) Liver cancers induced by MeIQx in rat (D) and by IQ in monkey.

lium.[76] *N*-Hydroxyl metabolites of most HCAs, including PhIP, IQ and MeIQx, are poor substrates of human NAT1. However, human NAT2 catalyzes *O*-acetylation of *N*-hydroxyl derivatives of IQ, MeIQ, and PhIP.[77, 78] There are at least two and ten polymorphic genotypes of human NAT1 and NAT2, respectively. The NAT2 fast acetylator trait (*NAT2*4* wild allele) has often, but not consistently, been associated with an increased risk of colorectal cancers.[79, 80]

N-Acetoxy metabolites of HCAs are spontaneously converted to arylnitrenium ions ($R\text{-}NH^+$) and react with DNA to form adducts at the 8-position carbon of guanine bases. IQ and MeIQx also form adducts by binding to the N^2 position in guanine. Adduct structures clarified by Snyderwine and others[81–83] are illustrated in Fig. 5.

It would be interesting to examine the change in the cytochrome P450 molecular species leading to more efficient activation in atrophic gastritis with intestinal metaplasia than in the normal stomach.[85] It is apparent that intestinalized lesions of

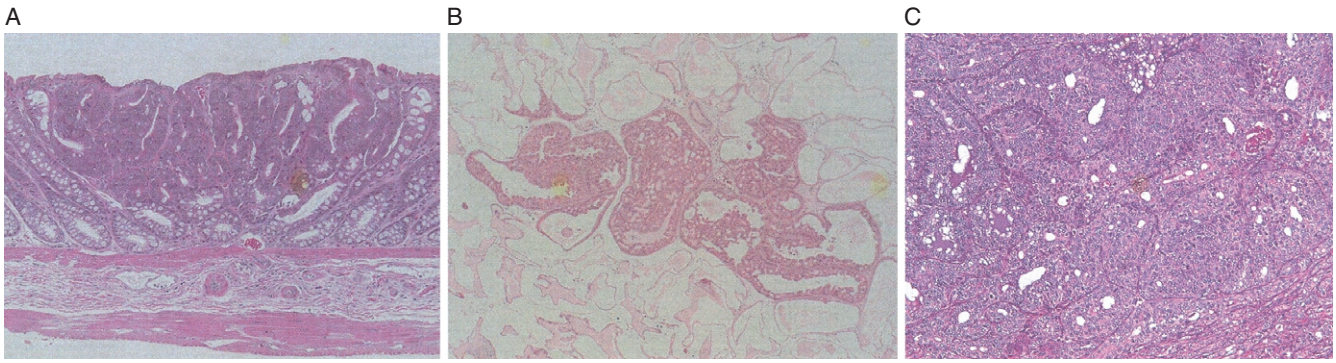

Fig. 4. Histological features of PhIP-induced colon, prostate and mammary gland cancers in rats. (A) Colon cancer, (B) prostate cancer, (C) mammary cancer. The picture for prostate cancer was kindly provided by Dr. Tomoyuki Shirai, Nagoya City University Medical School.

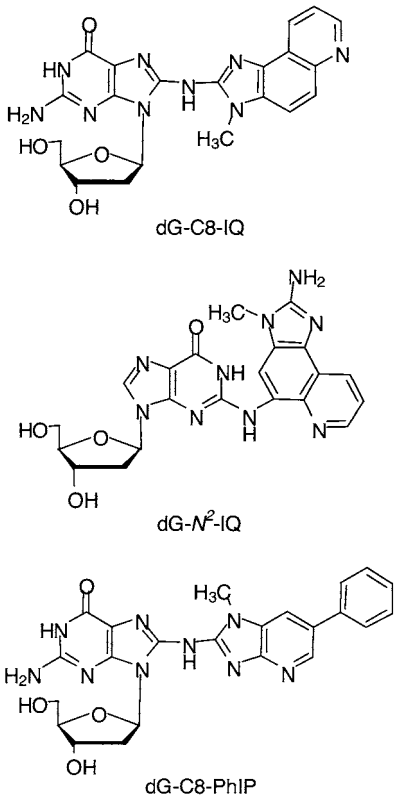

Fig. 5. Structures of major DNA adducts of IQ and PhIP. Major adducts of IQ were identified as *N*-(deoxyguanosin-8-yl)-IQ and 5-(deoxyguanosin-N^2-yl)-IQ,[81, 83] and of PhIP as *N*-(deoxyguanosin-8-yl)-PhIP.[82] The *anti* form is preferred for the dG-N^2 adduct of IQ, while the *syn* form is preferred for dG-C8-IQ. In the case of PhIP, the major structure for the dG-C8-PhIP adduct is the *syn* form.[84]

the stomach are produced by *Helicobacter pylori* infection, and gastric cancer often arises in areas with intestinalization.

