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Environmental and chemical carcinogenesis

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Abstract

People are continuously exposed exogenously to varying amounts of chemicals that have been shown to have carcinogenic or mutagenic properties in experimental systems. Exposure can occur exogenously when these agents are present in food, air or water, and also endogenously when they are products of metabolism or pathophysiologic states such as inflammation. It has been estimated that exposure to environmental chemical carcinogens may contribute significantly to the causation of a sizable fraction, perhaps a majority, of human cancers, when exposures are related to "life-style" factors such as diet, tobacco use, etc. This chapter summarizes several aspects of environmental chemical carcinogenesis that have been extensively studied and illustrates the power of mechanistic investigation combined with molecular epidemiologic approaches in establishing causative linkages between environmental exposures and increased cancer risks.

A causative relationship between exposure to aflatoxin, a strongly carcinogenic mold-produced contaminant of dietary staples in Asia and Africa, and elevated risk for primary liver cancer has been demonstrated through the application of well-validated biomarkers in molecular epidemiology. These studies have also identified a striking synergistic interaction between aflatoxin and hepatitis B virus infection in elevating liver cancer risk. Use of tobacco products provides a clear example of cancer causation by a life-style factor involving carcinogen exposure. Tobacco carcinogens and their DNA adducts are central to cancer induction by tobacco products, and the contribution of specific tobacco carcinogens (e.g. PAH and NNK) to tobacco-induced lung cancer, can be evaluated by a weight of evidence approach. Factors considered include presence in tobacco products, carcinogenicity in laboratory animals, human uptake, metabolism and adduct formation, possible role in causing molecular changes in oncogenes or suppressor genes, and other relevant data. This approach can be applied to evaluation of other environmental carcinogens, and the evaluations would be markedly facilitated by prospective epidemiologic studies incorporating phenotypic carcinogen-specific biomarkers.

Heterocyclic amines represent an important class of carcinogens in foods. They are mutagens and carcinogens at numerous organ sites in experimental animals, are produced when meats are heated above 180 °C for long periods. Four of these compounds can consistently be identified in well-done meat products from the North American diet, and although a causal linkage has not been established, a majority of epidemiology studies have linked consumption of well-done meat products to cancer of the colon, breast and stomach. Studies employing molecular biomarkers suggest that individuals may differ in their susceptibility to these carcinogens, and genetic polymorphisms may contribute to this variability. Heterocyclic amines, like most other chemical carcinogens, are not carcinogenic per se but must be metabolized by a family of cytochrome P450 enzymes to chemically reactive electrophiles prior to reacting with DNA to initiate a carcinogenic response. These same cytochrome P450 enzymes—as well as enzymes that act on the metabolic products of the cytochromes P450 (e.g. glucuronyl transferase, glutathione *S*-transferase and others)—also metabolize chemicals by inactivation pathways, and the relative amounts of activation and detoxification will determine whether a chemical is carcinogenic. Because both genetic and environmental factors influence the levels of enzymes that metabolically activate and detoxify chemicals, they can also influence carcinogenic risk.

Many of the phenotypes of cancer cells can be the result of mutations, i.e., changes in the nucleotide sequence of DNA that accumulate as tumors progress. These can arise as a result of DNA damage or by the incorporation of non-complementary nucleotides during DNA

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synthetic processes. Based upon the disparity between the infrequency of spontaneous mutations and the large numbers of mutations reported in human tumors, it has been postulated that cancers must exhibit a mutator phenotype, which would represent an early event in cancer progression. A mutator phenotype could be generated by mutations in genes that normally function to guarantee genetic stability. These mutations presumably arise via DNA damage by environmental or endogenous agents, but it remains to be determined whether the acquisition of a mutator phenotype is a necessary event during tumor progression.

Keywords: Aflatoxin; Liver cancer; Hepatitis viruses; Tobacco carcinogens; Lung cancer; Heterocyclic amines; Colon cancer; Carcinogen metabolism; Genetic polymorphism; Mutator phenotype

1. Validation of a causal relationship between aflatoxin exposure and hepatocellular carcinoma risk in humans: a molecular epidemiology paradigm demonstrating the power of biomarkers

The Monographs Program on the Evaluation of Carcinogenic Risks to Humans of the International Agency for Research on Cancer (IARC) publishes authoritative carcinogenic risk assessments based on examination by experts of all relevant information to assess the strength of available evidence that exposures to the chemicals could alter the incidence of cancer in humans. To date, these evaluations have identified a total of 88 agents, mixtures and exposures that are classified in Group 1, "carcinogenic to humans". Included are: 64 agents and groups of agents (22 drugs; 14 environmental chemicals; 14 radiation; 10 viruses, bacteria and parasites; and 4 inorganic fibers); 12 mixtures; and 13 exposure circumstances [http://monographs.iarc.fr/]. Nearly all of these risks were first identified through observational epidemiology, then verified by supplementary studies in animals and other experimental systems. The mold-produced aflatoxins are among the few environmental chemicals in this list that were first identified as carcinogens in animals, and subsequently shown to pose carcinogenic risks to humans through epidemiologic studies. Extensive research has produced a comprehensive database addressing risks resulting from the high prevalence of their contamination of major food staples in many parts of the world, together with their carcinogenic potency in animals. Indeed, the aflatoxin-liver cancer risk relationship is among the most extensively documented examples demonstrating the significance of a widely disseminated environmental chemical carcinogen as a determinant of increased risk for a major form of cancer. Continuing research efforts stimulated by their discovery in the early 1960s produced an extensive body of evidence regarding human health risks resulting from aflatoxin exposure. Collectively, epidemiologic data together with evidence from many types of experimental models defines the role of aflatoxin exposure in HCC causation. It is informative to review this information for perspective regarding the multifactorial etiology of the disease and, in particular, the critical role of well-validated molecular biomarkers in establishing the causal exposure-risk relationship.

Chronic infections by the hepatitis B (HBV) or hepatitis C (HCV) viruses are major risk factors for the great majority of HCC cases worldwide [1]. They also are largely responsible for the geographical pattern of HCC incidence, HBV being the dominant cause in developing countries of subSaharan Africa and Asia, while HCV is the major risk factor in developed countries with a high or intermediate incidence. Evidence supporting these conclusions has recently been summarized [2]. Carrier rates of HBV in African and Asian populations may be as high as 20%, and the infection is acquired early in life as a result either of perinatal infection by a carrier mother or by horizontal passage from infectious siblings. Males acquiring carrier status early in life are at very high risk of developing HCC, with a lifetime relative risk (RR) of 100 calculated for Taiwanese men. Thus, chronic infection with HBV has been stated to be the single most common cause of global HCC [2]. The importance of HCV infection in the causation of HCC has been recognized more recently, and interactive effects between the carcinogenicity of HBV and HCV have been demonstrated in most, but not all, populations in which it has been studied. Mechanisms through which these infections cause HCC are still unknown, although both direct and indirect actions are thought to be involved.

