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15 Chemical Carcinogenesis

Ainsley Weston, PhD • Curtis C. Harris, MD

Human chemical carcinogenesis is a multistage process that results from exposures, usually in the form of complex chemical mixtures, often encountered in the environment or through our lifestyle and diet (Table 15-1).[1-4] A prime example is tobacco smoke, which can cause cancers at multiple sites including the lung, the bladder, and the head and neck.[5-7] Although most chemical carcinogens do not react directly with intracellular components, they are activated to carcinogenic and mutagenic electrophiles by metabolic processes evolutionarily designed to rid the body of toxins and to modify endogenous compounds. Electrophilic chemical species are naturally attracted to nucleophiles like deoxyribonucleic acid (DNA) and protein, and through covalent bonding to DNA genetic damage results. Once internalized, carcinogens are subject to competing processes of metabolic activation and detoxification, although some chemical species can act directly. There is considerable variation among the human population in these competing metabolic processes, as well as the capacity for repair of DNA damage and cellular growth control. This is the basis for interindividual variation in cancer risk, and is a reflection of gene-environment interactions, which embodies the concept that heritable traits modify the effects of chemical carcinogen exposure.[8] Such variations in constitutive metabolism and DNA repair contribute to the relative susceptibility of individual members of the population to chemical exposures. For example, only 10% of tobacco smokers develop lung cancer, albeit that tobacco use accounts for other fatal conditions, including chronic obstructive pulmonary disease, stroke, and heart disease. Within the conceptual framework of multistage carcinogenesis, the primary genetic change that results from a chemical-DNA interaction is termed *tumor initiation*.[9,10] Thus, initiated cells are irreversibly altered and are at a greater risk of malignant conversion than are normal cells. The epigenetic effects of tumor promoters facilitate the clonal expansion of the initiated cells.[10] Selective, clonal growth advantage causes a focus of preneoplastic cells to form. These cells are more vulnerable to tumorigenesis because they now present a larger, more rapidly proliferating, target population for the further action of chemical carcinogens, oncogenic viruses, and other cofactors. Additional genetic and epigenetic changes continue to accumulate.[10,11] The activation of oncogenes, and the inactivation of tumor suppressor and DNA-repair genes, leads to genomic instability or the so-called *mutator phenotype* and an acceleration in the genetic changes taking place.[12,13] This scenario is followed by malignant conversion, tumor progression, and metastasis. The underlying molecular mechanisms that govern chemical carcinogenesis are becoming increasingly understood, and the insights generated are assisting in the development of better methods to investigate human cancer risk and susceptibility.[14] The results of such studies are intended to mold strategies for prevention and intervention. Moreover, insights into the normal operations of so-called *gatekeeper* genes,[15] like the tumor suppressor *TP53*, have provided an opportunity to develop new, targeted, therapeutic approaches.[16,17]

Table 15-1 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
	Isopropyl alcohol	–
Skin (scrotum)	Cutting oil	–
	Coal soot[a]	–
Liver (angiosarcoma)	Aflatoxin B1	HBV, HCB
	Vinyl chloride	Alcohol
Bladder	Aromatic amines (eg, 4-ABP and benzidine)	–
	Aromatic amines from tobacco smoke[b]	–
ALL	Benzene	–
Lymphatic and hemapoietic malignancies	Ethylene oxide	–

Note: A comprehensive treatise on the evaluation of the carcinogenic risk of chemicals to humans can be found in the ongoing International Agency for Research on Cancer monograph program initiated in 1971.[4]

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

[b] Strong circumstantial evidence.[58]

Abbreviations 4-ABP, 4-aminobiphenyl; ALL, acute lymphoblastic leukemia, BCME, bischloromethyl ether; HBV, hepatitis B virus, HCV, hepatitis C virus

Multistage Carcinogenesis

Carcinogenesis can be divided conceptually into four steps: tumor initiation, tumor promotion, malignant conversion, and tumor progression (Fig. 15-1). The distinction between initiation and promotion was recognized through studies involving both viruses and chemical carcinogens.[9,18] This distinction was formally defined in a murine skin carcinogenesis model in which mice were treated topically with a single dose of a polycyclic aromatic hydrocarbon (ie, initiator), followed by repeated topical doses of croton oil (ie, promoter),[9] and this model has been expanded to a range of other rodent tissues, including bladder, colon, esophagus, liver, lung, mammary gland, stomach, and trachea.[19] During the last 50 years, the sequence of events comprising chemical carcinogenesis has been systematically dissected and the paradigm increasingly refined, and both similarities and differences between rodent and human carcinogenesis have been identified.[20,21] Carcinogenesis requires the malignant conversion of benign hyperplastic cells to a malignant state, and invasion and metastasis are manifestations of further genetic and epigenetic changes.[22-24] The study of this process in humans is necessarily indirect and uses

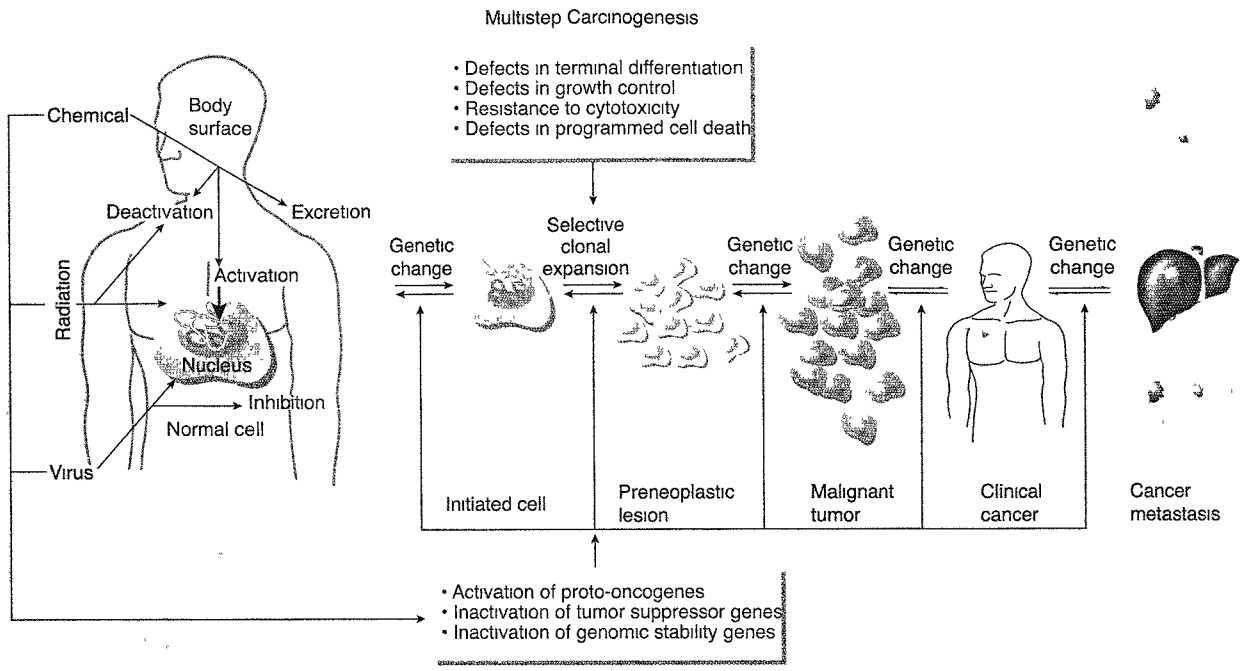

Figure 15-1 Multistage chemical carcinogenesis can be conceptually divided into four stages. tumor initiation, tumor promotion, malignant conversion, and tumor progression The activation of proto-oncogenes and inactivation of tumor suppressor genes are mutational events that result from covalent damage to DNA caused by chemical exposures. The accumulation of mutations, and not necessarily the order in which they occur, constitutes multistage carcinogenesis *Source*: From Refs. 26,99.