Measurement of HCAs in foods and HCA adducts in DNA and protein in the human body

Various convenient methods for quantification of HCAs are now being used worldwide. The simplest approach is to employ blue cotton absorption from crude homogenates and to subject the eluates to HPLC and LC/MS. However, several sophisticated and sensitive methods are also available.[86] One method currently being developed allows *N*-dimethylaminomethylene derivatives of HCAs to be analyzed by gas chromatography with nitrogen-phosphorus-selective detection.[87] Table 5 summarizes data on HCA contents in various foods. MeIQx and PhIP have also been detected in commercial pet foods.[90] The presence of HCAs, including MeIQx, PhIP, Trp-P-1 and Trp-P-2, in urine samples from healthy volunteers eating a normal diet, but not from in-patients receiving parenteral alimentation, has been reported[91] and PhIP has been detected in the milk of healthy women.[92]

The presence of DNA adducts with HCAs in humans further points to appreciable exposure.[93] DNA adduct levels in experimental animals after chronic administration of carcinogenic doses of HCAs reach plateau levels of around several adducts per 10^7 nucleotides.[67, 94] Surprisingly, similar levels of PhIP adducts were detected in mammary epithelial cells obtained from some human milk samples.[93] HCAs also produce adducts with proteins[95, 96] but most of them are not stable. Magagnotti *et al.* reported a good correlation between PhIP doses (0.1–10 mg/kg) and PhIP adduct levels (of the order of fmol/mg protein) in rat serum albumin and hemoglobin.[97]

The issue of comutagenicity: endogenous formation of heterocyclic amines

During studies of purification of mutagens, including HCAs from tryptophan pyrolysate, a sudden loss of mutagenicity was observed in certain fractionation steps. Remixing of the fractions restored the original mutagenic activity. Further study demonstrated the occurrence of comutagenicity.[98] Non-mutagenic aniline and non-mutagenic norharman (β-carboline) yield aminophenylnorharman in the presence of S9 mix of rat liver,[99] a type of HCA which, when further activated by S9 mix to hydroxyaminophenylnorharman and finally converted to an acetoxy derivative, produces DNA adducts and induces mutations, as shown in Fig. 6.[100] In rodents, *in vivo* formation of aminophenylnorharman from aniline and norharman has been proven[101] and, as expected from the mutagenicity, carcinogenicity was observed in rats.

Comutagenicity can also be observed with aromatic amines other than aniline, such as *o*-toluidine and diphenylamine,[102] and thus naturally occurring compounds or drugs and their metabolites might present a risk.

Risk of HCAs for development of human cancer: comparison of human dietary intake with carcinogenic doses in animals

Doses of individual HCAs that produce tumors in 50% of animals under standard experimental conditions are listed in Table 6.[103] Reliable data on the concentrations of HCAs in cooked foods and the daily intake of HCAs by humans are given in Table 7.[104–106] Comparison of the carcinogenic dose in rodents and the actual human daily intake suggests that the latter is definitely too low for cancer production to be explicable in terms of HCAs alone.

Table 5. Amounts of HCAs in cooked foods

Food	Cooking method	HCA (ng/100 g)								Reference
		PhIP		MeIQx		4,8-DiMeIQx		7,8-DiMeIQx		
		Flesh	Skin	Flesh	Skin	Flesh	Skin	Flesh	Skin	
Salmon	Grilled	29	593	10	59	0	0	0	414	88
Salted fish	Grilled	37	700	8	59	0	9	0	446	88
Bacon	Fried	30–450		nd–2370		20–140		nd		42
Pork	Barbecued	420		40		10		nd		42
Chicken breast	Grilled	2700–4800		nd–900		nd–200		nd		42
London broiled steak		18,200		300		nd		nd		89

nd: not detected.