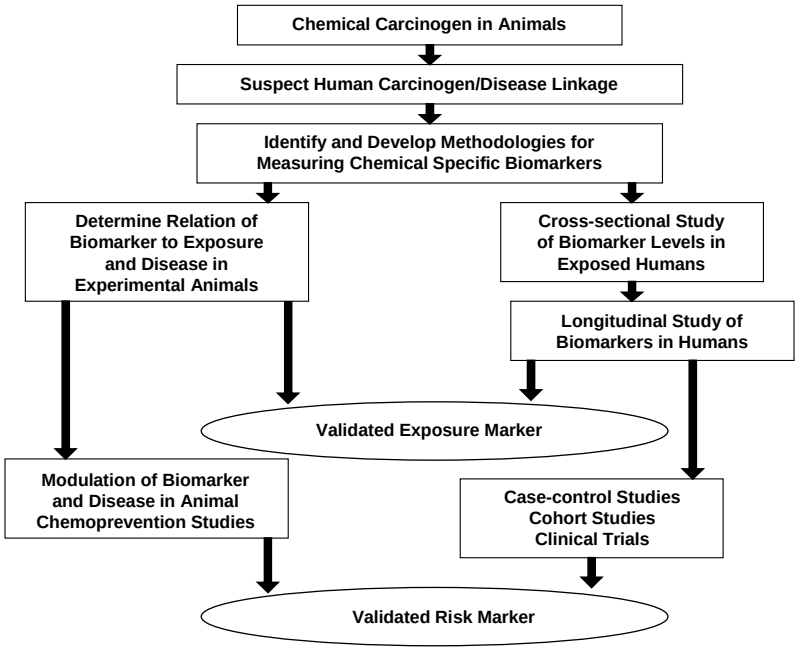
The potency of HBV infection overshadowed recognition of the significance of aflatoxin exposure as a cause of HCC, evidence of which has mounted over a period of several decades. Aflatoxins belong to a large group of mycotoxins, toxic metabolites that contaminate food and feed commodities during growth of certain spoilage molds. In addition to causing acute toxicity, aflatoxins are also liver carcinogens in experimental animals and extensive quality control measures are necessary to minimize levels in human foods. Aflatoxin-contaminated feed was discovered to be a liver carcinogen in rats even before the active agent was isolated and characterized. Subsequent experiments with chemically pure toxin showed that HCC was induced in sensitive species when aflatoxin B_1 (AFB_1), the major component of mixtures typically found in food raw materials, was fed at levels as low as 15 ppb (μg/kg) in the diet. Bioassays in various species offish, birds, rodents and sub-human primates eventually revealed that AFB_1 is a liver carcinogen in all animals tested. Although there is wide variation in sensitivity, no completely refractory species has been

identified. These data clearly implicate aflatoxin as a potential liver carcinogen in humans, and the plausibility of this implication is supported by much additional experimental evidence [3]. Aflatoxin is strongly mutagenic in test systems ranging from bacteria to human cells in culture, requiring metabolic activation by cytochrome P450; pathways of aflatoxin metabolism are similar in cells and tissues of susceptible animals and humans, including the epoxidation pathway resulting in covalent binding to DNA; the DNA adduct profile, with the aflatoxin-N^7-guanine adduct (AFB_1-N^7-gua) representing the major adduct, is identical in animal and human cells mutagenized by aflatoxin; adduct level in liver DNA is quantitatively related to aflatoxin dose and to tumor yield; and chemoprevention of DNA adduct formation inhibits tumorigenesis in experimental animals.

A paradigm for validating causal relationships utilizing molecular epidemiology is outlined in Scheme 1 [7]. The carcinogenic potency of aflatoxin in animals together with their frequent contamination of human foods stimulated cross-sectional epidemiologic investigations to assess relationships between exposure and incidence of HCC. Collectively, studies conducted in subSaharan Africa and Asia between 1965 and 1985 revealed a highly significant association between AFB_1 intake, calculated from analysis of foods as consumed, and HCC incidence estimated from cancer registry data [4]. Based on these epidemiologic data, together with the body of experimental evidence outlined above, AFB_1 was designated as a known human carcinogen (group 1) by IARC in 1993 [5]. Parenthetically, it is noteworthy that this designation was assigned in the absence of information about the prevalence of HBV infection in the populations studied.

Statistical association of carcinogen exposure with cancer incidence does not provide direct evidence of a cause-effect relationship. Consequently, despite the mounting database supporting its biological plausibility, the significance of aflatoxin as a risk factor for hepatocellular carcinoma in humans remained uncertain. Lack of capability to assess aflatoxin exposure of individuals within study populations was recognized as a serious limitation of the above epidemiologic studies. In response to this need, aflatoxin-DNA and serum albumin adducts were developed and validated as biomarkers capable of detecting exposure on an individual basis in large numbers of subjects [6,7]. In rats and other experimental animals, aflatoxin is metabolized in liver to oxidized derivatives, including AFM_1,that are excreted in urine and bile, with a small proportion (about 1% of the ingested dose) being activated through the epoxide to form covalent adducts in DNA. AFB_1-N^7-gua, the major adduct, is efficiently removed from DNA and excreted in urine where it, as well as other metabolites, can be detected by a sensitive analytical procedure involving immunoaffinity/HPLC purification and fluorescence detection. Aflatoxin adducts are also formed in serum albumin and can be detected by immunoassay. Measurements of urinary and serum aflatoxin adduct levels reflect recent (72 h) or chronic (11 days) exposures, respectively. Formation of aflatoxin-DNA adducts in liver, excretion of the urinary adduct and formation of the serum albumin adduct are highly correlated, and measurements of the two biomarkers provide complementary exposure information.

Early studies in populations consuming contaminated diets showed that humans had the metabolic capacity to produce aflatoxin metabolites previously detected in



Scheme 1. A paradigm for validating causal relationships between carcinogen exposure and cancer risk utilizing molecular epidemiology.

experimental animals. Subsequent dose-response studies in small groups of subjects in the PRC and The Gambia, West Africa, areas where aflatoxin contamination of foods is prevalent, measured both dietary aflatoxin intake and levels of urinary aflatoxin biomarkers [6,7]. Urinary AFB_1-N^7-gua and AFM1 excretion showed a dose-dependent relationship to aflatoxin intake, and additional studies showed a similar relationship with adducts in serum albumin. Importantly, the kinetics of formation and excretion of AFB_1-N^7-gua in urine were found to be similar in humans and rats. Combined data from studies in rats and exposed humans therefore indicated that urinary and serum adduct levels were valid biomarkers of exposure and biologically effective dose, strengthening the validity of cross-species extrapolation in assessment of HCC risk posed by aflatoxin ingestion. The validated biomarkers were employed in an evaluation of HBV and aflatoxin as independent and interactive risk factors for HCC in >18,000 male residents of Shanghai [8,9]. Data from this nested case–control study revealed a statistically significant increase in the RR of 3.4 for HCC cases in whom aflatoxin biomarkers, but no evidence of HBV infection, were detected. For HBsAg-positive individuals without aflatoxin biomarkers, the RR was 7, whereas for those positive for both aflatoxin and HBV biomarkers the RR was 59. These findings have been confirmed in subsequent studies of similar design in Taiwan [10], and in a subsequent prospective study in the PRC [11], strengthening the conclusion that aflatoxin is a causative agent for HCC and substantially amplifies the risk created by HBV infection. These data indicate independent causal relationships between the presence of aflatoxin-and HBV-specific biomarkers and the risk of HCC, and also demonstrate the power of validated aflatoxin biomarkers to define a previously unrecognized carcinogen–viral interaction in the induction of the disease.