information from lifestyle or occupational exposures to chemical carcinogens. 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.[25] Partial scheduling of specific genetic events in this process has been possible for some cancers. Examples of sequential genetic and epigenetic changes that occur with the highest probability are those found in the development of lung cancer[26,27] and colon cancer.[28,29]

Tumor Initiation

The early concept of tumor initiation indicated that the initial changes in chemical carcinogenesis are irreversible genetic damage. However, recent data from molecular studies of preneoplastic human lung and colon tissues implicate epigenetic changes as an early event in carcinogenesis. DNA methylation of promoter regions of genes can transcriptionally silence tumor suppressor genes.[24] 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 modifying the molecular structure of DNA that can lead to a mutation during DNA synthesis. Most often, this is brought about by forming an adduct between the chemical carcinogen or one of its functional groups and a nucleotide in DNA[7,19] (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 number of tumors that develop.[30-33] 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 may be a necessary, but not a sufficient, prerequisite for tumor initiation (the concept of so-called nongenotoxic carcinogens is also explored under "Carcinogen Metabolism"). DNA adduct formation that causes either the activation of a proto-oncogene or the inactivation of a tumor suppressor gene can be categorized as a tumor-initiating event (see "Tumor Progression," "Oncogenes and Tumor Suppressor Genes" in this chapter).

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, clonal expansion of initiated cells, produces a larger population of cells that are at risk of further genetic changes and malignant conversion.[24,27,28] Tumor promoters are generally nonmutagenic, are not carcinogenic alone, and often (but not always) are 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. Croton oil (isolated from *Croton tiglium* seeds) is used widely as a tumor promoter in murine skin carcinogenesis, and the mechanism of action for its most potent constituent, 12-*O*-tetradecanoylphorbol-13-acetate, which occurs via protein kinase C activation, is arguably the best understood among tumor promoters.[34] Chemicals or agents capable of both tumor initiation and promotion are known as complete carcinogens, eg, benzo[*a*]pyrene and 4-aminobiphenyl. Identification of new tumor promoters in animal models has accelerated with the sophisticated development of model systems designed to assay for tumor promotion. Furthermore, ligand-binding properties can be determined in recombinant protein kinase C isozymes that are expressed in cell cultures.[35] 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, polychlori-

nated biphenyls (PCBs), teleocidins, cyclamates, estrogens and other hormones, bile acids, ultraviolet light, wounding, abrasion, and other chronic irritation (ie, saline lavage).[19] In addition, protein kinase C is activated and cellular diacylglycerol elevated in laboratory animals maintained on high-fat diets.[36,37]

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 significant than frequently repeated administrations, and if the tumor promoter is discontinued before malignant conversion has occurred, premalignant or benign lesions may regress Tumor promotion contributes to the process of carcinogenesis by the expansion of a population of initiated cells, with a growth advantage, that 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.[38] The relatively low probability of malignant conversion can be increased substantially by the exposure of preneoplastic cells to DNA damaging agents,[19,39] 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 malignant cells to acquire more aggressive characteristics over time. Also, metastasis may involve the ability of tumor cells to secrete proteases that allow invasion beyond the immediate primary tumor location. A prominent characteristic of the malignant phenotype is the propensity for genomic instability and uncontrolled growth.[40] During this process, further genetic and epigenetic changes can occur, again including the activation of proto-oncogenes and the functional loss of tumor suppressor genes Frequently, proto-oncogenes 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 (ie, the 12th, 13th, 59th, or 61st codons), and members of the *myc*, *raf*, *HER2*, and *jun* multigene families can be overexpressed, sometimes involving amplification of chromosomal segments containing these genes. Some genes are overexpressed if they are translocated and become juxtaposed to a powerful promoter, eg, the relationship of *bcl-2* and immunoglobulin heavy chain 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 a 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 the determining factor.[26-28] Recent evidence from microarray expression analysis of human cancers supports an alternative, and not mutually exclusive, mode of tumor progression. Gene expression profiles of a primary cancer and its metastases are similar, indicating that the molecular progression of a primary cancer is generally retained in its metastases.[41,42] These results have clinical implications in molecular diagnosis of primary cancers and therapeutic strategies.

Gene-Environment Interactions and Interindividual Variation

A cornerstone of human chemical carcinogenesis is the concept of gene-environment interactions (Fig. 15-2).[8] Potential interindividual susceptibility to chemical carcinogenesis may well be defined by genetic variations in the host elements of this compound system. Functional polymorphisms in human proteins that have, or may have, a role in chemical carcinogenesis include enzymes that metabolize (ie, activate and detoxify) xenobiotic substances, enzymes that repair DNA damage, cell surface receptors that activate the phosphorylation cascade and cell-cycle control genes (ie, 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 gene-environment interaction. The interindividual variation in carcinogen metabolism and macromolecular adduct formation arising from such processes was recognized >25 years ago.[43,44] The cytochrome P450 (CYP) multigene family is largely responsible for the metabolic activation and detoxication of many different chemical carcinogens in the human environment.[45] Cytochrome P450s are Phase I enzymes that act by adding an atom of oxygen onto the substrate, they are induced by polycyclic aromatic hydrocarbons and chlorinated hydrocarbons [46] Phase II enzymes act on oxidized substrates and also contribute to xenobiotic metabolism. 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 dehydrogenases, aldehyde and steroid dehydrogenases, quinone reductases, NADPH diaphorase, azo reductases, aldoketoreductases, transaminases, esterases, and hydrolases. The pathways of activation and detoxification are often competitive, providing yet further potential for individual differences in propensity for

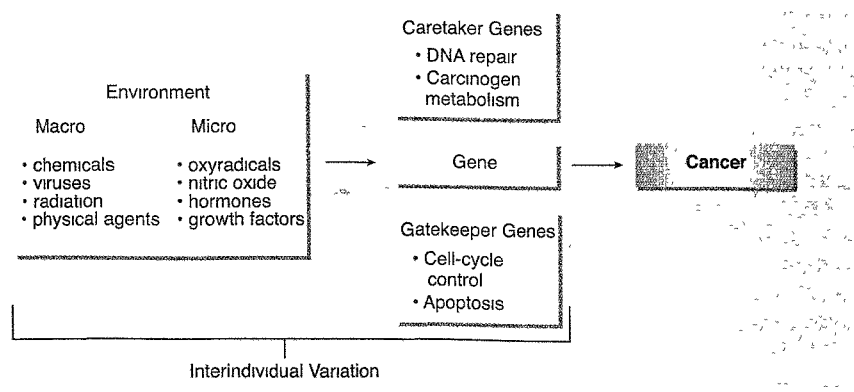

Figure 15-2 The concept of gene–environment interaction is multifaceted: (1) environmental chemicals are altered by the products of metabolic genes; (2) environmental chemicals disrupt the expression (induce or inhibit) of carcinogen metabolizing genes; and (3) environmental exposures cause changes (mutations) in cancer-related genes. The cancer-related genes have been classified as gatekeeper (eg, APC) and caretaker genes (eg, MSH1 and MLH1) The interaction of these genes with external and internal environmental agents can lead to the derangement of regulatory pathways that maintain genetic stability and cellular proliferation.

carcinogen metabolism to DNA damaging species. This scenario is further complicated by a second facet of gene-environment interaction that leads to enzyme induction or inhibition. In this case, environmental exposures alter gene expression, and genes responsible for carcinogen metabolism can be upregulated or repressed by certain chemical exposures.