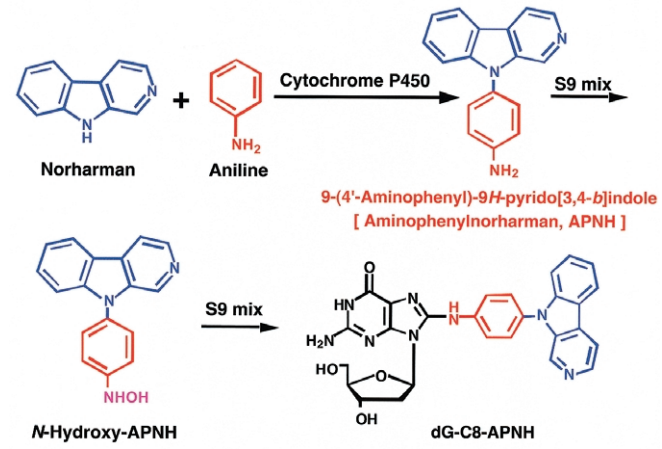

Fig. 6. Structures of aminophenylnorharman (APNH) and its DNA adduct, *N*-(Deoxyguanosin-8-yl)-APNH. The comutagens, norharman and aniline, are converted to APNH through the action of cytochrome P450, and APNH is further metabolized to a proximate form, *N*-hydroxy-APNH and then *N*-acetoxy-APNH.[99] It is considered that the latter form is spontaneously degraded to the arylnitrenium ion, which reacts with DNA.

In animal experiments, however, simultaneous co-administration of different kinds of carcinogens can result in additive or synergistic effects.[107] Moreover, the carcinogenic potency of HCAs is markedly enhanced in the presence of tumor promoters and agents that cause cell proliferation. When a limited amount of Trp-P-2 was painted on the skin of the back of mice, skin tumors only developed after subsequent painting of 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[108] While it is still unlikely that the group of HCA compounds present in food account for a major proportion of cancers in human beings, considering their high metabolic activation capacity, and the finding of very high levels of PhIP-DNA adducts in epithelial cells from human milk, further extensive studies are needed before definitive conclusions can be drawn regarding the importance of HCAs in human carcinogenesis.

Carcinogenic potency could easily be modulated by other dietary factors, making risk estimation difficult. In addition, there are genotoxic substances other than HCAs that enter the human body through food intake and/or by alternative routes and cause mutations. *Apc* and *β-catenin* mutations are frequently observed in HCA-induced colon cancers, but, in contrast, *p53* and K-*ras* mutations are rarely observed.[51, 109, 110] Squamous cell tumors induced by MeIQ and IQ[109] and mammary gland cancers induced by PhIP[111] are frequently associated with Ha-*ras* mutations. De-regulation of the cyclin D1/Cdk4 retinoblastoma pathway was recently reported to play a central role in PhIP mammary carcinogenesis.[112] Mutations in genes related to repair of DNA damage would be expected to result in genomic instability, with more rapid accumulation of mutations in somatic cells. Of course, precancerous cells, in which numbers of mutations relevant to carcinogenesis have already been accumulated, would be more susceptible to transformation to cancer by further induction of mutations by HCAs. Genomic instability was observed in the rat mammary adenocarcinomas induced by PhIP.[113, 114]

Table 6. TD_{50} of major HCAs in mice and rats

	TD_{50} (mg/kg/day)	
	Rats	Mice
PhIP	2.2	64.6
MeIQx	0.7	11
IQ	0.7	14.7
MeIQ	0.1	8.4
AαC	—	15.8
MeAαC	6.4	5.8

Other mutagenic compounds include arylhydrocarbons, *N*-nitrosamines, plant alkaloids, mycotoxins, and nitroarenes, as well as oxidative agents (such as peroxides of fatty acids), metals and naturally occurring radioactive molecules such as radon and potassium.

Modulation of carcinogenic activity

Carcinogenic activity of HCAs can be enhanced or suppressed by various dietary factors. For example, a high-fat diet efficiently enhanced colon carcinogenesis when administered with a relatively small amount of PhIP to F344 male rats.[115] Similarly, PhIP induced mammary tumors at a high incidence in female SD rats fed a high-fat diet.[116] On the other hand, docosahexaenoic acid (DHA), a polyunsaturated ω3 fatty acid in fish oil, significantly reduced PhIP-induced aberrant crypt focus (ACF) formation in the colon of rats,[117] possibly by decreasing levels of prostaglandin E_2. Conjugated linoleic acids (CLA), present in several foods, can also suppress IQ-induced ACF development in the rat colon.[118] Epidemiologically, it has been demonstrated that frequent *miso* soup and isoflavone consumption is associated with a reduced risk of breast cancer.[119] Consistent with these data, an isoflavone mixture (genistein:daidzein=4:1) clearly suppressed PhIP-induced rat mammary carcinogenesis.[120] Moreover, it is reported that green tea catechins inhibit PhIP-induced mammary carcinogenesis and Glu-P-1-induced hepatocarcinogenesis in rats.[121, 122] Indole-3-carbinol, which is present in cruciferous vegetables, was shown to suppress ACF development in rats given PhIP or IQ,[123–125] and its modification of the metabolic activation pathway of HCAs is probably associated with its beneficial effects. CYP1A2 plays a major role in metabolic activation of HCAs and administration of caffeine at 500 and 1000 ppm in drinking water for 2 weeks significantly increased levels of this enzyme. Concurrent administration of caffeine and PhIP resulted in a significant increase of ACF formation in the rat colon.[126] In addition, lyophilized cultures of *Bifidobacterium longum* in yo-