Mechanisms through which aflatoxin exerts its carcinogenicity and multiplicative interaction with HBV infection in HCC induction have not yet been elucidated, and are subjects of active current research. One pathway through which aflatoxin may contribute to HCC risk relates to its capacity to induce G:C to T:A transversions as a predominant mutation. This type of mutation has been identified at high frequency in the p53 tumor suppressor gene, with specific clustering at the third base of codon 249, in HCC occurring in populations exposed to high levels of dietary aflatoxin [12]. Few such mutations were present in HCC of patients residing in regions of low aflatoxin exposure. Significantly, AFB_1 has been shown preferentially to induce the p53 codon 249 G:C to T:A mutation in cultured human hepatocytes. These results provide further support for the plausibility of aflatoxin as an etiological factor in HCC.

Evidence that aflatoxin increases risk of HCC in patients with HBV hepatitis would suggest that measures to reduce exposure to aflatoxin might be beneficial to men with HBV hepatitis and could be evaluated in suitably designed protocols. Reduction of exposure per se through dietary modification is difficult, but protection through modulation of aflatoxin metabolism may be more readily achieved. It has recently been reported [13] in a phase II chemoprevention trial being conducted in Qidong, PRC, that intermittent or sustained administration of oltipraz, an inducer of phase 2 metabolizing enzymes, significantly increased biomarkers of aflatoxin detoxification. These results highlight the feasibility of this or related approaches as a chemopreventive strategy in populations at high risk for HCC.

2. Evaluation of tobacco carcinogens: a model for environmental carcinogenesis

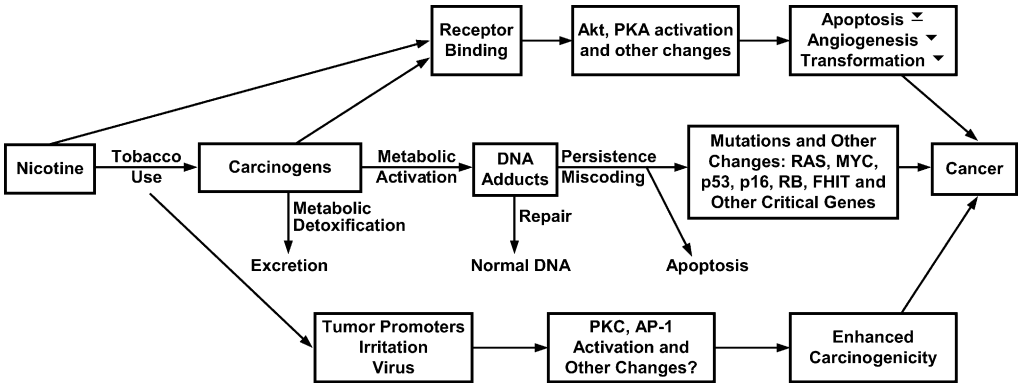
Tobacco products provide a clear example of cancer causation by a life-style factor involving carcinogen exposure. There are over one billion smokers and hundreds of millions of smokeless tobacco users worldwide. Tobacco use is by far the most widespread link between exposure to known carcinogens and death from cancer, and like the aflatoxin-HCC relationship, can be considered a model for understanding mechanisms of cancer induction by exogenous chemical carcinogens.

2.1. Tobacco products and cancer

The IARC Monograph entitled ‘Tobacco Smoke and Involuntary Smoking’, to be published in 2004, concluded the following based on an evaluation of the world’s literature [14]. Cigarette smoking increases the risk of all histological types of lung cancer. It causes cancer of the oral cavity, and this risk is greatly increased by the use of smokeless tobacco or by alcohol consumption in combination with smoking. Cigarette smoking is also causally associated with laryngeal, oropharyngeal and hypopharyngeal cancer, and increases the risks for sinonasal and nasopharyngeal cancer. Cigarette smoking is causally associated with cancer of the esophagus, both squamous cell carcinoma and adenocarcinoma. Furthermore, cigarette smoking causes cancer of the stomach, liver, and pancreas, as well as transitional cell carcinomas of the bladder, ureter and renal pelvis, and renal cell carcinoma. Finally, cigarette smoking is also a cause of squamous cell cervical carcinoma and myeloid leukaemia, and the risk of colorectal cancer can also be increased by smoking. Environmental tobacco smoke (ETS) causes lung cancer. Smokeless tobacco products are established causes of oral cavity cancer [15].

2.2. Tobacco carcinogens and cancer

The central role of tobacco carcinogens and their DNA adducts in tobacco-induced cancer is illustrated in Scheme 2 [16]. Carcinogens are the key connection between nicotine addiction and cancer. Nicotine addiction is the major reason why people continue to use tobacco products. While nicotine itself is not carcinogenic, each cigarette or dip of smokeless tobacco contains a mixture of carcinogens, tumor promoters,



Scheme 2. Scheme linking nicotine addiction and cancer via tobacco carcinogens.

and co-carcinogens. Most tobacco carcinogens require metabolic activation to exert their carcinogenic effects; there are competing detoxification pathways and the balance between metabolic activation and detoxification differs among individuals and affects cancer risk. This is shown in the central track of Scheme 2 [16].

Metabolic activation leads to the formation of DNA adducts, which are carcinogen metabolites bound covalently to DNA. DNA adducts are absolutely central to the carcinogenic process. If their formation is inhibited or blocked, so is carcinogenesis [16]. If DNA adducts escape cellular repair mechanisms and persist, they may lead to miscoding, resulting in permanent mutations. Cells with damaged DNA may be removed by apoptosis, or programmed cell death. If a permanent mutation occurs in a critical region of an oncogene or tumor suppressor gene, it can lead to activation of the oncogene or deactivation of the tumor suppressor gene. Multiple events of this type lead to aberrant cells with loss of normal growth control and ultimately to cancer. These events are also shown in the central track of Scheme 2. The chronic barrage of DNA damage by tobacco carcinogens in people who use tobacco products is completely consistent with the multiple genetic changes observed in tobacco-induced cancers and with our current understanding of the role of genetic aberrations in cancer induction.

