12 CHEMICAL CARCINOGENESIS

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Chemical carcinogenesis is a multi-stage process that begins with exposure, usually to complex mixtures of chemicals that are found in the human environment (Table 12.1).[1–3] Once internalized, carcinogens frequently are subjected to competing metabolic pathways of activation and detoxication, although some reactive environmental chemicals can act directly. Variations among individuals in the metabolism of carcinogens, together with differences in DNA-repair capacity and response to tumor promoters, govern the relative risk of an individual.[4] The initial genetic change that occurs as the result of chemical–DNA interaction is termed *tumor initiation*. Thus, initiated cells are irreversibly altered and are at greater risk of malignant conversion than are normal cells. The epigenetic effects of tumor promoters facilitate the clonal expansion of the initiated cell.[5] This selective, clonal growth advantage results in the formation of a focus of preneoplastic cells. These cells are more vulnerable to progress toward tumorigenesis because they present a larger, more rapidly proliferating target population for the further action of chemical carcinogens, oncogenic viruses, and other cofactors. Additional genetic changes occur, and consequently, the accumulation of mutations, which may activate proto-oncogenes and inactivate tumor-suppressor genes, leads to malignant conversion, tumor progression, and metastasis. The underlying genetic mechanisms that regulate chemical carcinogenesis are becoming increasingly well understood, and the insights generated have assisted in the development of methodologies designed to assess human cancer risk and susceptibility factors. The results of these latter studies are further intended to mold strategies for cancer prevention.

MULTI-STAGE CARCINOGENESIS

Carcinogenesis can be divided conceptually into four steps: tumor initiation, tumor promotion, malignant conversion, and tumor progression (Fig. 12.1). The distinction between initiation and promotion was recognized through studies involving both viruses and chemical carcinogens.[6,7] This distinction was formally defined in a murine skin carcinogenesis model where mice were treated topically with a single dose of a polycyclic aromatic hydrocarbon (i.e., initiator), followed by repeated topical doses of croton oil (i.e., promoter).[6] This mechanism also has been shown to operate in a range of other rodent tissues, including the bladder, colon, esophagus, liver, lung, mammary gland, stomach, and trachea.[5] During the last 50 years, the sequence of events comprising chemical carcinogenesis has been systematically dissected and the paradigm increasingly refined. It is now recognized that carcinogenesis requires the malignant conversion of hyperplastic cells from a benign to malignant state, and that invasion and metastasis are manifestations of further genetic and epigenetic changes.[8] Study of this process in humans is necessarily indirect. Measures of age-dependent cancer incidence have shown, however, that the rate of tumor development is proportional to the sixth power of time, suggesting that at least four to six independent steps are necessary.[9] Partial scheduling of specific genetic events in this process for some cancers has been possible. Sequential genetic changes occurring during the development of cancer of the head and neck[10] or colon cancer[11] are examples.

TUMOR INITIATION Tumor initiation results from irreversible genetic damage. For mutations to accumulate, they must arise in cells that proliferate and survive the lifetime of the organism. A chemical carcinogen causes a genetic error by modification of the molecular structure of DNA that can lead to a mutation during DNA synthesis. Most often, this is brought about by formation of an adduct between the chemical carcinogen or one of its functional groups and a nucleotide in DNA.[5] (The process by which this occurs for the major classes of chemical carcinogens is discussed in detail under Carcinogen Metabolism). In general, a positive correlation is found between the amount of carcinogen–DNA adducts that can be detected in animal models and the resulting number of tumors that develop.[12–14] Thus, tumors rarely develop in tissues that do not form carcinogen–DNA adducts. Carcinogen–DNA adduct formation is central to theories of chemical carcinogenesis, and it can be considered to be a necessary, but not a sufficient, prerequisite for tumor initiation. DNA adduct formation that results in either the activation of a proto-oncogene or the inactivation of a tumor suppressor gene can be considered to be a tumor initiating event (see Tumor Progression, Oncogenes, and Tumor-Suppressor Genes).

TUMOR PROMOTION Tumor promotion comprises the selective clonal expansion of initiated cells. Because the accumulation rate of mutations is proportional to the rate of cell division, or at least the rate at which stem cells are replaced, it follows that clonal expansion of initiated cells produces a larger population of cells that are at risk of further genetic changes and malignant conversion.[8,15] Tumor promoters generally are nonmutagenic, not carcinogenic alone, and often (but not always) able to mediate their biologic effects without metabolic activation. These agents are characterized by their ability to reduce the latency period for tumor formation after exposure of a tissue to a tumor initiator or to increase the number of tumors formed in that tissue. In addition, they induce tumor formation in conjunction with a dose of an initiator that is too low to be carcinogenic alone. Chemicals or agents capable of both tumor initiation and promotion are known as *complete carcinogens*; examples are benzo[*a*]pyrene and 4-aminobiphenyl.

Croton oil (isolated from *Croton tiglium* seeds) has been used widely as a tumor promoter in murine skin carcinogenesis, and the mechanism of action for its most potent constituent, 12-tetradecanoylphorbol-13-acetate, via activation of protein kinase C is arguably the best understood among tumor promoters.[14] Protein kinase C is a calcium-phospholipid-dependent enzyme family that, when activated, causes phosphorylation of critical substrates and stimulates a cascade of epigenetic changes that can lead to cell growth.[16,17] Among the changes observed in cells treated with 12-O-tetradecanoylphorbol-13-acetate are altered ion flux across the cell membrane, altered hormone binding, and inhibition of cell-cell communi-

Table 12.1. Selected Examples of Human Chemical Carcinogenesis

Organ System (specific pathology)	Chemical Carcinogen	Cocarcinogen
Lung	Metals: As, Be, Cd, Cr, Ni	
	BCME*	
(Small cell and squamous cell)	Tobacco smoke	Asbestos
	Diesel exhaust	
Pleural mesothelium	Asbestos	
Oral cavity	Smokeless tobacco	
	Betel quid	Slaked lime [$Ca(OH)_2$]
Esophagus	Tobacco smoke	Alcohol
Nasal sinuses	Snuff	Powdered glass
	Isopropylalcohol	
Skin	Cutting oil •	
(Scrotum)	Coal soot†	
Liver	Aflatoxin B_1	HBV*
(Angiosarcoma)	Vinyl chloride	Alcohol
Bladder	Aromatic amines (e.g., 4-ABP* and benzidine)	
	Aromatic amines from tobacco smoke‡	
ALL*	Benzene	
Lymphatic and hemapoietic malignancies	Ethylene oxide	

*BCME = *Bis* chloromethyl ether; HBV = hepatitis B virus; 4-ABP = 4-aminobiphenyl; ALL = acute lymphoblatic leukemia.

† Early report of occupational chemical carcinogenesis from 225 years ago.[1]

‡ Strong circumstantial evidence.[2] A comprehensive treatise on the evaluation of the carcinogenic risk of chemicals to humans can be found in the ongoing IARC monograph program initiated in 1971.[3]