Table 7. Daily intake of HCAs

Study	Subject number		Amount		Reference
European Prospective Investigation into Cancer and Nutrition		344	Median	103 ng/day	104
Japanese Public Health Center	Man	18,290	Mean for men	66 ng/day	106
	Woman	20,745	Mean for women	58 ng/day	
USA, Case (colorectal cancer) control study in Arkansas	Case	155	Mean for cases	364 ng/day	105
	Control	380	Mean for controls	261 ng/day	

gurt have been found to inhibit colon, mammary and liver carcinogenesis by IQ.[127]

Chlorophyllin is a stable and soluble derivative of chlorophyll, and this has been demonstrated to suppress IQ and PhIP-induced carcinogenesis in rats.[128, 129] Here, the mechanisms involve reduced absorption of HCAs from the intestine, probably due to interactions between these two types of planar molecules, and passage of unmetabolized HCAs in the feces.

Genetic determinants for HCA-induced carcinogenesis

As is now widely recognized, genetic backgrounds of individuals have a substantial impact on the development of cancers in response to exposure to various environmental carcinogens. The history of our research seeking genetic determinants of chemical-induced carcinogenesis started almost 20 years ago.[130, 131] Focusing on colon cancers, Demant's group in the Netherlands determined several quantitative trait loci (QTLs), including *Scc1* on mouse chromosome 2, using a DMH-induced model in mice,[132] and *Scc1* was recently identified as *protein tyrosine phosphatase receptor type J (Ptprj)*.[133] The secretory phospholipase A2 (*Pla2g2a*) gene has also been shown to serve as a modifier of the development of intestinal tumors in the $Apc^{Min/+}$ mice.[134, 135] Furthermore, we have observed differential susceptibility to the development of ACFs, precancerous lesions of the colon, and colon cancers due to PhIP among various rat strains.[136] The Buffalo strain is highly sensitive to PhIP, while the Fischer 344, Brown-Norway and Wistar strains are moderately susceptible, and ACI is resistant. QTL analysis using 290 backcross progeny of $(F344\times ACI)F_1\times ACI$ revealed the presence of a susceptibility locus on rat chromosome 16.[137] A long-term carcinogenesis experiment also demonstrated higher susceptibility of F344 rats as compared to the ACI strain. Resistant traits were also identified on several chromosomes and identification of candidate genes for these, which is currently on-going in our laboratory, should benefit us from a prophylactic point of view, namely for population-based cancer prevention of neoplasia induced by environmental HCAs.

Ways to lessen the intake of HCAs

Various easy and efficient ways to prevent the production of HCAs are available. Their generation mainly depends on an increase in temperature and heating time, and on dehydration of the meat.[138–140] Therefore, prolonged cooking and broiling of meat, and direct exposure to a naked flame should be avoided. Flipping hamburgers every minute for 7 min results in less than one-tenth the level of HCA contained after flipping once with a cooking time of 8.9 min.[141, 142] Usage of microwave ovens can be recommended.[42]

The formation of HCAs is inhibited by green tea extract, EGCG, β-carotene and γ-tocopherol,[143, 144] but this is not practical in the human situation. It is more realistic to avoid consuming charred parts produced on meat surfaces. For instance, charred black material on barbecued meat can be removed with a knife and the surface or skin of dried fish after broiling can be discarded by, for example, skilled use of chopsticks.[35]