A third facet of gene-environment interaction occurs when the chemical alters gene structure. Once a procarcinogen is metabolically activated to an ultimate carcinogenic form, it can bind covalently to cellular macromolecules, including DNA. This DNA damage can be repaired by several mechanisms.[47-49] Differences in rates and fidelity of DNA repair potentially influence the extent of carcinogen adduct formation (ie, biologically effective dose) and, consequently, the total amount of genetic damage. The consequences of polymorphisms in genes controlling the cell cycle (serine/threonine kinases, transcription factors, cyclins, cyclin-dependent kinase inhibitors, and cell surface receptors) are much less clear. However, molecular epidemiologic evidence suggests that certain common variants of these types of genes have a role in susceptibility to chemical carcinogenesis.[50,51] The evaluation of polymorphisms as potential biomarkers of susceptibility in the human population is discussed under "Implications for Molecular Epidemiology, Risk Assessment, and Cancer Prevention."

Carcinogen Metabolism

Polycyclic aromatic hydrocarbons (PAHs), eg, benzo[*a*]pyrene (BP), were the first carcinogens to be chemically isolated.[52] They are composed of variable numbers of fused benzene rings that form from incomplete combustion of fossil fuels and vegetable matter; they are common environmental contaminants. PAHs are chemically inert, and require metabolism to exert their biologic effects.[53] This multistep process involves the following: initial epoxidation (cytochrome P450), hydration of the epoxide (epoxide hydrolase), and subsequent epoxidation across the olefinic bond (Fig. 15-3).[45] The result is the ultimate carcinogenic metabolite, in the case of BP it is *r*7,*t*8-dihydroxy-*c*9,10 epoxy-7,8,9,10-tetrahydroxybenzo[*a*] pyrene (benzo[*a*]pyrene-7,8-diol 9,10-epoxide, BPDE).[54] The biology of cytochrome P450 (eg, CYP1A1) metabolism has been elucidated providing a molecular basis for inducibility and interindividual variation and variations in cytochrome levels among humans have been documented.[55] The arene ring of

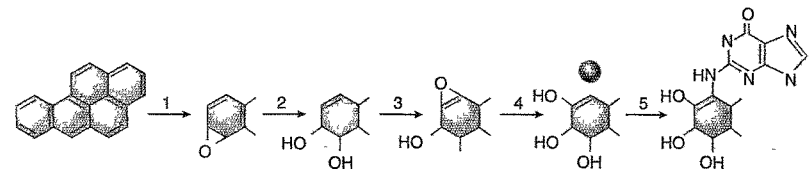

Figure 15-3 Metabolic activation of benzo[*a*]pyrene (1) Cytochrome P450 (CYP1A1) catalyses initial epoxidation across the 1-2, 2-3, 4-5, 7-8 (shown), 9-10 and 11-12 positions (2) With the exception of the 1-2 and 2-3 oxides that convert to phenols, epoxide hydrolase may catalyze the formation of dihydrodiols. (3) Benzo[*a*]pyrene-7, 8-dihydrodiol is further metabolized at the olefinic double bond by cytochrome P450 (CYP1B1 and CYP3A4) to form avicinal diol-epoxide (r7, t8-dihydroxy-c9, 10 epoxy-7,8,9,10-tetrahydroxybenz[*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 exocyclic amino group of deoxyguanosine

BPDE opens spontaneously at the 10th position, revealing a carbonium ion that can form a covalent addition product (adduct) with cellular macromolecules, including DNA. Several DNA adducts can be formed, the most abundant being at the exocyclic amino group of deoxyguanosine ([7R]-*N*2-[10-{7ß,8a,9a-trihydroxy-7,8,9,10-tetrahydro-benz[*a*] pyrene}yl]-deoxyguanosine; BPdG). One electron oxidation has been suggested as an alternative pathway of PAH activation, here a radical cation forms at the meso position (L-region). The resulting DNA adducts at the C8 of guanine (BP-6-C8Gua and BP-6-C8dGua), the N7 of guanine and adenine (BP-6-*N*7Gua and BP-6-*N*7Ade) likely undergo spontaneous depurination (Fig. 15-4). Firm evidence for the exfoliation of these adducts in urine has been provided.[56,57]

Aromatic amines are found in cigarette smoke, diesel exhaust, industrial environments and certain cooked foods. The compound, 4-aminobiphenyl, is thought to be responsible for bladder cancer among tobacco smokers and rubber industry workers.[58] In addition, nitrated polycyclic aromatic hydrocarbons are environmental contaminants that are related to aromatic amines by nitroreduction. Aromatic amines can be converted to an aromatic amide that is catalyzed by an acetyl coenzyme A-dependent acetylation.[59] The acetylation phenotype varies among the population. Persons with the rapid acetylator phenotype are at a higher risk of colon cancer, whereas, those who are slow acetylators are at risk of bladder cancer.[60] This latter association may result from the fact that activation of aromatic amines by N-oxidation is a competing pathway for aromatic amine metabolism. The N-hydroxylation products when protonated (eg, in the urinary bladder) are reactive and can cause DNA damage.

An initial activation step for both aromatic amines and amides is N-oxidation by CYP1A2. The reactions

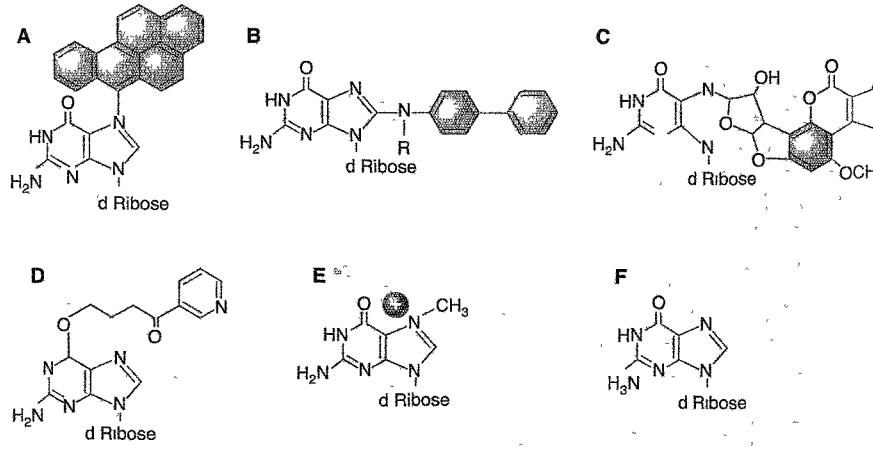

Figure 15-4 Examples of carcinogen–DNA adducts (**A**) N7(benzo[*a*]pyren-6-yl)guanine; (**B**) *N*-(deoxyguanosin-8-yl)-[acetyl]aminobiphenyl (when R= H the adduct is not acetylated [R can also be an acetyl group]); (**C**) 8,9-dihydro-8-(N5-formyl- 2′, 5′, 6′-triamino-4′-oxo-N5-pyrimidyl)-9-hydroxy-aflatoxin B1; (**D**) O6-[4-Oxo-4(3-pyridyl)butyl]guanine, amutagenic lesion formed by the metabolism of the tobacco-specific nitrosamine, NNK 73,74 ; (**E**) *N*7-methyldeoxygua-nosine; and (**F**) 3-methyladenosine. Adducts **E** and **F** can also result as the small alkyl products of NNK metabolism.[73,74]

Table 15-2 Chronic Inflammation and Infection Can Increase Cancer Risk

Disease	Tumor Site	Risk
Inherited		
Hemochromatosis	Liver	219
Crohn disease	Colon	3
Ulcerative colitis	Colon	6
Acquired		
Viral		
Hepatitis B	Liver	88
Hepatitis C	Liver	30
Bacterial		
Helicobacter pylori	Gastric	11
PID	Ovary	3
Parasitic		
Schistosoma hematobium	Urinary bladder	2-14
Schistosoma japonicum	Colon	2-6
Liver fluke	Liver	14
Chemical/physical/metabolic		
Acid reflux	Esophagus	50-100
Asbestos	Lung pleural	>10
Obesity	Multiple sites	1.3-6.5

"18% of human cancers, ie, 1 6 million per year, are related to infection " B Stewart and P Kleihues, World Cancer, Report, IARC Press, Lyon 2003, p. 57.