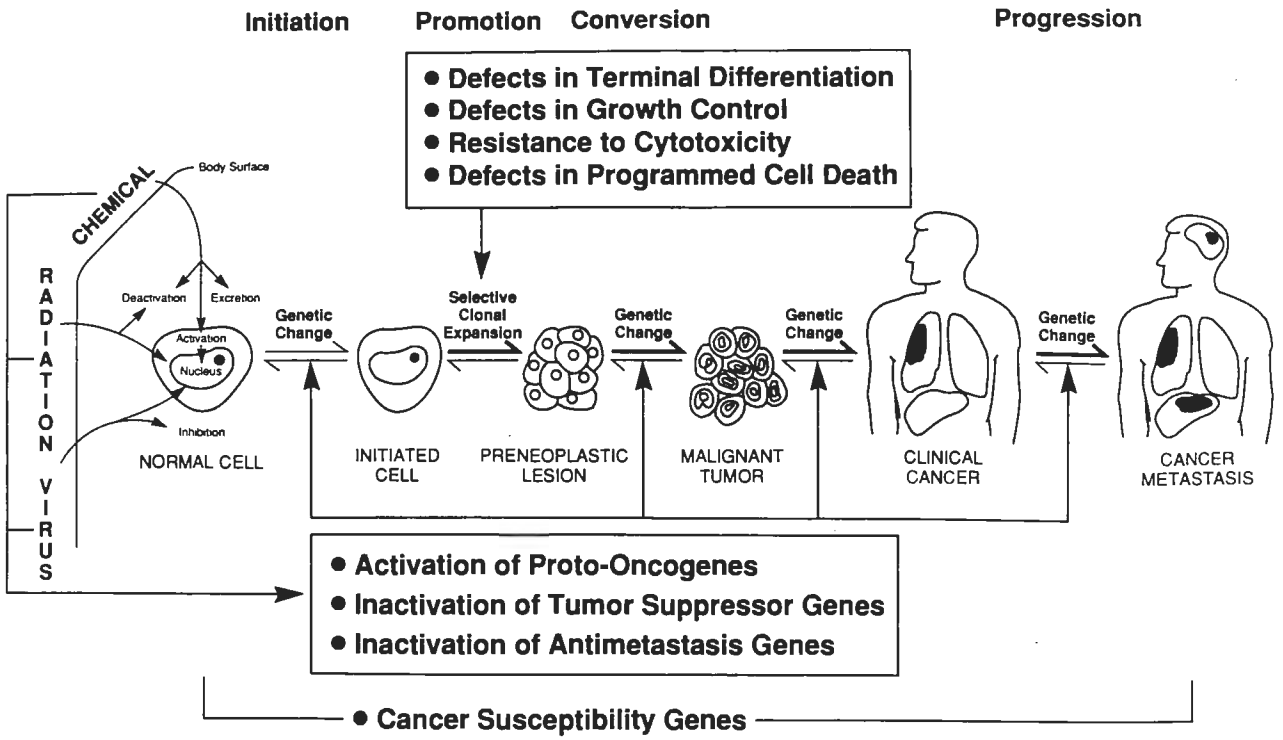

Figure 12.1. Multi-stage chemical carcinogenesis can be conceptually divided into four stages: tumor initiation, tumor promotion, malignant conversion, and tumor progression. Activation of proto-oncogenes and inactivation of tumor suppressor genes are mutational events that occur as the result of covalent damage to DNA caused by chemical exposures. The accumulation of mutations and not necessarily the order in which they occur constitutes multistage carcinogenesis.[64]

cation. With increasing recognition of redundancy in the signal transduction cascade, however, it is possible to appreciate that the effects of 12-O-tetradecanoylphorbol-13-acetate are even more diverse. Prostaglandin synthesis, which also is associated with tumor promotion, occurs because of stimulation of the arachidonic acid cascade that is mediated by protein kinase C. The cellular response to protein kinase C activation can result in the modification of differentiation or cell proliferation and is cell-type dependent. The cell-type–dependent differential response may be explained by the fact that protein kinase C is a multi-gene family, the members of which are differentially expressed among animal species and tissue types.

Identification of new tumor promoters in animal models has accelerated with the increasingly sophisticated development of model systems designed to assay for tumor promotion. Furthermore, ligand-binding properties also can be determined in recombinant protein kinase C isozymes that are expressed in cell cultures.[14] Chemicals, complex mixtures of chemicals, or other agents that have been shown to have tumor-promoting properties include dioxin, benzoyl peroxide, macrocyclic lactones, bromomethylbenzanthracene, anthralin, phenol, saccharin, tryptophan, dichlorodiphenyltrichloroethane (DDT), phenobarbital, cigarette-smoke condensate, polychlorinated biphenyls (PCBs), teleocidins, cyclamates, estrogens and other hormones, bile acids, ultraviolet light, wounding, abrasion, and other chronic irritations (i.e., saline lavage).[5] It also has been noted that protein kinase C is activated and cellular diacylglycerol elevated in laboratory animals maintained on high-fat diets.[18,19]

Malignant Conversion Malignant conversion is the transformation of a preneoplastic cell into one that expresses the malignant phenotype. This process requires further genetic changes. The total dose of a tumor promoter is less important than frequently repeated administrations, and if the administration of a tumor promoter is discontinued before malignant conversion has occurred, premalignant or benign lesions may regress. The contribution of tumor promotion to the process of carcinogenesis is the expansion of a population of initiated cells, which will then be at risk for malignant conversion. Conversion of a fraction of these cells to malignancy will be accelerated in proportion to the rate of cell division and the quantity of dividing cells in the benign tumor or preneoplastic lesion. In part, these further genetic changes may result from infidelity of DNA synthesis.[20] The relatively low probability of malignant conversion can be increased substantially by the exposure of preneoplastic cells to DNA-damaging agents,[5] and this process may be mediated through the activation of proto-oncogenes and inactivation of tumor-suppressor genes.

Tumor Progression Tumor progression comprises the expression of the malignant phenotype and the tendency of already malignant cells to acquire more aggressive characteristics with time. Metastasis also may involve the ability of tumor cells to secrete proteases that allow invasion beyond the immediate location of the primary tumor. A prominent characteristic of the malignant phenotype is the propensity for genomic instability and uncontrolled growth.[21] During this process further genetic changes can occur, again including the activation of proto-oncogenes and the functional loss of tumor-suppressor genes. Proto-oncogenes frequently are activated by two major mechanisms: in the case of the *ras* gene family, point mutations are found in highly specific regions of the gene (i.e., the 12th, 13th, 59th, or 61st codons), and members of the *myc*, *raf*, *her-2*, and *jun* multi-gene families can be overexpressed, sometimes involving amplification of chromosome segments containing these genes. Some genes are overexpressed if they are translocated and become juxtaposed to a powerful promoter (e.g., the relationship of *bcl-2* and immunoglobulin gene promoter regions in B-cell malignancies). Loss of function of tumor-suppressor genes usually occurs in a bimodal fashion, and most frequently involves point mutations in one allele and loss of the second allele by deletion, recombinational event, or chromosomal nondisjunction. These phenomena confer to the cells a growth advantage as well as the capacity for regional invasion and, ultimately, distant metastatic spread. Despite evidence for an apparent scheduling of certain mutational events, it is the accumulation of these mutations, and not the order or the stage of tumorigenesis in which they occur, that appears to be an important determining factor.[10,11]

GENE–ENVIRONMENT INTERACTIONS AND INTERINDIVIDUAL VARIATION

A cornerstone of human chemical carcinogenesis is the concept of gene–environment interactions. Potential interindividual susceptibility to chemical carcinogenesis may well be defined by genetic varia-

tions in the host elements of this compound system. Functional polymorphisms, among humans, of proteins that have, or may have, a role in chemical carcinogenesis include enzymes that metabolize (i.e., activate and detoxify) xenobiotic substances, enzymes that repair DNA damage, cell surface receptors that activate the phosphorylation cascade, and cell cycle control genes (i.e., oncogenes and tumor suppressor genes that are elements of the signal transduction cascade).

When chemicals or xenobiotics encounter biologic systems, they become altered by metabolic processes. This is an initial facet of the gene–environment interaction. Interindividual variation in carcinogen metabolism and macromolecular adduct formation arises from such processes and was recognized 20 to 25 years ago.[4,22] The cytochrome P-450 (CYP) multi-gene family is largely responsible for the metabolic activation and detoxication of many different chemical carcinogens in the human environment.[23–25] Cytochrome P-450s are phase I enzymes that act by adding an atom of oxygen onto the substrate; they also are inducible by polycyclic aromatic hydrocarbons and chlorinated hydrocarbons. Phase II enzymes act on oxidized substrates and also contribute to xenobiotic metabolism.[26] Some phase II enzymes are methyltransferases, acetyltransferases, glutathione transferases, uridine 5'-diphosphoglucuronosyl transferases, sulfotransferases, nicotinamide-adenine dinucleotide (NAD)- and nicotinamide-adenine dinucleotide phosphate (NADP)-dependent alcohol, aldehyde and steroid dehydrogenases, quinone reductases, NADPH diaphorase, azo reductases, aldoketoreductases, transaminases, esterases, and hydrolases.[27] The pathways of activation and detoxication are often competitive, providing yet further potential for individual differences in propensity for carcinogen metabolism. This scenario is further complicated by a process of enzyme induction or inhibition, where genes responsible for carcinogen metabolism can be upregulated or repressed by certain chemical exposures.[27,28]

A second facet of gene–environment interaction occurs when the chemical alters the gene. Once a procarcinogen has been metabolically activated to an ultimate carcinogenic form, it can bind covalently to cellular macromolecules, including DNA. This damage to DNA can be repaired by several mechanisms.[29,30] Differences in the rates and fidelity of DNA repair potentially influence the extent of carcinogen adduct formation (i.e., biologically effective dose) and, consequently, the total amount of genetic damage that accumulates. The consequence of polymorphisms in genes controlling the cell cycle (serine/threonine kinases, transcription factors, cyclins, cyclin-dependent kinase inhibitors, and cell surface receptors) is much less clear. However, molecular epidemiologic evidence suggests that certain common variants of these types of gene have a role in susceptibility to chemical carcinogenesis.[31,32] Evaluation of polymorphisms as potential biomarkers of susceptibility in the human population are discussed under Molecular Epidemiology.