The upper track of Scheme 2 shows that nicotine and tobacco-specific nitrosamines can bind directly to certain receptors leading to activation of cellular regulatory factors such as AKT. This can result ultimately in decreased apoptosis, increased angiogenesis, and increased cell transformation. These changes may enhance the effects of carcinogens and their DNA adducts. The lower track of Scheme 2 illustrates the contributions of cofactors such as tumor promoters and co-carcinogens in tobacco products, or irritation and viruses (for example, in the oral cavity) which may enhance the activity of tobacco carcinogens through a variety of mechanisms.

Tobacco products contain a diverse array of chemical carcinogens. Table 1 presents an overview of carcinogens in tobacco products. More than 60 known carcinogens have been detected in cigarette smoke. Several carcinogens listed in Table 1 have been detected only sporadically, but most are routinely found. All of the carcinogens in Table 1 have been formally evaluated by the IARC, and in each case, studies in either laboratory animals or in humans have provided sufficient evidence of carcinogenicity. There is a large range of potencies and concentrations among these carcinogens. In general, the stronger carcinogens such as polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and aromatic amines occur in lower amounts in cigarette smoke (1–200 ng per cigarette) than the weaker carcinogens such as acetaldehyde (nearly 1 mg per cigarette). The total amount of carcinogens in cigarette smoke add up to 1–3 mg per cigarette (similar to the amount of nicotine, 0.5–1.5 mg per cigarette), although most of this total is comprised of weaker carcinogens such as acetaldehyde, catechol, and isoprene.

Unburned tobacco, including cigarette tobacco, oral snuff, chewing tobacco, and other smokeless tobacco products,

Table 1
Carcinogens in smoke and unburned tobaco

Chemical class	No. of compounds	Representative carcinogens
Tobacco smoke		
PAH	14	BaP, dibenz[a,h]anthracene
Nitrosamines	8	NNK, NNN
Aromatic amines	12	4-Aminobiphenyl, 2-naphthylamine
Aldehydes	2	Formaldehyde, acetaldehyde
Phenols	2	Catechol
Volatile hydrocarbons	3	Benzene, 1,3-butadiene
Nitro compounds	3	Nitromethane
Other organics	8	Ethylene oxide, acrylonitrile
Inorganic compounds	9	Cadmium
Total	61	
Unburned tobacco		
Chemical class carcinogens	No. of compounds	Representative
PAH	1	BaP
Nitrosamines	6	NNK, NNN
Aldehydes	2	Formaldehyde, acetaldehyde
Inorganic compounds	7	Cadmium
Total	16	

Table 2
Evaluation of specific carcinogens as causes of lung cancer in smokers

Compound(s)	Presence in cigarette smoke	Pulmonary carcinogenicity in rodents	Human uptake	Human metabolism and adduct formation	Molecular changes in human genes	Overall score
Specific PAHs	4	4	4	3	3	18
Aza-arenes	3	3	1	1	2	10
NNK, *N*-nitrosodiethyl-amine	4	4	4	3	3	18
Metals	4	4	1	1	1	11
Miscellaneous organic compounds	4	3	1	1	1	10
Free radicals/oxidative damage	3	1	3	3	1	11

Scores: 1= inadequate data; 2 = weak or equivocal evidence; 3 = some evidence, limited studies; 4 = clear evidence, strong reproducible studies.

contains fewer carcinogens than cigarette smoke because most in smoke are formed during combustion (Table 1). Levels of PAH in unburned tobacco are typically low. Nitrosamines, particularly the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN), are by far the most prevalent strong carcinogens in unburned tobacco [15,18]. The levels of NNK and NNN in smokeless tobacco products are hundreds to thousands of times higher than those of carcinogenic nitrosamines in any other consumer product designed for ingestion [18].

2.3. Evaluating the role of specific carcinogens in tobacco-related cancer

When considering the relationship between tobacco carcinogen exposure and specific types of cancer, we have the "advantage" of a known exposure and a known cancer endpoint. We are also aided by the comprehensive characterization of tobacco product chemistry that is available in the literature. Tobacco use is unfortunately the largest voluntary carcinogen exposure experiment in history, and is still ongoing. The major disadvantage, from the point of view of relating specific carcinogens to tobacco-induced cancer, is that the exposures are always to mixtures of tobacco carcinogens, along with cofactors such as tumor promoters and cocarcinogens. This clearly complicates the task of relating particular tobacco carcinogens to specific cancer types.

Nevertheless, a weight of the evidence approach can be taken [17]. This is illustrated for cigarette smoke carcinogens and lung cancer in Table 2. The criteria used for evaluation are the presence of the compounds in cigarette smoke, their pulmonary carcinogenicity in laboratory animals, their human uptake, metabolism and adduct formation, their possible role in causing molecular changes in oncogenes or suppressor genes, and other relevant data. Using this approach and assigning a score to each group of compounds as illustrated in Table 2, the conclusion is that considerable evidence favors PAHs and NNK as major etiological factors in tobacco-induced lung cancer (Table 3).

PAHs are strong locally-acting carcinogens, and tobacco smoke fractions enriched in these compounds are carcinogenic [19–21]. PAH-DNA adducts have been detected in human lung samples, and mutations in the Tp53 gene isolated from lung tumors are similar to those produced in vitro by PAH diol epoxide metabolites. NNK is a strong systemic lung carcinogen in rodents, inducing lung tumors independently of its route of administration [22]. The strength of NNK is particularly great in the rat, in which total doses as low as 6 mg/kg (and 1.8 mg/kg when considered as part of a dose-response trend) have induced a significant incidence of lung tumors. This compares to an estimated 1.1 mg/kg dose of NNK in 40 years of smoking. DNA adducts derived from NNK or the related tobacco-specific nitrosamine NNN are present at a higher level in lung tissue from lung cancer patients than controls, and metabolites of NNK are found in the urine of people who use tobacco products or are exposed to ETS [23]. Epidemiologic data indicate that a systemic carcinogen causes lung cancer in cigar smokers who do not inhale; this is consistent with the tumorigenic properties of NNK. The changing histology of lung cancer, in which adenocarcinoma has now overtaken squamous cell carcinoma as the most common lung cancer type, is also consistent with the role of NNK, which produces primarily adenocarcinoma in rodents. NNK concentrations in

Table 3
Roles of specific tobacco carcinogens in tobacco-induced cancers in humans

Cancer type	Likely carcinogen involvement
Lung	PAH, NNK (major) 1,3-butadiene, isoprene, ethylene oxide, ethyl carbamate, aldehydes, benzene, metals
Larynx	PAH
Nasal	NNK, NNN, other nitrosamines, aldehydes
Oral cavity	
Smokers	PAH, NNK, NNN
Smokeless tobacco users	NNK, NNN
Esophagus	NNN, other nitrosamines
Liver	NNK, other nitrosamines, furan
Pancreas	NNK, NNAL
Cervix	PAH, NNK
Bladder	4-Aminobiphenyl, other aromatic amines
Leukemia	Benzene

mainstream smoke increased, while those of BaP decreased, as nitrate concentrations in tobacco increased over the period of 1959–1997 due to the use of tobacco blends containing higher levels of air-cured tobacco, use of reconstituted tobacco, and other factors.