Rheumatoid arthritis is an example of a chronic inflammatory disease without a marked increased cancer risk, eg, joint sarcoma.

Oncogenic human papilloma viruses are examples of cancer-prone chronic infections without inflammation

of *N*-hydroxyarylamines 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*-aryl-hydroxamic acids arise from the acetylation of *N*-hydroxyarylamines or N-hydroxylation of aromatic amides; they are not electrophilic and require further activation. The predominant pathway here occurs through acetyltransferase-catalyzed rearrangement to a reactive *N*-acetoxyarylamine. Sulfotransferase catalysis forms *N*-sulphonyloxy arylamides. This complex pathway results in two major adduct types, amides (acetylated) and amines (nonacetylated).

Heterocyclic amines form in food cooking from pyrolysis (>150°C) of amino acids, creatinine, and glucose. They have been recognized as food mutagens, shown to form DNA adducts and cause liver tumors in primates.[61] These compounds are activated by CYP1A2, and their metabolites form DNA adducts in humans. The *N*-hydroxy metabolites of heterocyclic amines like 2-amino-3-methyl-imidazo-[4,5-*f*]quinoline (IQ) can react directly with DNA. Enzymic O-esterification of *N*-hydroxy metabolites plays a key role in activating food mutagens, and because the *N*-hydroxy metabolites are good substrates for transacetylases these chemicals may be implicated in colorectal cancer.

Aflatoxins (B1, B2, G1, and G2), metabolites of *Aspergillus flavus*, contaminate cereals, grain, and nuts. A positive correlation exists between dietary aflatoxin exposure and the incidence of liver cancer in developing countries, where grain spoilage is high. Aflatoxins B1 and G1 have an olefinic double bond at the 8,9-position that can be oxidized by several cytochrome P450.[62] This implies that the olefinic 8,9-bond is the activation site. Further support for this mechanism comes from studies of DNA adducts and the prevalence of *TP53* 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.[63] This phenomenon is consistent with metabolic activation of aflatoxin B1 and the formation of depurinating carcinogen-deoxyguanosine adducts.

Carcinogenic *N*-nitrosamines are ubiquitous environmental contaminants and can be found in food, alcoholic beverages, cosmetics, cutting oils, hydraulic fluid, rubber, and tobacco.[64] Tobacco-specific *N*-nitrosamines (TSNs), eg, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), are not formed by pyrolysis, which accounts for the highly carcinogenic nature of snuff and chewing tobacco.[65] TSNs are not symmetric so both small alkyl adducts and large bulky adducts can be formed, eg, NNK metabolism gives rise to either a positively charged pyridyl-oxobutyl ion or a positively charged methyl ion, both of which are able to alkylate DNA.[65] Endogenous nitrosamines form when an amine reacts with nitrate alone or nitrite in the presence of acid. Thus, nitrite (used to cure meats) and l-cysteine, in the presence of acetaldehyde (from alcohol), form *N*-nitrosothiazolidine-4-carboxylic acid. *N*-nitrosodimethylamine undergoes α-hydroxylation, catalyzed primarily by the alcohol inducible CYP2E1, to form an unstable α-hydroxynitrosamine. The breakdown products are formaldehyde and methyl diazohydroxide. Methyl diazohydroxide and related compounds are powerful alkylating agents that can add a small functional group at multiple sites in DNA.

Nongenotoxic carcinogens may function at the level of the microenvironment by dysregulation of hormones and growth factors, or indirectly inducing DNA damage and mutations through the action of free radicals [66] These chemicals are none or poorly reactive and are resistant to activation through metabolism. They are also characterized by their persistence in biological systems and consequently tend to accumulate in the food chain. However, they can stimulate oxyradical formation by at least three mechanisms: organochlorine species interact with the Ah receptor which can lead to cytochrome P450 induction and associated oxyradical formation; interaction with other receptors, like IFN-γ, can stimulate elements of the primary immune response and again generate oxyradicals; and agents like asbestos can promote oxyradical formation through interaction with ferrous metal. The resulting oxyradicals can then damage DNA. Some of the so-called "nongenotoxic" carcinogens might more appropriately be considered to be "oxyradical triggers." Indeed, chronic inflammatory states, which involve oxyradical formation, can also be cancer risk factors.[66]

Chronic Inflammation and Cancer

More than a century ago, the German pathologist, Virchow proposed that inflammation was associated with cancer.[67] Infection and inflammation significantly contribute to about 25% of cancer cases worldwide (Table 15-3).[68] Free radicals, endogenous chemicals, are released during the inflammatory response. These reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated as a physiological protective response to pathogenic microorganisms and toxic agents. During chronic inflammation, eg, chronic viral hepatitis, and oxyradical overload conditions, eg, hemochromatosis, these free radicals can induce genetic and epigenetic changes including somatic mutations in cancer-related genes and posttranslational modifications in proteins involved in DNA repair, apoptosis, and arachidonic acid cascade (Fig. 15-5).[68] Epigenetic transcriptional silencing of cancer-related genes including p16, RUNX3, and MLH1, by DNA methylation of their promoter regions has been associated with chronic inflammation in ulcerative colitis and Barretts esophagus.[69,70] MicroRNA expression is also regulated by inflammatory cytokines and free radicals.[71-75] These non-protein coding small RNAs of about 22 base pairs regulate mRNA stability and translation into proteins.[71,76] MicroRNA genes are regulated by transcription factors including the p53 tumor suppressor protein[75] and are involved in carcinogenesis including tumor invasion and metastasis.[77,78] Not surprisingly, microRNAs are also clinical biomarkers associated with diagnosis, prognosis, and therapeutic outcome of cancer.[71,79-83]

DNA Damage and Repair

The DNA damage initials a complex network of signaling cascades.[84,85] The

Table 15-3 **Examples of Disease Susceptibility and Disease Syndromes Associated with Mutations in DNA-Repair Genes**