CARCINOGEN METABOLISM

The first chemically identified carcinogens were the polycyclic aromatic hydrocarbons.[28,33] They are composed of variable numbers of fused benzene rings that form from incomplete combustion of fossil fuels and vegetable matter, and they are common environmental contaminants. The polycyclic aromatic hydrocarbons are chemically inert and require metabolism to exert their biologic effects.[34] This is a multi-step process, involving initial epoxidation (cytochrome P-450, CYP1A1 an inducible isoform), hydration of the epoxide (epoxide hydrolase), and subsequent epoxidation across the remaining olefinic bond (principally CYP3A4). The result is the formation of the ultimate carcinogenic metabolite a diol-epoxide.[35] Variations among humans in the levels of the enzymes involved has been documented.[22] The biology of CYP1A1 metabolism has been elucidated, providing a molecular basis for inducibility and interindividual variation.[36–38]

Metabolic activation of benzo[*a*]pyrene leads to the formation of the bay-region benzo[*a*]pyrene-7,8-diol 9,10-oxide.[28,39] This vicinal diol-epoxide is asymmetric, and eight stereoisomers are possible. The reactivity of each isomer is variable, and the isomers are formed in varying proportions by metabolism. Biologic response to the different enantiomers in mammalian systems suggests that the *anti* forms are the most active mutagens and carcinogens and the *syn* forms are the least active.

The arene ring of benzo[*a*]pyrene-7,8-diol 9,10-oxide opens spontaneously at the 10-position, giving a highly reactive carbonium ion that can form a covalent addition product (i.e., adduct) with cellular macromolecules, including DNA. These adducts cause the DNA to be damaged, either by their persistence and consequent interference with replication or by aberrant DNA repair. The same basic tenet holds for carcinogen–DNA adducts of other chemical classes that may be activated by different metabolic pathways (Fig. 12.2).

Aromatic amines are another class of chemical carcinogens, and 4-aminobiphenyl is thought to be responsible for bladder cancer among workers in the rubber industry. This and many related compounds are components of cigarette smoke, diesel exhaust, and the pyrolysis of certain foods. In addition, nitrated polycyclic aromatic hydrocarbons are also environmental contaminants resulting from the incomplete combustion of vegetable matter and diesel fuel, and they are related to aromatic amines by nitroreduction.

The metabolic activation of aromatic amines is complex.[39] They can be converted to an aromatic amide that is catalyzed by an acetyl coenzyme A–dependent acetylation. The acetylation phenotype varies among the population. Persons with the rapid acetylator phenotype are at higher risk of colon cancer,[40,41] whereas those who are slow acetylators are at risk of bladder cancer.[42] This latter association may result from the fact that activation of aromatic amines by *N*-oxidation is a competing pathway for aromatic amine metabolism. Also the *N*-hydroxylation products, when protonated (by the acid conditions in the urinary bladder), form reactive electrophiles that bind covalently with DNA or proteins to produce macromolecular damage.

An initial activation step for both aromatic amines and amides is *N*-oxidation by CYP1A2. This cytochrome P-450 is inducible by phenobarbital, and because it is also responsible for the 3-demethylation of 1,3,7-trimethylxanthine (i.e., caffeine), the distribution of metabolic phenotypes in the population, as well as the disposition of an individual with respect to CYP1A2 metabolism, is relatively easy to determine.[43]

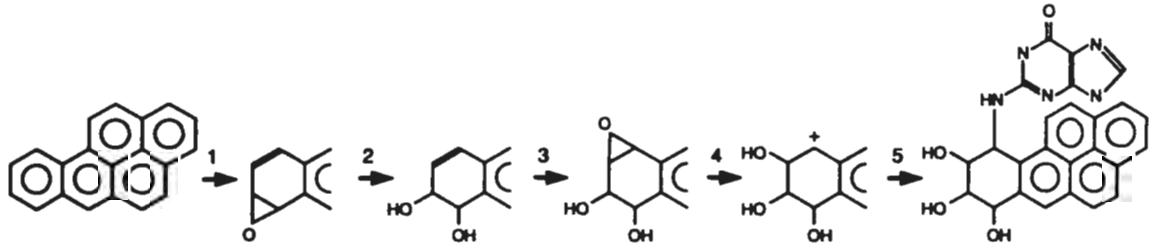

Figure 12.2. Metabolic activation of benzo[a]pyrene. (1) Cytochrome P-450 (CYP1A1) catalyzes initial epoxidation across the 1 - 2, 2 - 3, 4 - 5, 7 - 8 (shown), 9 - 10 and 11 - 12 positions. (2) With the exception of the 1 - 2 and 2 - 3 oxides which convert to phenols, epoxide hydrolase catalyzes the formation of dihydrodiols. (3) Benzo[a]pyrene-7, 8-dihydrodiol is further metabolized at the olefinic double bond by cytochrome P450 (CYP1A1 and CYP3A4) to form a vicinal diol-epoxide (r7, t8-dihydroxy-c9, 10 epoxy-7,8,9,10-tetrahydroxybenzo[a]pyrene). (4) The highly unstable arene ring opens spontaneously to form a carbocation. (5) This electrophic species forms a covalent bond between the 10 position of the hydrocarbon and the exocylic amino group of deoxyguanosine.

The reaction of *N*-hydroxy-arylamines with DNA appear to be acid catalyzed, but they can be further activated by either an acetyl coenzyme A–dependent O-acetylase or a 3'-phosphoadenosine-5'phosphosulfate–dependent O-sulfotransferase. The *N*-arylhydroxamic acids, which arise from the acetylation of *N*-hydroxy-arylamines or *N*-hydroxylation of aromatic amides, are not electrophilic; therefore, they require further activation. The predominant pathway for this occurs through acetyltransferase-catalyzed rearrangement to a reactive *N*-acetoxy-arylamine. Sulfotransferase catalysis results in the formation of *N*-sulfonyloxy arylamides. This complex pathway results in two major adduct types: amides (i.e., acetylated) and amines (i.e., nonacetylated).

The heterocyclic amines are formed during the preparation of cooked food, primarily from the pyrolysis (> 150°C) of amino acids, creatinine, and glucose. They have been recognized as food mutagens,[44] and they have been shown to form DNA adducts and cause liver tumors in primates.[45] Compared with other carcinogens, their metabolism is less well understood, but *N*-hydroxylation is considered to be a necessary step. Because they are similar in structure to the aromatic amines, it is not surprising that they can be activated by CYP1A2. The *N*-hydroxy metabolites of 3-amino-1-methyl-5*H*-pyrido[4,3-b]indole (Trp-P-1), 2-amino-6-methyldipyrido[1,2-a:39, 29-d]imidazole (Glu-P-1), and 2-amino-3-methyl-imidazo-[4,5-f]quinoline (IQ) can react directly with DNA. Unlike, however, the aromatic amines, this reaction is not facilitated by acid pH. Enzymic O-esterification of *N*-hydroxy metabolites is important in the activation of these food mutagens, and the *N*-hydroxy metabolites also are good substrates for transacetylases. This suggests a possible role for these chemicals in the etiology of colorectal cancer in combination with the rapid acetylator phenotype.