Using this weight of the evidence approach, the role of various carcinogens as causes of specific tobacco-induced cancers other than lung can also be estimated (Table 3). The particulate phase of cigarette smoke causes tumors of the larynx in hamsters—this may be attributed to PAH. Tp53 gene mutations identified in tumors of the human larynx support a role for PAH in the development of this cancer. Nitrosamines, as well as acetaldehyde and formaldehyde, induce nasal tumors in rodents and are likely causes of smoking-associated nasal tumors. Based on animal studies, PAH, NNK and NNN are the most likely causes of oral cancer in smokers. NNK and NNN, perhaps together with enhancing agents, may cause oral cancer in smokeless tobacco users, because they are the most prevalent strong carcinogens in these products and they induce oral tumors in rats, when co-administered. Nitrosamines are the most effective esophageal carcinogens known, and NNN, which produces tumors of the esophagus in rats, is the most prevalent nitrosamine carcinogen in cigarette smoke.

NNK, several other cigarette smoke nitrosamines, and furan are effective hepatocarcinogens in rats. NNK and its major metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) are the only known pancreatic carcinogens to which people who use tobacco products are exposed, and biochemical data from studies with human tissues support their role in smoking related pancreatic cancer. Biochemical studies demonstrate that NNK and PAH can reach the cervix in humans, and are metabolically activated there. DNA adducts derived from BaP and other hydrophobic compounds have been detected in cervical tissue of smokers. Therefore, these compounds may contribute to the etiology of cervical cancer in smokers, in combination with human papilloma virus. 4-Aminobiphenyl and 2-naphthylamine are known human bladder carcinogens, and considerable data from human studies support the role of these and other aromatic amines as the major cause of bladder cancer in smokers. The most probable cause of leukemia in smokers is benzene, which occurs in large quantities in cigarette smoke, and is a known cause of acute myelogenous leukemia in humans.

The criteria used here for evaluation of the roles of specific tobacco carcinogens in tobacco-induced cancer, namely the presence of the compound in tobacco products; its carcinogenicity in laboratory animals; its human uptake, metabolism and adduct formation; its possible role in causing molecular changes in oncogenes or suppressor genes; and other relevant data can all be applied to environmental carcinogens. It is only necessary to substitute “presence of the compound in tobacco products” for “presence of the compound in the environment”. In this way, evaluation of tobacco carcinogens serves as a model for evaluation of environmental carcinogens.

The major gap in this evaluation scheme is the relative paucity of prospective epidemiologic studies that have used molecular biomarkers of specific tobacco carcinogens, such as carcinogen-DNA adducts, carcinogen-protein adducts, and carcinogen metabolites in blood or urine. While several studies have examined levels of DNA adducts in smokers with respect to development of certain cancers, they used nonspecific methods such as immunoassay and ^{32}P-postlabelling, results of which cannot be traced to individual carcinogens. Prospective epidemiologic studies that incorporate specific tobacco carcinogen biomarkers would provide the most convincing evidence that a given carcinogen was related to cancer development. Furthermore, such studies ultimately could provide information that might be incorporated into a predictive model of individual cancer susceptibility. Such models would be extremely useful in tobacco-related cancer control.

2.4. Summary

Tobacco carcinogens and their DNA adducts are absolutely central to cancer induction by tobacco products. The contribution of specific tobacco carcinogens to tobacco-induced cancer can be evaluated by a weight of the evidence approach. Examples were given for various carcinogens and tobacco related cancers, e.g. the role of PAH and NNK in lung cancer. Factors considered in this approach include the presence of the compound in tobacco products, its carcinogenicity in laboratory animals, its human uptake, metabolism and adduct formation, its possible role in causing molecular changes in oncogenes or suppressor genes, and other relevant data. All of these factors can be applied to evaluation of environmental carcinogens. It is only necessary to change “presence of the compound in tobacco products” to “presence of the compound in the environment”. These evaluations would be markedly facilitated by prospective epidemiologic studies that incorporate phenotypic carcinogen-specific biomarkers, but few such studies have been carried out to date.

3. Heterocyclic amine carcinogens in our diet: etiological agents for human cancer?

Human risk estimates and cancer etiology attributed to the consumption of mutagens and carcinogens in our food are difficult to evaluate, as these toxicants come from numerous sources in our diet. As discussed above, mycotoxins, such as aflatoxin B_1 are formed by fungi growing on poorly stored grain products and can be strong liver carcinogens, especially in individuals infected by the hepatitis B virus. PAH such as benzo[a]pyrene, as combustion products, are present in wood fires or flame grilling and can be deposited on food from fat dripping onto the coals during this type of cooking. These compounds are generally potent

carcinogens in experimental animals, but are quite ubiquitous in our environment.

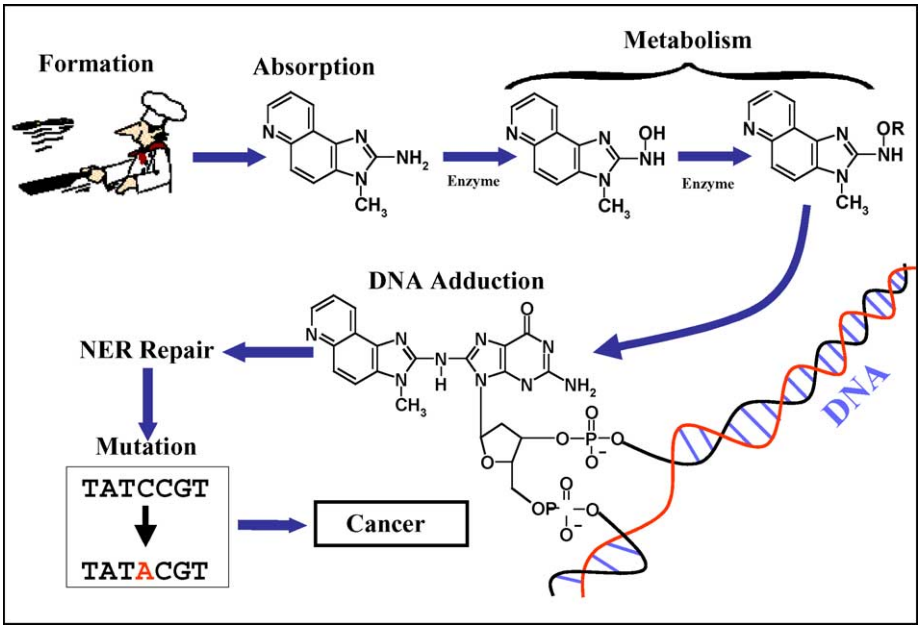
Another important class of carcinogens in food is the heterocyclic amines. These compounds are potent mutagens and moderately potent carcinogens at numerous organ sites in rodents and in the liver of non-human primates. They are produced when muscle foods are heated above 180 °C for long periods of time. The levels in meat products can reach hundreds of parts per billion (ppb), but are generally lower in chicken and beef cooked to a well-done state. By virtue of their frequent presence in meat, they present a problem in widespread exposure in the diet of non-vegetarians. At least sixteen, and possibly more, different heterocyclic amines have been isolated from cooked foods. Four of these compounds can consistently be identified in well-done meat products from the North American diet. The majority (more than 75%) of epidemiology studies designed to evaluate the health significance of these animal carcinogens have linked consumption of well-done meat products to cancer of the colon, breast and stomach. However, a causal linkage has not been firmly established, since some well-designed studies found no statistically significant positive correlation between consumption of diets containing heterocyclic amines and incidence of colon or other cancers. Studies employing DNA adducts and urine metabolites as molecular biomarkers suggest that individuals may differ in their susceptibility to these carcinogens. Polymorphisms in metabolic activation and detoxification, as well as DNA repair genes may contribute to this variability. Variable exposures and genetic differences in a large number of genes, each probably having small impact (i.e., low penetrance) complicate estimation of individual risks. Additional variables are introduced by modulation of internal dose and exposure by interactions with other foods in the diet as well as individual differences in adsorption of the carcinogens. It is therefore essential to put these variables into perspective, in assessment of risks to the general population attendant to consumption of dietary carcinogens.