Gene	Function	Pathology of Cancer
Cancer susceptibility		
MMR[a]		
MLH1	Damage recognition	HNPCC2[b], glioma
MLH2	DNA binding	HNPCC1, ovarian cancer
MSH3	–	Endometrial cancer
MSH6	Sliding clamp	Endometrial cancer, HNPCC1
PMS1	Damage recognition	HNPCC3
PMS2	Repair initiation	HNPCC4, glioblastoma
NER		
BRCA-1	Directs *p53* transcription toward DNA-repair pathways	Breast cancer, ovarian cancer
RB1	Cell-cycle restriction	Retinoblastoma, breast cancer, and progression osteosarcoma
DSB		
BRCA-2	Regulation of RAD51	Breast cancer, pancreatic cancer
HR		
RAD54	Helicase	Colon cancer, breast cancer, NHL
Other		
TP53 (DSB, NER, HR)	Cell-cycle control; exonuclease; apoptosis; DNA binding	Colon cancer, common somatic defect in human cancer in general; inherited in Li-Fraumeni syndrome and some breast cancers
hOgg1 (Various)	Glycosylase	Cancer susceptibility
Xeroderma pigmentosum (XP)		
NER		
XPD	DNA helicase	Skin and neurologic, but later onset than XPA
XPB	DNA helicase	Skin lesions
XPG	Endonuclease	Acute sun sensitivity, mild symptoms; late skin cancer
XPC (and BER)	Exonuclease	Mental retardation; skin sensitivity; microcephaly
DDB1 and DDB2	Binds specific DNA damage	XPE—Mild skin sensitivity
XPA	Damage sensor	XPA—Skin and neurologic problems: the most severe XP
XPC	Damage sensor	XPC—Skin, tongue, and lip cancer
XPE	Damage sensor	XPE—Neurologically normal
PRR		
POLH	Polymerase	XPV—Mild to severe skin sensitivity, neurologically normal
Other syndromes		
NER		
Cockaynes		
CSB	ATPase	Cutaneous, ocular, neurologic, and somatic abnormalities; short stature, progressive deafness, mental retardation, neurologic degeneration, early death; sometimes presents together with XPB
Juberg-Marsidi		
ATRX	Putative helicase	Thalassemia/mental retardation
SB		
Nijmegen		
NBS1	Nibrin, cell-cycle regulation	Microencephaly; mental retardation; immunodeficiency; growth retardation, radiation sensitivity; predisposition to malignancy
Ataxia-telangiectasia		
ATM	Phosphorylation	Neurologic deficiencies, manifest by inability to coordinate muscle actions; skin and corneal telangiectases. Leukemia, lymphoma, and other malignancies (breast cancer?)
MRE11(Ataxia-like)	Exonuclease	DNA damage sensitivity; genomic instability; telomere shortening; aberrant meiosis; severe combined immunodeficiency
PRKDC	Ser/Thr kinase	SCID
Bloom's		
BLM	DNA helicase	High rate of spontaneous lymphatic and other malignancy; high-rate SCE[c]
Fanconi anemia		
FANCA-G	Protein control	Multiple congenital malformations; chromosome breaks; pancytopenia Telomere shortening
Werner		
WRN	DNA helicase/exonuclease	Premature senility, short stature, exonuclease rapidly progressing cataracts, loss of connective tissue and muscle, premature arteriosclerosis, increase risk of malignancy
RecQ4	DNA helicase	Osteosarcoma; premature aging

[a] **Repair mechanisms** BER, base excision, DSB, double-strand break, HR, homologous recombination, MMR, mismatch, NER, nucleotide excision, PRR, postreplication, SB, strand break
[b] **Diseases:** HNPCC, hereditary nonpolyposis colon cancer, NHL, non-Hodgkin lymphoma.
[c] **Other abbreviations** SCE, sister chromatid exchange; SCID, severe combined immunodeficiency

chemical structure of DNA can be altered by a carcinogen in several ways: the formation of large bulky aromatic adducts, small alkyl adducts, oxidation, dimerization, and deamination. In addition, double- and single-strand breaks can occur. Chemical carcinogens can cause epigenetic changes, such as altering the DNA methylation status that leads to the silencing of specific gene expression.[86] A complex pattern of carcinogen-DNA adducts likely results from a variety of environmental exposures, because of the mixture of different chemical carcinogens present

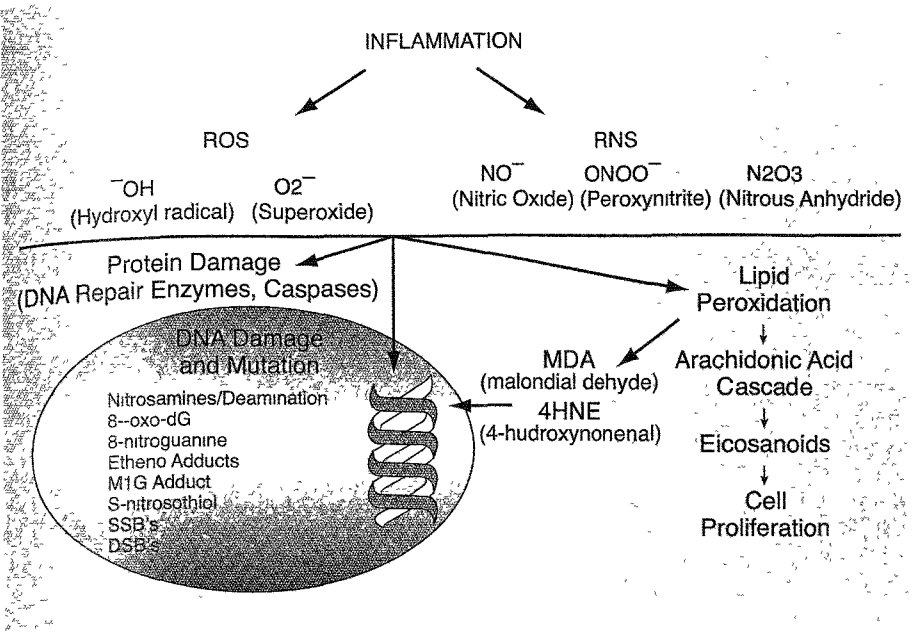

Figure 15-5 Several reactive oxygen (ROS) and reactive nitrogen species (RNS) are generated during chronic inflammation The reactive species can induce DNA damage, including point mutations in cancer-related genes, and modifications in essential cellular proteins that are involved in DNA repair, apoptosis, and cell cycle, either directly or indirectly through the activation of lipid peroxidation and generation of reactive aldehydes, eg, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE).[161]

BPDE reacts with the exocyclic (N2) amino group of deoxyguanosine and resides within the minor groove of the double helix; it is typical of polycyclic aromatic hydrocarbons. This adduct, BPdG, is probably the most common, persistent adduct of benzo[*a*]pyrene in mammalian systems, but others are possible. Adducts like BPdG are thought to induce *ras* gene mutations, which are common in tobacco-related lung cancers.[87] Aromatic amine adducts are more complex, because they have both acetylated and nonacetylated metabolic intermediates, and 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 (see Fig. 15-4).[59]

Aflatoxin B1 and G1 activation through hydroxylation of the olefinic 8,9-position results in adduct formation at the N7-position of deoxyguanosine. These are relatively unstable with a half-life of ~50 h at neutral pH; depurination products have been detected in urine.[88] The aflatoxin B1-*N7*-deoxyguanosine adduct also can undergo ring opening to yield two pyrimidine adducts; alternately, aflatoxin B1-8,9-dihydrodiol could result, restoring the DNA molecular structure if hydrolysis of the original adduct occurs.[89]

DNA alkylation 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), which explains why *O6*-methyldeoxyguanosine is a promutagenic lesion and *N7*-methyldeoxyguanosine is not.[64,90]

Another potentially mutagenic cause of DNA damage is the deamination of DNA-methylated cytosine residues. 5-Methylcytosine comprises ~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 cause a mutation (TpA).[91] Deamination of cytosine also can generate a C to T transition if uracil glycosylation and G-T mismatch repair are inefficient.