Aflatoxins (aflatoxin B_1, B_2, G_1, and G_2) are metabolites of *Aspergillus flavus*. These fungal mutagens contaminate improperly stored cereals, grains, and nuts. A positive correlation exists between dietary aflatoxin exposure and incidence of liver cancer in the developing countries, where grain spoilage is high. Aflatoxins are activated by several cytochrome P-450s, including CYP2A3, CYP2A6, and CYP3A4.[23,46] Aflatoxin B_1 and G_1 have an olefinic double bond at the 8,9-position, and they are more mutagenic and carcinogenic than aflatoxin B_2 and G_2, which are saturated and have an ethylenic bond at this position. This implies that the olefinic 8,9-bond is the site of activation. Further support for this mechanism comes from studies of DNA adducts and the prevalence of *p53* mutations in liver cancer. In people with liver cancer from parts of China and Africa where food spoilage caused by molds is high, G:C to T:A transversions in codon 249 are frequent.[47] This phenomenon is consistent with metabolic activation of aflatoxin B_1.

Carcinogenic *N*-nitrosamines are ubiquitous environmental contaminants and can be found in food, alcoholic beverages, cosmetics, cutting oils, hydraulic fluid, rubber, and tobacco.[48] Tobacco-specific *N*-nitrosamines such as 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone are carcinogenic in a wide range of animal species, and they may account for the carcinogenic nature of snuff and chewing tobacco.[49] Endogenous nitrosation also can occur because of the reaction of an amine with nitrate alone or nitrite in the presence of acid. Thus, nitrite (used in curing meats) and L-cysteine in the presence of acetaldehyde (a metabolite of alcohol) form *N*-nitrosothiazolidine-4-carboxylic acid. The *N*-nitrosamines are activated primarily by CYP2E1, which is inducible by alcohol.

N-nitrosodimethylamine undergoes *a*-hydroxylation to form an unstable *a*-hydroxynitrosamine. The breakdown products are formaldehyde and methyl diazohydroxide. The alkyl groups of compounds, such as methyl diazohydroxide, are good leaving groups and, thus, are powerful methylating agents that can add a small functional group (small alkyl adduct as opposed to the bulky aryl adducts formed by the carcinogens discussed earlier) at more than 10 different sites in DNA. The tobacco-specific nitrosamines are not symmetric and also can form bulky adducts; 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone metabolism gives rise to either a positively charged pyridyloxobutyl ion or a positively charged methyl ion, both of which are able to alkylate DNA.[48,49]

DNA DAMAGE AND REPAIR

There are several ways in which the chemical structure of DNA can be altered by a carcinogen, including the formation of bulky aromatic-type adducts, alkylation (generally small adducts), oxidation, dimerization, and deamination. Chemical carcinogens also can cause epigenetic changes, such as alteration in DNA methylation status, that can lead to silencing of specific gene expression.[50] Carcinogen–DNA adducts vary in their promutagenic potential; the binding of benzo[*a*]pyrene-7,8-diol 9,10-epoxide to the exocyclic (N2) amino group of deoxyguanosine forms an aromatic adduct that resides within the minor groove of the double helix, and it is typical of polycyclic aromatic hydrocarbons (Plate 5, Fig. 12.3). Although this adduct appears to be the most common form, by far, of DNA damage induced by benzo[*a*]pyrene in mammalian systems, others are possible, including covalent binding of metabolites to deoxyadenosine.[28,39] Aromatic amine adducts are more complex, not only because they have both acetylated and nonacetylated metabolic intermediates but also because they form covalent bonds at the C8-, N2-, and sometimes O6-positions of deoxyguanosine as well as deoxyadenosine. The major adducts, however, are C8-deoxyguanosine adducts, which reside predominantly in the major groove of the DNA double helix.[39]

Although the evidence for the activation of aflatoxins B_1 and G_1 through hydroxylation of the olefinic 8,9-position is circumstantial, the structures of the adducts are known. They are formed at the N7-position of deoxyguanosine. They are relatively unstable and have a half-life of approximately 50 hours at neutral pH, with resulting depurination. The aflatoxin B1-N7-deoxyguanosine adduct also can undergo ring opening, to yield two pyrimidine adducts; alternativly, aflatoxin B_1-8,9-dihydrodiol could result. This latter possibility could restore the molecular structure of DNA if hydrolysis of the original adduct occurs, but a potentially promutagenic lesion would result if formation of the 8,9-dihydrodiol results from degradation of ring-open adduct forms.[51]

Alkylation of DNA can occur at many sites, either following the metabolic activation of certain *N*-nitrosamines or directly by the action of the *N*-alkylureas (*N*-methyl-*N*-nitrosourea) or the *N*-nitrosoguanidines. The protonated alkyl-functional groups that become available to form lesions in DNA generally attack the following nucleophilic centers: adenine (N1, N3, and N7), cytosine (N3), guanine (N2, O6, and N7), and thymine (O2, N3, and O4). Some of these lesions are known to be repaired (O6-methyldeoxyguanosine), while others are not (N7-methyldeoxyguanosine).[48,49] Furthermore, O6-methyldeoxyguanosine is a promutagenic lesion, whereas N7-methyldeoxyguanosine is not.

Oxy-radical damage can result in the modification of DNA to form thymine glycol or 8-hydroxydeoxyguanosine adducts. Three major pathways have been identified. Exposure to organic peroxides (catechol, hydroquinone, and 4-nitroquinoline-*N*-oxide) leads to this type of oxyradical damage; however, oxyradicals and hydrogen peroxide can be generated in lipid peroxidation and the catalytic cycling of some enzymes.[52] Cells also can be stimulated to produce peroxisomes by treatment with certain drugs and plasticizers.[53] Exposure to tumor promoters can indirectly increase oxyradical formation, and perhaps the best known relationship is that between the phorbol esters and inflammatory cells. In this system, mediated through protein kinase C and the subsequent activation of a membrane-localized pyridine nucleotide-dependent oxidase, oxyradical formation is highly correlated with the relative potencies of the different phorbol esters.[54] Correspondingly, promoters that do not stimulate the protein kinase C signal transduction cascade do not affect oxyradical production.

Another potentially mutagenic cause of DNA damage is the deamination of methylated cytosine residues in DNA. 5-methylcytosine comprises approximately 3% of deoxynucleotides. In this case, deamination at a CpG dinucleotide gives rise to a TpG mismatch. Repair of this lesion most often restores the CpG; however, repair may also cause a mutation (TpA).[55] Deamination of cytosine also can generate a C-to-T transition, if uracil glycosylation and G-T mismatch repair are inefficient. Oxyradicals can enhance the rate of deamination, so the activity of inducible nitric oxide synthase and production of high concentrations of nitric oxide could contribute to DNA damage by this mechanism.

DNA repair enzymes act at sites of DNA damage caused by chemical carcinogens, and six major mechanisms are known: direct DNA

repair, nucleotide excision repair, base excision repair, double-strand break repair, mismatch repair, and postreplication repair.[29,30] These have been characterized in lower organisms; such as yeast and bacteria. However, among recent advances is the cloning of more than 70 human genes involved in five of these DNA repair pathways.[56] These genes are responsible for the fidelity of DNA repair, and defects in these genes provides a biologic mechanism for the genetic defect leading to increased mutations or the mutator phenotype.[20]

Direct DNA repair is effected by DNA-alkyltransferases. These enzymes catalyze translocation of the alkyl moiety from an alkylated base (e.g., O6-methyldeoxyguanosine) to a cysteine residue at their active site, in the absence of DNA strand scission. Thus, one molecule of the enzyme is capable of repairing one alkyl lesion in DNA. The human O6-methyldeoxyguanosine DNA-methyltransferase has been mapped to chromosome 10q24.33 - qter.[56]

In nucleotide excision repair of DNA, lesion recognition, preincision, incision, gap-filling, and ligation are required. Therefore, it is not surprising that the so-called *excinuclease complex* comprises 16 or more different proteins. Large distortions caused by bulky DNA adducts (e.g., BPDE-dG and AAF-dC) are recognized by specific proteins that recognize DNA damage (e.g., *XPA*). Removal of the DNA damage is achieved by the action of the endonucleases (e.g., *XPF*, *XPG* and *FEN*). Then using the intact strand as a template, a patch is constructed by 5' to 3' polymerization (*polδ* and *polε*), and ligation of the free ends then occurs (p102). This type of transcription repair is strand specific, that is, the transcribed strand in a gene is preferentially repaired by comparison to the nontranscribed or DNA coding strand.[29] Nucleotide excision repair is a vital mechanism in humans and lack of this function results in xeroderma pigmentosum (XP).