A related chemical, acrylamide, has recently been identified in starch-based foods such as potato chips and French fries cooked using high temperature deep-frying and baking methods. This compound is weakly- or non-mutagenic in in vitro tests and weakly carcinogenic to experimental animals, but in comparison to the heterocyclic amines are present in large (part-per million) quantities in these starch-derived products. These compounds do not have the strong genotoxic properties of the other food-borne carcinogens discussed in this Chapter, such as aflatoxin B_1, benzo[a]pyrene and the heterocyclic amines, but the exposure levels may be quite high.

In terms of mode of action, current knowledge about the heterocyclic amines indicates that the underlying mechanisms are comparable to those involved in the carcinogenicity of many other environmental carcinogens (see Scheme 3). Exposure, metabolism, DNA damage, repair of the damage, mutations and tumors all result from or are impacted by the etiological agent. For heterocyclic amines all these steps fit into the etiological pathway.

4. Extrapolation of animal carcinogenesis studies to humans—which human?

Most chemical carcinogens are not carcinogenic per se but must be metabolized by a family of cytochrome P450



Scheme 3. There are a number of complex steps between carcinogen formation and cancer. They should make mechanistic sense and contribute to the parts of an etiological puzzle before implying causation of human cancer.

enzymes to chemically reactive electrophiles prior to reacting with DNA to initiate a carcinogenic response. These same cytochrome P450 enzymes – as well as enzymes that act on the metabolic products of the cytochromes P450 (e.g. glucuronyl transferase, glutathione *S*-transferase and others) – also metabolize chemicals by inactivation pathways, and the relative amounts of enzymes that metabolically activate and detoxify the chemical will determine whether it is carcinogenic. Because both genetic and environmental factors influence the levels of enzymes that metabolically activate and detoxify chemicals, these factors can influence carcinogenic risk.

4.1. Species differences in the metabolism of carcinogens and drugs

Large interspecies differences exist in the rates of metabolism of foreign chemicals and in the profile of metabolites formed. Since species differences in the metabolism of carcinogens can influence the carcinogenic response, these differences are important to consider in extrapolation of animal carcinogenesis data to humans. For instance, the *N*-hydroxylation of *N*-2-fluorenylacetamide (FAA; 2-acetylaminofluorene) to a proximate carcinogenic metabolite occurs in rats, mice and humans but not in the guinea pig or Steppe lemming [24]. Accordingly, FAA is carcinogenic in rats and mice but not in the guinea pig or Steppe lemming. Since humans *N*-hydroxylate FAA, it is likely that FAA would be carcinogenic in humans. The metabolism of FAA to the inactive ring hydroxylated 7-OH FAA and its carcinogenic N-OH-FAA metabolite in several animal species as measured by urinary excretion is shown in Table 4. Since FAA was once considered for human use as a pesticide, it is fortunate that the initial carcinogenicity study with this compound was done in the rat instead of the guinea pig. These and other related observations indicate that it is important to evaluate the potential carcinogenicity of chemicals in an animal species with the same profile of metabolic capabilities as humans. Comparative studies on the metabolic profile of new chemicals by experimental animals and humans can be done with liver microsomes, purified cytochromes P450 and/or in vivo. Extrapolation of animal data to humans should take into consideration the large interindividual differences that occur in the metabolism of chemicals in the human population.

Table 4
Comparison of the metabolism of FAA in several species

Species	Carcinogenicity	Percent of dose	
		N-OH–FAA	7-OH–FAA
Guinea pig	–	0	72
Steppe lemming	–	Trace	42
Rat	+	0.3–15	19–27
Mouse	+	1.8–2.3	16–20
Rabbit	+	13–30	15–29
Hamster	+	15–20	35–39
Dog	+	5.2	0.9
Cat	+	1.5	11
Monkey	?	1.8–2.7	9–18
Man	?	4–14	25–30

Data compiled by Weisburger et al. [24].

4.2. Interindividual differences in the metabolism of drugs and carcinogens

There are large person-to-person differences in the rates of metabolism of drugs and carcinogens. Large differences in the metabolic activation of benzo[a]pyrene 7,8-dihydrodiol and aflatoxin B_1 to mutagenic metabolites and in the metabolism of benzo[a]pyrene to noncarcinogenic phenolic metabolites by ten surgical liver biopsy samples from different individuals are shown in Fig. 1 [25,26].

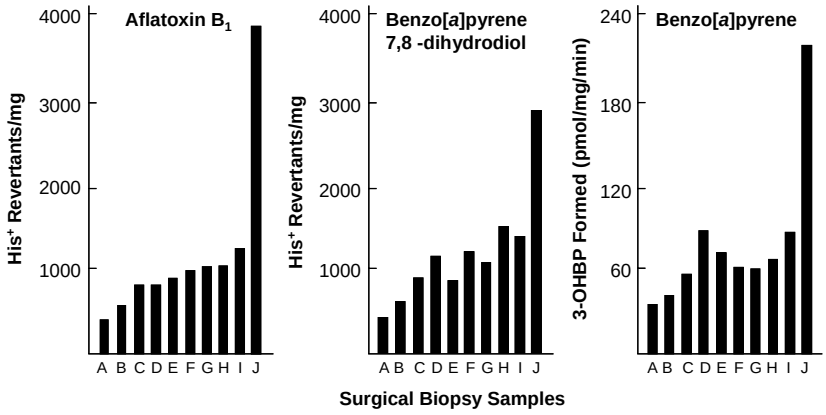


Fig. 1. Interindividual differences in the metabolism of carcinogens by surgical biopsy samples of 10 different human livers. The metabolism of benzo[a]pyrene (BP) to fluorescent phenols, expressed as 3-hydroxy-BP (3-HOBP), and the metabolism of aflatoxin B_1 and BP 7,8-dihydrodiol to mutagens were determined by incubation of the substrate with liver microsomes and NADPH. Each liver biopsy was taken for medical reasons, and only histologically normal samples were used for the metabolism studies (taken from refs. [25,26]).