Oxyradical damage can form thymine glycol or 8-hydroxydeoxyguanosine adducts. Exposure to organic peroxides (catechol, hydroquinone, and 4-nitroquinoline-*N*-oxide) leads to oxyradical damage; however, oxyradicals and hydrogen peroxide can be generated in lipid peroxidation and the catalytic cycling of some enzymes, as well as environmental sources (eg, tobacco smoke).[68,92] Certain drugs and plasticizers can stimulate cells to produce peroxisomes, and oxyradical formation is mediated through protein kinase C when inflammatory cells are exposed to tumor promoters like phorbol esters.[93,94] Oxyradicals can contribute to deamination through induction of NO synthetase.[95]

Maintenance of genome integrity requires mitigation of DNA damage. Thus, diminished DNA-repair capacity is associated with carcinogenesis, birth defects, premature aging, and foreshortened life span. DNA-repair enzymes act at DNA damage sites caused by chemical carcinogens, and six major mechanisms are known: direct DNA repair, nucleotide excision repair, base excision repair, nonhomologous end joining (double-strand break repair), mismatch repair, and homologous recombination (postreplication repair).[48,91]

In the presence of nonlethal DNA damage, cell-cycle progression is postponed for repair mechanisms. This highly coordinated process involves multiple genes. A DNA-damage recognition sensor triggers a signal transduction cascade and downstream factors direct G1 and G2 arrest in concert with the proteins operationally responsible for the repair process. Although there are at least six discrete repair mechanisms, within five of them there are numerous multiprotein complexes comprising all the machinery necessary to accomplish the step-by-step repair function.

Generically, DNA repair requires damage recognition, damage removal or excision, resynthesis or patch synthesis, and ligation. Recent advances have led to the cloning of more than 130 human genes involved in five of these DNA-repair pathways. A list of these genes and their specific functions was published elsewhere.[96] These genes are responsible for the fidelity of DNA repair, and when they are defective the mutation rate increases. This is the mutator phenotype.[97] Mutations in at least 30 DNA-repair associated genes have been linked to increased cancer susceptibility or premature aging (Table 15-4).[96] Moreover, the role of common polymorphisms in some of these genes are associated with increased susceptibility in a gene-environment interaction scenario (this is discussed under "Implications for Molecular Epidemiology, Risk Assessment, and Cancer Prevention"). Indeed, molecular epidemiologic evidence suggests that tobacco-smoking-related lung cancer is associated with a polymorphism in the nucleotide excision repair gene, XPC (ERCC2).[98]

Direct DNA repair is effected by DNA alkyltransferases. These enzymes catalyze translocation of the alkyl moiety from an alkylated base (eg, *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 DNA alkyl lesion, in a suicide

Table 15-4 Mutational Spectra of TP53 in Human Cancers[a]

Carcinogen Exposure	Neoplasm	Mutation
Aflatoxin B1	Hepatocellular carcinoma	Codon 249 (AGG 6 AGT)
Sunlight	Skin carcinoma	Dipyrimidine mutations (CC 6 TT) on nontranscribed DNA strand
Tobacco smoke	Lung carcinoma	G:C 6 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 6 ATG)
Vinyl chloride	Hepatic angiosarcoma	A:T 6 T:A transversions

[a]For reviews see Refs, 116, 124, 127, 166

mechanism. The inactivation of this mechanism by promoter hypermethylation is associated with Kras G to A mutations in colon cancer.[99]

In DNA nucleotide excision repair, lesion recognition, preincision, incision, gap-filling, and ligation are required, and the so-called excinuclease complex comprises 16 or more different proteins. Large distortions caused by bulky DNA adducts (eg, BPDE-dG and 4ABP-dC) are recognized (XPA) and removed by endonucleases (XPF, XPG, FEN). A patch is then constructed (pol, pol e) and the free ends are ligated.

Base excision repair also removes a DNA segment containing an adduct; however, small adducts (eg, 3-methyladenine) are generally the target so that there is overlap with direct repair. The adduct is removed by a glycosylase (hOgg1, UDG), an apurinic endonuclease (APE1 or HAP1) degrades a few bases on the damaged strand, and a patch is synthesized (pol ß) and ligated (DNA ligases: I, II, IIIa, IIIß, and IV).

DNA mismatches occasionally occur, because excision repair processes incorporate 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). The mechanism for correcting 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 to the mismatch and an unmethylated adenine in a GATC recognition sequence, it removes the whole intervening DNA sequence. The parental template strand is then used by the polymerase to fill the gap

Double-strand DNA breaks can occur from exposure to ionizing radiation and oxidation. Consequences of double-strand DNA breaks are the inhibition of replication and transcription, and loss of heterozygosity. Double-strand DNA break repair occurs through homologous recombination, where the 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.[95]

Postreplication repair is a damage-tolerance mechanism and it occurs in response to DNA replication on a damaged template. The DNA polymerase stops at the replication fork when DNA damage is detected on the parental strand. Alternately, 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 a helical nucleoprotein (RAD51); 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.

Persistent non-repaired DNA damage blocks the replication machinery. Cells have evolved translesion synthesis (TLS) DNA polymerases to bypass these blocks.[100] Most of these TLS polymerases belong to the recently discovered Y-family, have much lower stringency than replicative polymerases, and thus are error prone. An increased mutation frequency is an evolutionary trade-off for cellular survival.

Mutator Phenotype

Cancer cells contain substantial numbers of genetic abnormalities when compared with normal cells. These abnormalities range from gross changes such as nondiploid number of chromosomes, ie, aneuploidy, and translocations or rearrangements of chromosomes, to much smaller changes in the DNA sequence including deletions, insertions, and single nucleotide substitutions. Therefore, carcinogenesis involves *errors* in (1) chromosomal segregation; (2) repair of DNA damage induced by either endogenous free radicals or environmental carcinogens; and (3) DNA replication Loeb originally formulated the concept of the mutator phenotype in 1974[101] to account for the high numbers of mutations in cancer cells when compared to the rarity of mutations in normal cells. Recent advances in the molecular analysis of carcinogenesis in human cells and animal models have refined the mutator phenotype[13] concept that is also linked to the clonal selection theory proposed by Nowell (Fig. 15-6).[102]

Oncogenes and Tumor Suppressor Genes

Chronic exposures to carcinogens, accumulation of mutations, development of the mutator phenotype, and clonal selection during several decades result in cancer Although the phenotypic traits of individual cancers are highly variable, commonly acquired capabilities include limitless replicative potential, self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, tissue invasion, sustained angiogenesis, and metastasis.[103,104] These phenotypic traits reflect a complex molecular circuitry of biochemical pathways and protein machines within cancer cells.[21]

The genes encoding the proteins within the cancer-associated molecular circuitry are of many functional classes and, historically, have been conceptually divided into oncogenes and tumor suppressor genes.[21,104] Detailed descriptions of oncogenes and tumor suppressor genes are found in Chapters 4 to 7. The *ras* oncogene and the *TP53* tumor suppressor gene will be used as examples of molecular targets of chemical carcinogens

Activated *ras* genes predominate as the family of oncogenes to be isolated from solid tumors that are 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. The first direct evidence of proto-oncogene activation by a chemical carcinogen was obtained from in vitro studies.[105] A wild-type recombinant clone of the human *Ha-ras* gene (pEC) was modified with benzo[*a*]pyrene diolepoxide. The treated plasmid was then used to transfect murine NIH-3T3 cells with the result that the transformed cell foci contained the same 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 carcinogen