Base excision repair also removes a segment of DNA containing an adduct; however, small adducts (e.g., 3-methyladenine) are generally the target so that there is overlap with direct repair. Removal of the adducted base is brought about by a glycosylase (e.g., *hOgg1*, *UDG*) and repair of the damaged strand is accomplished by the combined action of an apurinic endouclease (e.g., *HAP1*) that degrades a few bases on the damaged strand and a polymerase that synthesizes a "patch" in the 5' to 3' direction, using the undamaged strand as a template (e.g., *polβ*). The patch is then ligated by one of a number of ligases (DNA ligases: I, II, IIIα, IIIβ, IV).

DNA mismatches occasionally occur because of excision repair processes and involve incorporation of unmodified or conventional, but noncomplementary, Watson-Crick bases opposite each other in the DNA helix. Transition mispairs (G-T or A-C) are repaired by the mismatch repair process more efficiently than transversion mispairs (G-G, A-A, G-A, C-C, C-T, and T-T), probably because of differential recognition of the mispairings. Repair efficiency of mispairings also depends on their oligonucleotide environment for the same reason. Thus, mispairings in the G-C–rich regions are repaired more efficiently than those in the A-T–rich regions. The mechanism for the correction of mispairings is similar to that for nucleotide excision repair and resynthesis described earlier, but it generally involves the excision of large pieces of the DNA containing mispairings. Because the mismatch recognition protein is required to bind simultaneously the mismatch and an unmethylated adenine in a G-A-T-C recognition sequence, it removes the whole intervening DNA sequence. The parental template strand is then used by the polymerase to fill the gap. Relatively common mutations in *hPMS1, hPMS2, hMLH1, hMSH2, hMSH3* have been shown to predispose to human hereditary nonpoliposis colon cancer and glioma.[56]

Double-strand DNA breaks can occur from exposure to ionizing radiation and oxidation. Consequences of double-strand DNA breaks are inhibition of replication and transcription and loss of heterozygosity. Double-strand DNA break repair occurs through homologous recombination, joining of the free ends is mediated by a DNA-protein kinase in a process that also protects the ends from nucleolytic attack. The free ends of the DNA then undergo ligation by DNA ligase IV. Genes known to code for DNA-repair enzymes that participate in this process include: *XRCC4, XRCC5, XRCC6, XRCC7, hRAD51b, hRAD52, RPA* and *ATM*.[56]

Postreplication repair is a damage tolerance mechanism, and it occurs in response to replication of DNA on a damaged template. The DNA polymerase stops at the replication fork when DNA damage is detected on the parental strand. Alternatively, the polymerase proceeds past the lesion, leaving a gap in the newly synthesized strand. The gap is filled in one of two ways: either by recombination of the homologous parent strand with the daughter strand in a process that is mediated by the RecA protein; or, when a single nucleotide gap remains, mammalian DNA polymerases insert an adenine residue. Consequently, this mechanism may lead to recombinational events as well as base mispairing.

The rate, but not the fidelity, of DNA repair can be measured by adduct removal or unscheduled DNA synthesis. Substantial variations among individuals have been found in these rates.[4,57] Markedly reduced rates of excision repair are found in individuals with xeroderma pigmentosum, and these individuals are at known risk of ultraviolet light–induced skin cancer. Among the general population, however, an approximately five-fold variation in the rates of excision repair have been found in lymphocytes treated with carcinogens in vitro. An association also has been found between the reduced capacity of mononuclear leukocytes in vitro to repair aromatic amine adducts in individuals who have first-degree relatives with cancer. Up to 40-fold variations among humans in the activity of O6-alkylguanine-DNA alkyltransferase have been reported as well. DNA repair rates are inhibited by aldehydes, alkylating agents, and some chemotherapeutic drugs. Decreased DNA repair capacity also has been noted in the fibroblasts of patients with lung cancer, compared with those patients with melanoma or noncancer controls. For benzo[*a*]pyrene-7,8-diol 9,10-epoxide–DNA adducts, a unimodal distribution of repair rates is observed in lymphocytes, but interindividual variation has been found to be substantial.[57]

BIOLOGIC RESPONSE TO TUMOR PROMOTERS

The biologic effects of tumor-promoters lead to selective clonal expansion of initiated cells and are reversible, suggesting that the mechanism is epigenetic. These effects can be mediated through activation of the protein kinase C pathway. Resistance of initiated cells to phorbol ester–mediated terminal differentiation may relate to alteration in the expression of protein kinase C (via the initiating event). Evidence, to date, supports this molecular model for the differential effects of tumor promoters between normal and initiated cells.[14]

Phorbol esters produce different effects in different cell types. This may be explained by the expression of different classes of protein kinase C receptors among cell types. In addition, multiple protein kinase C genes and mRNA species have been identified in mammalian tissues, and different rodent strains vary in their sensitivity to promoting agents.[14] Diversity in the elements that comprise the complex protein activation cascade likely are differentially expressed among the population, either because polymorphisms or differential exposures to environmental agents and variation in individual response to tumor promoters could result. Because of their role in differentiation, the protein kinase C gene family represent a target for anticancer therapy.[58]

ONCOGENES AND TUMOR-SUPPRESSOR GENES

Activation of proto-oncogenes and loss of tumor-suppressor genes are genetic changes associated with carcinogenesis. The study of mechanisms by which chemical carcinogens cause these changes is an active area of interdisciplinary cancer research. It clearly is reasonable that chemical–DNA interactions and carcinogen–DNA adduct formation (either direct, in the case of polycyclic aromatic hydrocarbons, or indirect, in the case of oxyradicals) lead to this type of genetic change in human cancer, because these changes have been detected in tumors with a recognized chemical etiology. Furthermore, both the recognition that carcinogen–DNA interaction is an important step in carcinogenesis and the results of short-term mutagenesis assays led to the conclusion that chemically induced DNA damage is an early step in the carcinogenic process.

Proto-oncogenes are normal cellular genes that control cell growth (i.e., proliferation), specialization (i.e., differentiation) and death (i.e., apoptosis). Almost all proto-oncogenes encode a protein component of

the signal transduction cascade. This integrated, multi-process system is responsible for the smooth, orderly, and specific transmission of extracellular signals to the nucleus, and this process regulates gene transcription with respect to replication.[59] When proto-oncogenes are activated, they are termed *oncogenes*. Oncogenes exert a positive driving force for cell growth by their failure to desist in response to the absence of stimulation.[60] The discovery of oncogenes, their role in cell transformation, and the realization that these genes arise from the activation of normal cellular genes (proto-oncogenes) is also discussed. That there are several possible mechanisms by which proto-oncogenes may be activated should be emphasized. These are overexpression of the gene product, leading to an increased concentration of the protein (dosage hypothesis); expression of the gene at an inappropriate time or context, which could occur because of a mutation in the regulatory region of the gene (unscheduled gene expression); expression of a proto-oncogene in an inappropriate cell type; and structural alteration of the gene product. The primary mechanisms by which chemicals cause oncogene activation are discussed later.

Activated *ras* genes predominate as the family of oncogenes to be isolated from solid tumors induced by chemicals in laboratory animals. Members of the *ras* gene family code for proteins of molecular weight 21,000 (p21); these proteins are membrane bound, have GTPase activity, and form complexes with other proteins. The *ras* genes code for small G-proteins (guanine nucleotide binding) that exert a powerful proliferative response through the signal transduction cascade. They have been referred to as a molecular switch that, when mutated, freezes in the "on" position. Activated *ras* binds to raf, a protein kinase, and through this mechanism recruits other mitogen-activated protein kinases to cause cell proliferation. Disruption of this signalling pathway holds promise for future therapeutic strategies.[60–62] The first direct evidence of proto-oncogene activation by a chemical carcinogen was obtained from in vitro studies.[63] A wild-type recombinant clone of the human *Ha-ras* gene (pEC) was modified with benzo[*a*]pyrene-diol-epoxide. The treated plasmid was then used to transfect NIH-3T3 cells, with the result that the transformed cell foci produced contained the same specific point mutations (in either codon 12 or 61) known to exist in activated *ras* genes isolated from human tumors including the bladder (pEJ).