Person-to-person differences in the metabolism of drugs and carcinogens are caused by both genetic and environmental factors. Many studies have demonstrated polymorphisms in genes that code for specific drug-metabolizing enzymes, and mutations in these genes can lead to impaired drug metabolism and altered drug action in patients [27]. Examples of environmental factors that influence the metabolism of drugs and carcinogens in humans include diet, smoking, alcohol ingestion, drug administration, ingestion of herbal remedies, exposure to environmental pollutants and disease states [26,28]. Because of these considerations, determination of an individual's ability to metabolically activate and detoxify chemical carcinogens requires both phenotyping and genotyping methods. Availability of simple methods for large scale testing is currently limited, and accordingly, it will be important to develop simple, rapid and accurate methods for both genotyping and phenotyping individuals that will contribute to assessment of an individual's ability to metabolically activate and detoxify environmental chemical carcinogens.

4.3. Effect of environmental context on carcinogenesis by chemicals

Whether a chemical is a carcinogen, an anticarcinogen or neither depends on the environmental context. Although 3-methylcholanthrene is a carcinogen in many experimental animal models, it inhibits the hepatocarcinogenic effects of 3′-methyl-4-dimethylaminoazobenzene and FAA by enhancing the metabolic detoxification of these chemicals [29–31]. Although TCDD (dioxin) causes liver tumors in rats and is a tumor promoter in 7,12-dimethylbenz[a]anthracene-initiated HRS/J hairless mice [32,33], TCDD inhibits the formation of spontaneous breast tumors in rats [32] and inhibits initiation of tumor formation by both 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene in mouse skin by enhancing the metabolic detoxification of these hydrocarbons [34,35]. In additional studies, topical applications of 1α,25-dihydroxyvitamin D_3 or all-*trans* retinoic acid inhibit TPA-induced tumor promotion in 7,12-dimethylbenz[a]anthracene-initiated mouse skin, but these compounds enhance 7,12-dimethylbenz[a]-anthracene-induced complete carcinogenesis in mouse skin (Table 5) [36–40].

Epidemiology studies indicate that daily supplements of 20–30 mg of β-carotene are associated with an increased risk of lung cancer in smokers who also drink alcoholic beverages [41]. In contrast to the adverse effects of β-carotene in smokers who drink alcoholic beverages, daily supplements of 25 mg of β-carotene inhibit the recurrence of colorectal adenomas in persons who do not smoke or drink alcoholic beverages [42]. These results indicate that a chemical may be carcinogenic in one experimental setting and an anticarcinogen in another, demonstrating the importance of environmental context as a variable in assessing carcinogenic hazard.

Table 5
Effects of 1α,25$(OH)_2D_3$ and all-*trans* retinoic acid on tumor promotion by TPA and complete carcinogenesis by DMBA

Experiment	Treatment	% Tumor bearing animals	Tumors/mouse (mean ± S.E.)
1	TPA	92	20.0 ± 2.5
	TPA + VD_3 (0.5 nmol)	63	3.9 ± 1.0
	TPA + RA (2.0 nmol)	33	1.3 ± 0.5
2	DMBA	63	1.20 ± 0.26
	DMBA + VD_3 (0.5 nmol)	100	5.67 ± 0.76
	DMBA + RA (0.5 nmol)	80	2.57 ± 0.43
	DMBA + RA (25 nmol)	93	8.40 ± 1.13

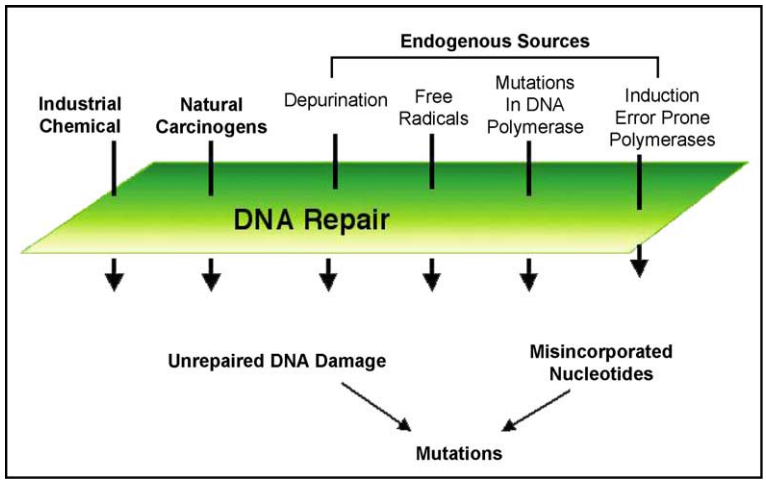
In experiment 1, CD-1 mice previously initiated with 200 nmol of 7,12-dimethylbenz[a]anthracene (DMBA) were treated topically with all-*trans* retinoic acid (RA) or 1α,25-dihydroxyvitamin D_3(VD_3) together with 5 nmol TPA twice a week for 15 weeks. In experiment 2, animals were treated with VD_3, RA or solvent vehicle 1 h prior to treatment with 50 nmol DMBA twice a week for 16 weeks. Mice that were treated twice weekly with VD_3 or all-*trans* retinoic acid in the absence of DMBA did not develop any tumors (taken from refs. [37,38,40]).

5. Multiple mutations in cancers: sources and consequences

5.1. Cancer is a chronic disease

In the case of solid tumors there is a 20–40-year interval from the time of exposure of an individual to a chemical or viral carcinogen until the clinical detection of a tumor. By the time a tumor is apparent, cancer cells have acquired the ability to divide where normal cells ought not, to invade adjacent cellular architectures, to metastasize and to kill the host. Many of these phenotypes can be the result of mutations that accumulate as tumors progress. Mutations can be defined as a change in the nucleotide sequence of DNA. These can arise as a result of DNA damage or by the incorporation of non-complementary nucleotides during DNA synthetic processes. Sources of DNA damage can be broadly divided into two categories: those that result from exogenous agents such as chemicals, viruses, and irradiation and those cause by reactive molecules generated by normal cellular processes. Normal cellular processes that damage DNA include the generation of reactive oxygen and nitrogen species, alkylation, depurination, and cytidine deamination [43]. The magnitude of DNA damage by normal cellular processes is enormous; for example, it has been estimated that approximately ten thousand depurinated sites are generated per cell per day [44] and an even larger number of alterations result from the generation of reactive oxygen species [45] (Scheme 4).