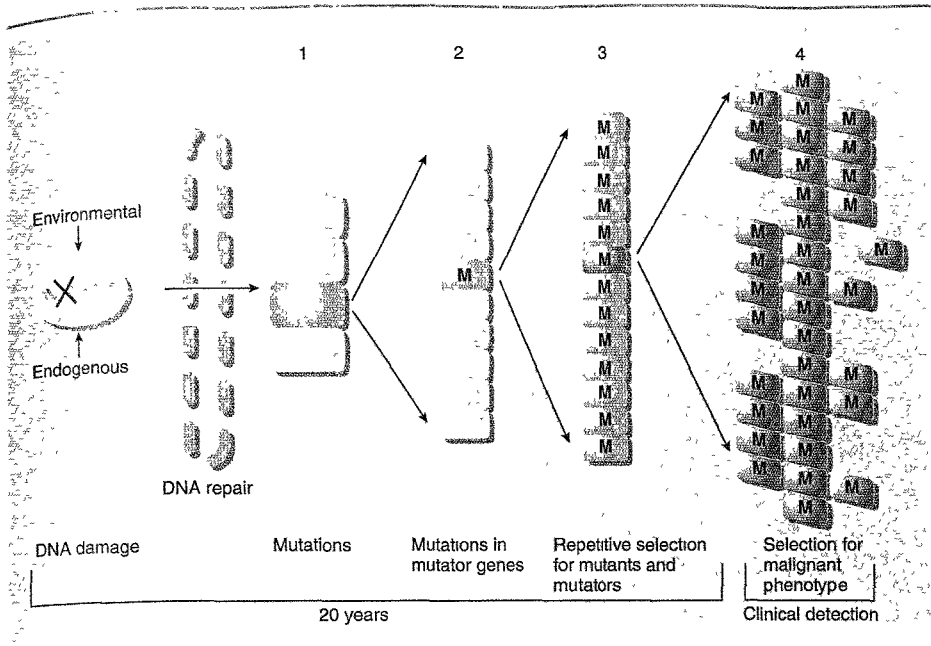

Figure 15-6 Mutation accumulation during tumor progression (1) Random mutations result when DNA damage exceeds the cell's capacity for error-free DNA repair. (2) These random mutations can result in clonal expansion and mutations in mutator genes (M). (3) Repetitive rounds of selection for mutants yield coselection mutants in mutator genes. (4) From this population of mutant cancer cells, there is selection for cells that escape the host's regulatory mechanisms for the control of cell replication, invasion, and metastasis. *Source* Modified from Ref 13

esis and surveys of different types of human tumors that arise from a variety of environmental exposures, *ras* mutations have been found.[35,106,107] For example, tobacco smoke can mutate *K-ras* during the molecular pathogenesis of human lung adenocarcinoma.[108] 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 mutations 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 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 methylmethanesulfonate, α-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 selective clonal growth advantage. The types of mutations that are found in chemically activated *ras* genes cause conformational changes that alter protein binding (GTPase-activating protein) in such a way that the *ras*-MAP kinase pathway is permanently activated. 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.[107] These and other results implicate *ras* mutations in chemical carcinogenesis. Similarly, malignant transformation occurred when immortalized human bronchial epithelial cells were transfected with an activated *ras* gene.[109,110] *Ki-ras* gene mutations are also one of many changes that can arise either early or late in the development of colorectal carcinoma.[111] These findings indicate that the accumulation of mutations, and not necessarily the order in which they occur, contributes to multistage carcinogenesis. Furthermore, the stage of carcinogenesis in which each mutation occurs is not necessarily fixed. In the model for human colorectal carcinoma, *ras* mutations most often occur during malignant conversion, but can be an early event (ie, tumor initiation), but 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 vs acute exposure, or they may be a function of tissue type.

The *TP53* tumor suppressor gene is central in the response pathway to cellular stress.[112] For example, DNA damage caused by chemical carcinogens activates the p53 tumor suppressor protein by posttranslational modification to transduce signals to "guard the genome"[113] by engaging cell-cycle checkpoints and enhancing DNA repair, and as a fail-safe mechanism, to cause replicative senescence or apoptotic death.[114,115] Mutations in the *TP53* gene or inactivation of its encoded protein by viral oncoproteins generally lead to a loss of these cellular defense functions. Not surprisingly, *TP53* mutations are common in human cancer.[116-120]

Molecular analysis of *TP53* can give clues to the environmental etiology of cancer (Table 15-4). It is implicit from the preceding text (see "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 resulting mutation. A dramatic example of this phenomenon is the previously mentioned *TP53* codon 249 mutation, which is detected in almost all aflatoxin-related hepatocellular carcinomas.[117,121,122] 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 B1 mutations. As discussed earlier, aflatoxin B1-8,9-oxide causes a promutagenic lesion by covalently binding to the N7 position of deoxyguanosine. Alternately, cells bearing the codon 249 lesion may have an important selective growth advantage. Evidence that a combination of these factors is responsible has been presented as well.[121] Another prominent example where circumstantial evidence points to specific molecular events is that of *TP53* mutations indicative of pyrimidine dimer formation in ultraviolet light–related skin cancers.[123] In the case of tobacco smoking and lung cancer, G:C to T:A transversions indicate the formation of adducts from activated bulky carcinogens (eg, polycyclic aromatic hydrocarbons).[116,124,125]

Assessment of Causation by the Bradford-Hill Criteria

Results obtained from molecular epidemiologic studies can be used for the assessment of causation. Using the "weight of the evidence" principle, Bradford-Hill

proposed criteria in the assessment of cancer causation, including strength of association (consistency, specificity, and temporality) and biologic plausibility.[126] These criteria can be applied for the analysis of data obtained in molecular epidemiologic studies.[127] Cigarette smoking has been established as a major risk factor for the incidence of lung cancer (Table 15-5). Codons 157, 248, and 273 of *TP53* are designated as mutational hotspots in lung cancer. The majority of mutations found at these codons are G to T transversions. Furthermore, besides lung cancer, codon 157 also constitutes one of the hotspots for G to T transversions in breast, and head and neck cancers. In smoking-associated lung cancer, the occurrence of G to T transversions has been linked to the presence of benzo[*a*]pyrene in cigarette smoke. Interestingly, codon 157 (GTC to TTC) mutations are not found in lung cancer from never smokers.[116-118] A dose-dependent increase in *TP53* G to T transversion mutations with cigarette smoking has been reported in lung cancer.[128] Benzo[*a*]pyrene diolepoxide, the metabolically activated form of benzo[*a*]pyrene, has been shown to bind to guanosine residues in codons 157, 248, and 273, which are mutational hotspots in lung cancer.[129] Also, cigarette-smoke condensate or benzo[*a*]pyrene neoplastically transforms in vitro human bronchial epithelial cells.[130] In general, molecular and epidemiologic data provide only circumstantial evidence for causation. Bradford-Hill criteria provide a framework for the logical consideration of converging lines of evidence in cancer etiology.

Table 15-5 **Assessment of Causation by the Bradford-Hill Criteria[a]**

Hypothesis: The chemical carcinogen, benzo[α]pyrene, in tobacco smoke can cause TP53 hotspot mutations at codons 157, 248, and 273 in human lung carcinogenesis

Strength of Association	Biologic Plausibility
Consistency	Tobacco smoke and benzo[*a*]pyrene are mutagens
Cigarette smoking or exposure to coal smoke is associated with a dose-response	Benzo[α]pyrene is metabolically activated and forms benzo[*a*]pyrene diolepoxide-DNA adducts in human bronchus in vitro (75-fold interindividual variation)
Increase in *TP53* mutations (G to T transversions in human lung cancer)	
Specificity	Benzo[α]pyrene diol-exposide binds to Gs in codons 157, 248, and 273, which are *TP53* mutational hotspots
Codon 157 (GTC 6 TTC) mutations are uncommon in other types of cancer, including in lung cancer from never smokers	Benzo[α]pyrene exposure to human cells in vitro produces codon 248 (CGG ≥CTG) *TP53* mutations
Temporality	Cigarette-smoke condensates or benzo[*a*]pyrene can neoplastically transform human bronchial epithelial cells in the laboratory
TP53 mutations can be found in bronchial dysplasia	