In animal models of chemical carcinogenesis and surveys of different types of human tumors that arise from a variety of environmental exposures, *ras* mutations have been found.[14,64,65] In rodents, polycyclic aromatic hydrocarbons (3-methylcholanthrene,7,12-dimethylbenz[*a*] anthracene, and benzo[*a*]pyrene) have been used repeatedly to produce both benign tumors and malignant carcinomas. A large proportion of these premalignant and malignant lesions have mutations in either the 12th or 61st codons. Similarly, treatment of rats with either 7,12-dimethylbenz[*a*]anthracene or *N*-methyl-*N*-nitrosourea resulted in the development of mammary carcinomas containing *ras* codon 12 or 61 mutations. These types of mutation also have been observed in mouse skin after initiation with 7,12-dimethylbenz[*a*]anthracene and tumor promotion with 12-O-tetradecanoylphorbol-13-acetate. Mutations in *ras* have been found in mouse liver after treatment with vinyl carbamate, hydroxydehydroestragole, or *N*-hydroxy-2-acetylaminofluorene. The same point mutations also have been found in murine thymic lymphomas after treatment with *N*-methyl-*N*-nitrosourea or γ-radiation and in other rodent skin models after treatment with either methylmethanesulphonate, *a*-propiolactone, dimethylcarbamyl chloride, or *N*-methyl-*N*9-nitro-*N*-nitrosoguanidine.

These data indicate that chemical carcinogens may produce site-specific mutations based, in part, on nucleoside selectivity of the ultimate carcinogen. Persistence of a specific mutation, however, also depends on the amino acid substitution, in that the function of the mutant protein is altered to confer on the cell a clonal growth advantage. The types of mutations that are found in chemically activated *ras* genes cause conformational changes that alter nucleotide binding to the p21 protein in such a way that the p21 GTPase activity is not reduced. Data support the hypothesis that *ras* activation is associated with malignant conversion as well as tumor initiation. Transfection of activated *ras* genes into benign papillomas that did not contain a constitutively activated *ras* gene caused malignant progression.[66]

Similarly, malignant transformation occurred when human bronchial epithelial cells were transfected with an activated *ras* gene,[67,68] as well as when both *c-raf-1* and *c-myc* were overexpressed in immortalized bronchial epithelial cells.[62] In addition, *Ki-ras* gene mutations are one of a number of changes that can arise either early or late in the development of colorectal carcinoma.[64] These findings indicate that it is the accumulation of mutations, and not necessarily the order in which they occur, that contributes to multi-stage carcinogenesis. Furthermore, the stage of carcinogenesis in which each mutation occurs is not necessarily fixed. It appears that in the model for human colorectal carcinoma, *ras* mutations most often occur during malignant conversion but can be an early event (i.e., tumor initiation); in the rodent skin models, *ras* mutations appear to be primarily a tumor-initiating event. These differences may reflect the type of exposure, both in terms of chemical class and chronic versus acute exposure, or they may be a function of tissue type.

Loss of the function of genes that may suppress the tumor phenotype was considered as a theoretic possibility in regard to retinoblastoma more than 20 years ago.[69] Firm experimental evidence for the existence of tumor suppressor genes was provided by analysis of the molecular genetics of pediatric tumors (retinoblastoma, Wilms' tumor, rhabdomyosarcoma, and bilateral acoustic neurofibromatosis). Examination of DNA restriction fragment length polymorphisms by Southern hybridization shows the loss of a restriction fragment from the tumor of a constitutive heterozygote, if that genetic locus has been affected by certain mutational events (deletion, translocation, nondisjunction, mitotic recombination). This type of genetic analysis is termed *loss of heterozygosity* (LOH). In fact, in pediatric tumors, studies that showed loss of the normal allele and duplication of the inherited, defective allele provided the first proof of mitotic recombination in humans.

Loss of a tumor suppressor gene is generally characterized by a mutation in one copy of the gene and loss of the homologous copy. Examples of genes with these characteristics have been located on specific chromosomes: the retinoblastoma gene (*RB*) (13q14); the Wilms' tumor gene (*WT-1*) (11p13); the *p53* gene (17p13); the von Hippel-Lindau disease gene (*VHL*) (3p25); the neurofibromatosis genes (*NF1* and *NF2*) (17q11 and 22q, respectively); the adenopolyposis coli gene (*APC*) (5q21); the fragile histidine triad (*FHIT*) (3p14.2) and the $p16^{INK4}$ gene (9p21).[70–74] Concerning tumor suppressor genes, most available data are derived from studies of *p53*. Point mutations in the *p53* gene that give rise to amino acid changes or chain termination are observed in approximately 50% of human cancers and are frequent in lung as well as colon cancer.[47,72,75]

The role of tumor suppressor genes (i.e., *p53* in homeostasis) is to prevent tissue overgrowth, nullify cells with damaged genomes, and metastasis. These controlling functions, even in the presence of already severely damaged cells that are being driven by activated proto-oncogenes (oncogenes), may be thwarted by a fully functional p53 protein. It is of further importance to recognize that the p53 function may be compromised by viral infection (e.g., human papillomavirus). The role of p53 in the life cycle of the cell is becoming increasingly well understood, and the p53 protein has been shown to have broad functionality in cellular processes. These include cell cycle control, DNA repair, differentiation, genomic plasticity, and apoptosis (i.e., programmed cell death).[73,74]

Molecular analysis of the *p53* gene may gives clues to environmental etiology of cancer (Table 12.2). It is implicit from the preceding text (DNA damage and repair) that the covalent binding of activated carcinogens to DNA is not random. Therefore, the formation of a particular DNA lesion, to some extent, may be deduced from the mutation that resulted. The *p53* gene mutations in many human cancers could provide the clues. A dramatic example of this phenomenon is the previously mentioned codon 249 mutation, which is detected in almost all aflatoxin-related hepatocellular carcinomas.[47] The striking nature of this association could arise by two distinct mechanisms. First, the third base in codon 249 (AGG) may be unusually susceptible to activated aflatoxin B_1 mutations. Indeed, it was discussed earlier that aflatoxin B_1-8,9-oxide causes a promutagenic lesion by covalent

binding to the N7-position of deoxyguanosine. Alternatively, cells bearing the codon 249 lesion may have a selective growth advantage. Evidence that a combination of these factors is responsible has been presented as well.[76]

Another prominent example where circumstantial evidence points to specific molecular events is that of *p53* mutations indicative of pyrimidine dimer formation in ultraviolet light–related skin cancers.[77] In the case of tobacco smoking and lung cancer, G:C to T:A transversions indicate formation of adducts from activated bulky carcinogens (e.g., polycyclic aromatic hydrocarbons).[47] However, we should be cautious in our interpretation because the two major confounding factors in this approach are that different carcinogens can lead to the formation of identical mutations, and that most environmental chemical carcinogens are highly complex mixtures (e.g., tobacco smoke and diesel exhaust). These and other examples of mutational spectra at the *p53* gene locus and others have been comprehensively reviewed.[47,72,73]

The general mechanism for the loss of heterozygosity that occurs in tumors that have a familial origin may be different from that occurring in chemically induced cancer. Mitotic recombination is a common feature of pediatric neoplasms. The carcinogenic effects of the clastogens found in cigarette smoke, however, appear to be mediated, in part, more typically through chromosomal deletions. These deletions are primarily terminal, but they are to a lesser extent interstitial.[78] Furthermore, given the complexity of tobacco smoke (a mixture of mutagens, carcinogens, and promoters), these and other mutations likely result from both direct (adduct formation) and indirect (oxyradical formation) damage to DNA. Determination of these types of disease-associated mutational spectra, which include both oncogenes and tumor suppressor genes, eventually may be useful in defining causal chemical exposure.