Against this extensive DNA damage is an armamentarium of DNA repair systems with overlapping specificities. These systems continuously monitor the genome and repair sites of DNA damage. So far, over 130 DNA repair gene products have been identified [46]. Pathways of DNA repair include base-excision repair, nucleotide excision repair,



Scheme 4. Equilibrium between DNA damage and DNA repair. Above the screen are listed exogenous and endogenous sources of DNA damage. Below the screen are the small number of DNA alterations that escape DNA repair and result in mutagenesis.

transcription-coupled repair, mismatch repair, and even direct reversal of DNA damage. In addition, cell cycle control genes such as p53 monitor cell replication, halting the cycle and as a result allowing additional time for DNA repair, when required. Loss of checkpoint controls result in aberrant DNA synthesis or mitotic segregation. The high efficiency of these mechanisms for DNA repair guarantees that only a few of the tens of thousands of DNA lesions are still present at the time of DNA replication that have the potential to cause mutations.

In normal cells DNA replication and chromosomal segregation are exceptionally accurate processes. Measurements of mutagenesis of cells grown in culture yield values of approximately 2×10^{-10} single base-substitutions/nucleotide in DNA/cell division or 1×10^{-7} mutations gene/cell division [47]. An even lower frequency has recently been demonstrated using stem cells in culture [48] and it is generally believed that tumors arise from stem cells. Taking into account this very low mutation frequency, it seems improbable that the spontaneous mutation rate in normal cells is sufficient to generate the large numbers of genetic alterations that are observed in human cancer cells. If one assumes that a cancer arises in a single stem cell, then the spontaneous mutation rate would only be adequate to account for less than one mutation per tumor.

Based upon the disparity between the infrequency of spontaneous mutations and the large numbers of mutations reported in human tumors, it was postulated that cancers must exhibit a mutator phenotype [49]. The expression of a mutator phenotype would be an early event in cancer progression. It could arise by mutations in different genes that normally function to maintain genetic integrity. Attractive candidates would be mutations in DNA polymerases that render them error-prone, mutations in DNA repair genes that render them inefficient or mutations in genes involved in chromosomal segregation. Mutations in these genes or in the pathways in which they function could exceed cellular capacity for DNA repair and result in the accumulation of multiple mutations throughout the genome. Amongst the thousands of mutations that ensue in a cancer would be mutations in oncogenes and tumor suppressor genes that confer a malignant phenotype. The concept of a mutator phenotype is not at variance with the two-hit model of Knudsen for the origin of retinoblastoma [50] or the limited number of phenotypes that have been postulated to be required for tumorigenesis [51]. A mutator phenotype could account for the large numbers of mutations present in cancers and provide a mechanism for the generation of mutations in a limited number of critical cancer related genes.

Mutations in cancer cells cover a wide spectrum, from chromosomal alterations that encompass millions of nucleotides to point mutations that involve only a few nucleotide substitutions. Multiple chromosomal alterations have been identified in most types of tumors and involve translocations, deletions, amplifications and aneuploidy. While there are diagnostic chromosomal aberrations that occur at high frequencies in certain tumors, there is also a striking heterogeneity of chromosomal alterations in cancer cells within most tumors. In some tumors there is evidence for a sequential order for mutations in key genes during tumor progression [52]. However, the order of chromosomal alterations has not been reported to be invariant or to occur within all cancer cells in a tumor. Measurements of the number of copies of segments of the genome in tumor cells (DNA copy number) and the loss of pieces of DNA (loss of heterozygosity) have established that many tumors harbor as many as 40 chromosomal alterations, each involving millions of genes [53]. Careful studies on isolated single tumor cells have documented that large numbers of changes that occur in each individual cancer cell [54]. The first direct evidence to support a mutator phenotype at the level of small changes in nucleotide sequence was provided by the demonstration that patients with hereditary non-polyposis colon cancer exhibited changes in the length of repetitive

nucleotide sequences in association with mutations in genes involved in mismatch repair [55]. It is presumed that these changes are due to slippage by DNA polymerases. Similar changes, but at lower frequencies, have been reported in a variety of tumors that are not known to be associated with mutations or silencing in mismatch repair genes [56].

A mutator phenotype could be generated by mutations in genes that normally function to guarantee genetic stability. These mutations presumably arise via random DNA damage by environmental or endogenous agents. One mechanism for the generation of a mutator phenotype would be via mutations in DNA repair genes that result in diminished function (Scheme 4). A number of mouse models have been constructed harboring deletions in DNA repair genes. Many of these result in a variety of cancers. Moreover, there are inherited human diseases with mutations in DNA repair genes. The most instructive example is xeroderma pigmentosum; mutations in genes that repair UV-induced DNA damage result in skin cancer in individuals exposed to UV-irradiation [57]. Enhanced mutagenesis can also be the result of increased misincorporation by DNA polymerases. A mouse model has been constructed involving the replacement of the major replicating DNA polymerase-delta with a mutant allele that lacks proofreading activity. These mice exhibit a high incidence of lymophomas as well as a variety of epithelial tumors. Thus, both mouse models [58] and human disease demonstrate that the expression of a mutator phenotype can be causally associated with cancer. These studies establish that a mutator phenotype is sufficient to result in malignancy but it remains to be demonstrated that it is a necessary requirement in humans.

It is instructive to consider the arguments that have been raised against a mutator phenotype hypothesis in cancer. First, most mutations are detrimental and thus a large increase in mutation frequency would be lethal. Second, is the proposal that aneuploidy is the initiating event in the conversion of normal cells to cancer cells [59]. Third, mathematical models of tumor progression in colon cancer can account for large numbers of mutations without invoking a mutator phenotype [60]. Finally, sequencing of cDNA in tumor cell lines has so far failed to reveal large numbers of mutations [61]. It is appropriate to point out that normal colon stem cells undergo large numbers of cell divisions and this is not a general property of many tissues that give rise to malignancies. The sequencing studies would not detect random events after the last round of clonal selection, and do not encompass non-synonymous substitutions and sequence changes in introns where mutation accumulation would be most pronounced.

It remains to be determined whether the acquisition of a mutator phenotype is a necessary event during tumor progression. The presence of multiple random mutations in human tumors has important implications. First, it provides a monitor for the malignant state of a tumor and may allow for stratification of tumors. Tumors harboring fewer mutations might be less likely to become resistant to chemotherapeutic agents. Second, it may be utilized to calibrate chronic exposure of individuals to carcinogens or to measure the susceptibility of different populations to different carcinogens. Third, the presence of thousands of random mutations in individual cancer cells, suggests that within a clinically detected tumor, comprising 10^8 cells, there are cancer cells that harbor mutations rendering them resistant to any chemotherapeutic agent. Lastly, if the acquisition or expression of a mutator phenotype is rate-limiting for tumor progression, then inhibiting mutation accumulation may delay carcinogenesis. Assuming a 20-year average progression to clinical cancer in adults, even a two-fold reduction in the rate of mutation accumulation would provide a significant clinical delay, as much as 20 years. The design and utilization of drugs that reduce DNA damage could delay the onset of cancer and thus significantly reduce cancer deaths.

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