Proposals for cancer prevention based on the science of HCAs

Armitage and Doll had already emphasized in 1954 that cancers of the lungs, intestine and breasts are established by several events, from analyses of age-occurrence curves.[145] In addition to the three main causes of human cancers, cigarette smoking, food components and inflammation/infection,[12, 13] air and water pollution, pesticides and insecticide residues, iatrogenic exposure to medicines, and food additives are claimed to make minor contributions. As food-related matters, over-eating and obesity may be particularly important factors for cancer causation. Avoidance of or quitting the smoking habit, appropriate diet intake in qualitative and quantitative terms, and establishment of hygienic conditions free from infections of *Helicobacter pylori*, hepatitis viruses and human papilloma viruses are effective cancer preventive measures. Cancer prevention should be the outcome of the integration of efforts to suppress multiple steps of carcinogenesis, involving multiple exposures to multiple carcinogenic factors, that are either environmental/xenobiotic or endogeneous/autobiotic. Thus, the holistic approach, namely life-style improvement seems important. This is the reason why we proposed twelve points for cancer prevention, and these recommendations were distributed in brochures all over Japan.[146, 147] In the case of HCAs, exposure levels are low, but they should still be reduced as far as possible, even though complete avoidance would be impossible.

IARC has rated many chemicals into the categories of human carcinogen, possible human carcinogen, and non-human carcinogen, based on information regarding chemical nature and pharmacobiology, as well as animal experimental and epidemiological data. IQ is in Group 2A "probably carcinogenic to humans" and MeIQ, MeIQx, PhIP are in Group 2B, "possibly carcinogenic to humans."[148] Accidents or other unusual occasions of exposure of humans to chemicals are sometimes important for evaluation, for instance, following factory explosions. Fortunately, there have been no industrial accidents associated with synthesis of HCAs so far, but if one occurred it would likely lead to their upgrading as human carcinogens. People tend to over-look the presence of HCAs in their daily routine lifestyle with the present ratings by the IARC. We would like to draw attention to this danger and the need for improvement. If we assume an intake of 10 μg of HCAs/day, lifetime exposure could easily reach around 300 mg. Awareness of the fact that humans could be exposed to such a large dose during an ordinary life-span may indeed have a significant psychological impact, and contribute substantially to the realization that HCAs are important as environmental carcinogens.

Epidemiology and HCA risk

Early epidemiological studies indicated a positive relation between stomach cancer and intake of broiled fish.[149] More precise epidemiological studies have demonstrated a higher intake of HCAs among American people than Europeans or Japanese, as shown in Table 7, and a positive correlation between PhIP exposure and mammary cancer incidence was demonstrated among Americans. Correlations between MeIQx or DiMeIQx intake and colon cancer incidence were also demonstrated in studies conducted in the USA.[79, 105, 150] Currently, red

meat intake is being claimed to be one factor responsible for a high incidence of colon cancer.[151] On the other hand, an European study found no relation among the type of cooking of meat, frequency of meat intake and cancer occurrence.[152] Precise quantification of HCA intake is, however, difficult, although there has been remarkable progress in methodology. Further well-planned cohort studies are on-going in the United States and other countries, and should be assisted by establishment of surrogate markers such as HCA adducts in DNA and protein in tissue, body fluid and urine.

Concluding comments

HCAs are readily produced by cooking meat in the kitchen and most people are exposed to appreciable, although very small, amounts of these unequivocal carcinogens.[153] A comparable situation was noted a couple of years ago, in that acrylamide was produced by frying potatoes and flour and roasting coffee.[154] Acrylamide induced chromosomal aberrations in mammalian cells *in vitro*, and tumors in rats in a long-term carcinogenicity test.[155] Heavy exposure to acrylamide on the occasion of strengthening a tunnel structure occurred among workers in the south of Sweden[156] and this has prompted more exhaustive risk analyses of the effects of the compound and the hazard to man. To reduce the formation and intake of acrylamide to zero is impossible, just as in the case of HCAs. Common points are that they are both produced in daily life. Regulatory agencies cannot proscribe events going on in the home, and complete avoidance is clearly not possible. Therefore, it is essential to limit the formation and exposure in everyday life by increasing the public's awareness.

We would like to close this review by mentioning that organic solvent extracts of broiled horse meat induced tumors in mammary glands when repeatedly painted on the backs of mice, as reported by Widmark in Lund University, Sweden, in 1939.[157] This was communicated personally to one of the authors (T.S.) by Prof. B. Holmstedt of the Karolinska Institute, many years after HCAs had been isolated and their carcinogenicity demonstrated. Original scientific achievements and important information are sometimes hidden by a stream of trendy science.

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