CLONAL EVOLUTION

The question of tumor clonality has been addressed primarily by examination of genetic markers. In particular, cytogenetic techniques for leukemia and LOH for cancer of the head and neck and colon cancer.[10,11,79] In chronic myelogenous leukemia, the early disease phase is characterized by a single reciprocal translocation, t(9;22), called the *Philadelphia chromosome*. This genetic change activates the c-*abl* proto-oncogene through the formation of a hybrid gene of c-*abl* with the break-point cluster region. The resulting gene product has elevated tyrosine kinase activity. The later stages of chronic myeloid leukemia are typified by overgrowth of one or more subclones that have additional karyotypic alterations.

In colorectal tumorigenesis, a paradigm has been developed in which accumulated alterations include at least one dominantly acting oncogene and several tumor suppressor genes.[64] These same studies provided evidence for the progressive nature of genetic changes in carcinogenesis. LOH in these and other types of tumors always results in loss of one of a pair of restriction fragments, that is, the same allele (e.g., *p53*) in all the cells is evidence of clonality. The clonal origin of colorectal tumors in female patients with cancer has been more convincingly demonstrated by differential methylation. Inactivation of the X chromosome (by methylation) during embryogenesis is random; therefore, polyclonal female tissues develop with an approximately equal complement of inactivated maternal and paternal X chromosomes. If the tissues are monoclonal, the same inactive X chromosome should be present in all the cells. By using a DNA restriction fragment length polymorphism–based strategy, all the colorectal tumors were found to be monoclonal[80].

In cancer of the head and neck, the question of clonality as it pertains to field cancerization was studied. Comparative molecular analysis found some paired tumors, arising in the same field, shared a common origin. These findings indicate that a single progenitor cell can replicate, expand, and populate contiguous regions in the process of clonal expansion.[10,79] However, the zonal limits of clonal expansion have not yet been adequately determined. Genetic studies have found at least 95% of human tumors to be monoclonal in origin.[80]

CHEMICAL AND VIRAL INTERACTIONS

A distinction between viral and chemical carcinogenesis was made more than 50 years ago paving the way for the multi-stage theory of carcinogenesis.[6,7] Certain viruses can integrate into the genome, depending on where they may activate the proto-oncogenes and/or inactivate the tumor suppressor genes. Because such viruses are able to act at every stage of carcinogenesis, it is reasonable that chemicals and viruses may have interactive effects. There now is good evidence from experiments with in vivo systems that viruses and chemicals also can interact in a synergistic manner.[81] The causation of cancer by purely chemical means previously has been discussed, and a number of studies clearly have demonstrated that certain forms of cancer have a viral etiology, including Burkitt's lymphoma and T-cell leukemia.

A number of human cancers now are considered to have both viral and chemical components to their etiology. Evidence exists for hepatitis B virus and aflatoxin B_1 or alcoholic beverages in hepatocellular carcinoma; Epstein-Barr virus and *N*-nitrosamines in nasopharyngeal carcinoma; and human papilloma virus and tobacco smoke components in cancers of the uterine cervix, oral cavity, and larynx.[82] In essence chemicals can act as tumor promoters following tumor initiation by viral agents, and viruses can act as promoters following chemical initiation. Cells that are pretreated with chemical carcinogens (benzo[*a*]pyrene, 4-nitroquinoline-*N*-oxide, 3-methylcholanthrene or thymidine analogues) have been shown to be morphologically transformed more easily by Simian virus 40 (SV40). Similarly, enhanced transformation by other viruses (adenovirus SA7, mutant adenovirus type 5, or herpes simplex virus type 2) also has been observed following pretreatment with several polycyclic aromatic hydrocarbons. In other cases, alkylating agents have been used (methylmethane sulfate) before infection with wild-type adenovirus 5 as a regimen to morphologically transform rat-embryo fibroblast cells in culture.

Human epithelial cells in vitro have proved to be more difficult for the study of chemical–viral interactions. Some reports exist where viruses (SV40 or Epstein-Barr) have been used first to immortalize the cells, and chemicals (3-methylcholanthrene or *N*-acetoxy-2-acetylaminofluorene) have been used to cause neoplastic transformation of the immortalized cells.[83] In Epstein-Barr virus–immortalized B lymphocytes, however, *N*-methyl-*N*-nitrosoguanidine treatment failed to cause neoplastic transformation. Taken together, these studies may indicate that immortalization, usually by the activation of telomerase, is required before malignant progression can occur, and that more than one gene is involved. Although, it is difficult to assess the relevance of such an immortalization step to human carcinogenesis in vivo, most human cancers contain an activated telomerase.[84]

IMPLICATIONS FOR MOLECULAR EPIDEMIOLOGY, RISK ASSESSMENT, AND CANCER PREVENTION

Molecular epidemiology has resulted from the confluence of several important disciplines.[85] It encompasses the detection of carcinogen–macromolecular adducts (DNA as a direct genotoxic measure and protein as a surrogate), normal DNA sequence variants (heritable vari-

Table 12.2. Mutational Spectra of p53 in Human Cancers*

Carcinogen Exposure	Neoplasm	Mutation
Aflatoxin B_1	Hepatocellular carcinoma	Codon 249 (AGG → AGT)
Sunlight	Skin carcinoma	Dipyrimidine mutations (CC → TT) on nontranscribed DNA strand
Tobacco smoke	Lung carcinoma	G:C → T:A mutations on nontranscribed DNA strand (frequently codons: 157, 248 and 273)
Tobacco and alcohol	Carcinoma of the head and neck	Increased frequency *p53* mutations (especially codons 157 and 248)
Radon	Lung carcinoma	Codon 249 (AGG → ATG)
Vinyl chloride	Hepatic angiosarcoma	A:T → T:A transversions

*For reviews see references 47, 72, 73, 95.

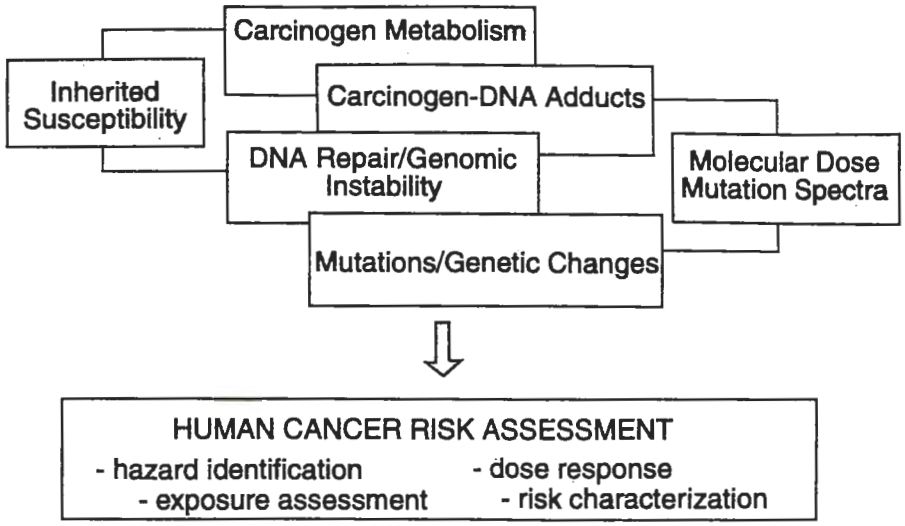

Figure 12.4. Facets of Molecular Epidemiology that investigate gene–environment interactions. Once the internalized chemical carcinogens are metabolized to reactive species that cause damage to DNA (carcinogen–DNA adducts). The innate ability to repair DNA damage may reduce or ablate the overall damage burden. Alternatively, genetic changes (mutations, clastogenesis) may occur. Carcinogen metabolism and DNA repair are categorizable genetic traits (host factors). DNA adducts (molecular dose) and mutational spectra are measures of exposure. Information from assays designed to investigate host factors and exposure measure can be used for human cancer risk assessment.

ations), and mutations in target genes (somatic changes). Therefore, these investigations use epidemiologic methods to investigate all aspects of gene–environment interactions and risk assessment in human populations (Fig. 12.4).

The biologically effective dose of a chemical carcinogen is governed by the amount that reaches a target tissue in a form that becomes activated in that tissue to a chemical species capable of causing lesions in DNA.[86] Humans are most commonly exposed to complex mixtures of chemicals. Human carcinogen dosimetry at the molecular level requires sensitive and specific methods for carcinogen–macromolecular adduct quantitation. The low levels of adducts that are present in human DNA samples challenge the detection limits of conventional assay systems, and complex mixtures of adducted materials confound simple assay systems.

The most commonly used methods that have been developed for carcinogen–DNA dosimetry in humans, specifically, the most commonly used techniques for adduct measurement, are ^{32}P-nucleotide postlabeling, immunoassays, fluorescence spectroscopy, electrochemical conductance, and gas chromatography/mass spectroscopy. Each of these techniques currently has its own advantages and limitations, and within the framework of epidemiologic surveys, multiple corroborative end-point analyses seem to provide the most useful information. These methodologies, their application, and their limitations are reviewed extensively elsewhere.[87]

The most convincing example that correlates carcinogen–DNA adduct levels with human environmental exposure and disease outcome is that of aflatoxin B_1. Exfoliated aflatoxin–N^7guanine adducts measured in urine samples, as well as aflatoxin–albumin adducts, were well correlated with both exposure and 6-hydroxycortisol levels, indicating a role for CYP3A4 in aflatoxin B_1 activation. Importantly, the presence of aflatoxin–N^7guanine adducts in urine was associated with liver cancer.[88,89] Measurements of polycyclic aromatic hydrocarbon–DNA adducts in the peripheral white blood cells of occupationally exposed people have shown that this approach to human biomonitoring is feasible. Further research and development are required, however, to establish reliable methods.[87] Studies to measure 4-aminobiphenyl–hemoglobin adducts have shown a dose–response relationship between the extent of smoking, type of tobacco used, and adduct levels.[90]

Interindividual variations in cancer susceptibility, and consequently meaningful human cancer risk assessment, involve determination of inherited host factors as well as exposure assessment. Most often these were determined by the use of indicator drugs (e.g., caffeine, debrisoquine, dextromethorphan, dapsone, and isoniazid), however, increasingly metabolic polymorphisms are determined by direct genetic assays.[38] This approach has further allowed for the determination of more diverse host factors, for which indicator drugs were not available. Thus, genetic indicators of propensity for carcinogen activation and detoxication, DNA repair capacity, and cell cycle control ability are all features of molecular epidemiologic studies that are complementary to adduct studies because of the implications for biologically effective dose following exposure.[87]

It is reasonable to suspect that cytochrome P-450 polymorphisms, involved in carcinogen activation, and glutathione-*S*-transferases, UDPglucuronosyltransferases, sulfotransferases, and *N*-actyltransferases, involved in both carcinogen activation and detoxication could explain variations in cancer susceptibility among the human population. It is fair to conclude that people absent protection of a functionally intact *GSTM1* gene are at increased risk of lung cancer from exposure to polycyclic aromatic hydrocarbons (PAHs) through tobacco smoke inhalation than those inheriting a functional variant of the gene.[38,91] Similarly, UDPglucuronosyltransferases (e.g., *UGT1A1, UGT1A9, UGT2B7*) have been implicated in cancer of the head and neck. Persons inheriting reduced activity variants of *NAT1* and *NAT2* genes, resulting in the slow acetylator phenotype, are at greater risk of aromatic amine induced bladder cancer, this may include persons exposed through tobacco smoke inhalation. Even though the inducible form of AHH (*CYP1A1* and *CYP1A2*) has long been suspected of increasing cancer susceptibility in PAH-exposed persons, molecular epidemiology studies remain inconclusive. Similarly, studies of *CYP2D6* metabolizer status and tobacco smoke–related lung cancer is similarly confusing. However, analysis of multiple traits, e.g., *CYP1A1* and *GSTM1*, in the same population may help to resolve these issues. Recent reviews confirm the current need for improved epidemiologic study design that integrates DNA adduct measures with indicators of metabolic capacity.[26,91–93]

A large number of DNA repair genes have been described recently, but only few polymorphisms have yet been described. Nevertheless, molecular epidemiologic studies have provided evidence that genetic variation in these attributes can be a human cancer risk factor.[56]

The components of cell cycle control comprise genes that code for cell membrane receptors, intermediate messengers, and transcription factors. An early molecular epidemiologic study that examined a potential link with an early intermediate messenger and cancer was that describing *HRAS-1* rare alleles.[94] For most cancers studied, ambiguous results have been recorded, but in the case of breast cancer, there have been 9 studies, all of which are positively correlated. Five are highly significant, 2 are borderline, and 2 are not significant.[32] Despite these and other positive findings (lung and bladder), a molecular mechanism remains elusive. Polymorphisms in *p53, BRCA-1* and *WAF-1* (*p21*) have also been studied. In general, no associations with increased cancer risk have been observed; however, several studies exist that have linked a specific *p53* haplotype with breast cancer risk.[31]

Molecular characterization of tumors, that is, molecular profiling, is emerging as an important tool that has both etiologic and clinical application. During chemical carcinogenesis, the genome becomes altered and mutations accumulate. These mutations become evident in genes responsible for growth control and cellular homeostasis (includ-

ing oncogenes, tumor suppressor genes, and some DNA repair genes) because corruption of these functions is part of the carcinogenesis process. In respect to chemical carcinogenesis, the most studied genes are the Kirsten-*ras* (K-*ras*) oncogene and *p53*. K-*ras* is mutated in approximately 30% of lung adencarcinomas and may prove to be an indicator of prognosis or a guide to treatment.[65] The *p53* tumor suppressor gene is mutated in many forms of human cancer, and it is the most commonly mutated gene yet known (e.g., mutations in *p53* are found in approximately 50% of lung cancers). Unlike *ras* gene mutations that are found in highly specific regions (codons 12, 13, 59 and 61), *p53* mutations occur more widely. The reason for this is presumably because positive growth advantage conveys only with specific *ras* mutations, and loss of *p53* suppressor function can occur with less specificity. However, for some malignancies, *p53* mutations have provided clues to cancer etiology. The best examples of this are mutations characteristic of aflatoxin exposure in liver cancer, radon or tobacco smoke in lung cancer, and UV light in skin cancer (see Table 12.2).[73,95] *p53* is further distinguished from other genetic lesions, in that several possible mutant phenotypes can exist. Mutations may simply lead to the absence of p53, an inactive mutant protein may exist, or the mutant might convey growth advantage. Several studies have investigated *p53* expression, and though its role in prognosis has not been clearly defined, it may be that it will provide a guide to treatment options.[96,97] All mutant cancer genes are natural targets of gene- or gene-directed therapy. One notable success in this area is the use of Herceptin. Herceptin (or trastuzumab) is a humanized mouse monoclonal antibody that has an affinity for HER-2, an epidermal growth factor receptor-related transmembrane receptor (p185). Herceptin preferentially mediates antibody-dependent cellular cytotoxicity in cancer cells that overexpress HER-2.[98,99]

The goal of molecular epidemiology is to identify risk factors for disease and outcome. Variations among humans in carcinogen biodistribution, metabolism, DNA adduct formation, DNA repair, and potential response to tumor promoters have important implications in determinating cancer risk. An increased understanding of the molecular basis of these differences and their connection with critical steps in carcinogenesis may assist in future predictions of disease risk before the clinical onset of disease.

The facets of molecular epidemiology of human cancer risk are the assessment of carcinogen exposure and inherited and acquired host cancer-susceptibility factors.[73,86] The interaction between these facets determines cancer risk. When combined with carcinogen bioassays in laboratory animals and classic epidemiology, molecular epidemiology can contribute to the four traditional aspects of cancer risk assessment: (1) hazard identification, (2) dose–response assessment, (3) exposure assessment, and (4) risk characterization. Important bioethical considerations accompany the identification of high-risk individuals; these include autonomy, privacy, justice, and equity. Benefits of the knowledge of risk for the individual may be offset by specific concerns relating to that individual's responsibility to family members and pychosocial anxiety regarding the genetic testing of children. Therefore, the uncertainty of current individual risk assessments and the limited availability of genetic counseling services dictate caution. In addition, it is widely held that genetic testing should be restricted to those situations that are amenable to preventative or therapeutic intervention.[100]

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