[a]For reviews see Refs 126 and 160

Implications for Molecular Epidemiology, Risk Assessment, and Cancer Prevention

Molecular epidemiology (use of biochemical and molecular biological methods to buttress epidemiological studies) has resulted from the confluence of several disciplines.[131] It encompasses the detection of carcinogen-macromolecular adducts (DNA as a direct genotoxic measure and protein as a surrogate), normal DNA sequence variants (heritable variations), 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. 15-7)

The biologically effective dose of a chemical carcinogen is governed by the amount that reaches a target tissue in a form that becomes activated to a chemical species capable of causing DNA lesions.[132] 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 for carcinogen-DNA dosimetry in humans are ^{32}P-nucleotide postlabeling, immunoassays, fluorescence spectroscopy, electrochemical conductance, liquid chromatography/electrospray ionization/tandem mass spectrometry (LC/ESI/MS/ MS), and gas chromatography/mass spectroscopy (GC/MS). 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.[3,32]

For exposure to tobacco smoke, GC/MS has provided a tool to measure aromatic amine protein adducts such as 4-aminobiphenyl hemoglobin. These studies have shown a dose-response relationship between the extent of smoking, type of tobacco used, and the adduct levels.[133] Similarly, tobacco-specific nitrosamine globin adducts have been used to monitor the dose in smokers and snuff dippers A corroborative approach to the measurement of benzo[*a*]pyrene-DNA adducts has been used in the monitoring of both tobacco and coal smoke exposure. In this study, both GC/MS and fluorescence line-narrowing spectroscopy were used to detect adducts exfoliated in urine.[57,134]

In the case of aflatoxin B1, levels of adducts exfoliated in human urine were measured by GC/MS 8,9-Dihydro-8-(*N*5-formyl-2',5',6'-triamino-4'-oxo-*N*5-pyrimidyl)-9-hydroxy-aflatoxin B1 (aflatoxin-*N*7 guanine) adducts correlated with environmental exposure and disease outcome. Similarly, aflatoxin-albumin adducts provided a corroborative surrogate. Both of these markers were also correlated with 6-hydroxycortisol levels, indicating a role for CYP3A4 in aflatoxin B1 activation Particularly, the presence of aflatoxin-*N*7 guanine adducts in urine was associated with liver cancer.[135,136] Based on these findings, a randomized clinical trial of the *interceptor molecule*, chlorophyllin (Derifil), was performed. The test drug or placebo was taken three times daily and urinary AFB1-*N*7-Gua was monitored by GC/MS. After 12 weeks, adduct levels were >100% higher among 90 persons taking the placebo than those (n = 90) taking chlorophyllin.[137]

Interindividual variation in cancer susceptibility, and, consequently, meaningful human cancer risk assessment, involve determination of inherited host factors as well as exposure assessment. Metabolic polymorphisms have been determined by the use of indicator drugs (eg, caffeine, debrisoquine, dextromethorphan, dapsone, and isoniazid); however, these assays are being replaced by direct genetic assays.[138-140] This approach has allowed the investigation of diverse host factors for which indicator drugs were not available, and it has been applied to a wide variety of cancers, including lung, head, and neck [141-143] Thus, genetic indicators of propensity for carcinogen activation and detoxification, DNA-repair capacity, and cell-cycle control are all features of molecular epidemiologic

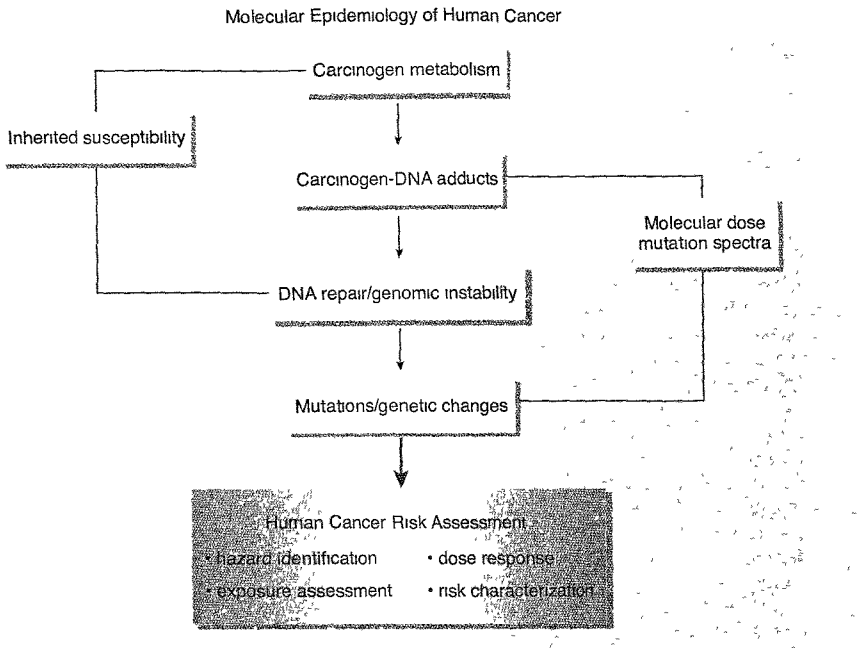

Figure 15-7 Facets of molecular epidemiology that investigate gene–environment interactions Once internalized, chemical carcinogens are metabolized to reactive species that cause DNA damage (carcinogen DNA adducts) The innate ability to repair DNA damage may reduce or ablate the overall damage burden. Alternately, 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 measure exposure can be used for human cancer risk assessment.

studies that are complementary to adduct studies because of the implications for a biologically effective dose after exposure.[3]

Cytochrome P450 polymorphisms, involved in carcinogen activation, and glutathione-S transferases, uridine diphosphate (UDP) glucuronosyltransferases, sulfotransferases, and N-acyltransferases, involved in both carcinogen activation and detoxification, could explain variations in cancer susceptibility among the human population Evidence that absent protection of a functionally intact *GSTM1* gene correlates with an increased risk of tobacco-related lung cancer.[144,145] Similarly, UDP glucuronosyltransferases (eg, *UGT1A1, UGT1A9, UGT2B7*) have been implicated in cancers of the head and neck. Persons inheriting reduced activity variants of *NAT1* and *NAT2* genes, resulting in the slow acetylator phenotype, are at a greater risk of aromatic amine-induced bladder cancer. This may include persons exposed through tobacco smoke inhalation.[60] Even though the inducible form of arylhydrocarbon hydroxylase (AHH) (*CYP1A1* and *CYP1A2*) has long been suspected of increasing cancer susceptibility in PAH-exposed persons, molecular epidemiologic studies remain inconclusive. Studies of *CYP2D6* metabolizer status and tobacco smoke-related lung cancer are similarly confusing.[8] However, analysis of multiple traits, eg, *CYP1A1* and *GSTM1*, in the same population may help to resolve these issues. Currently, there is a need for improved epidemiologic study design that integrates DNA adduct measures with indicators of metabolic capacity.[146-148]

Many DNA-repair genes have been described recently, and a growing number of polymorphisms have been identified for which molecular epidemiologic studies have provided evidence that genetic variation in these attributes can be a human cancer risk factor.[98,149-151] Typically, these types of molecular epidemiological studies initially focus on high exposure groups such as workers, patients taking therapeutic drugs, and tobacco smokers. Several polymorphisms in DNA-repair genes have now been implicated in tobacco-related neoplasams.[152]

Molecular characterization of tumors, ie, molecular profiling, is an important tool that has both etiologic and clinical application. Molecular profiling is a rapidly advancing area that is being propelled by DNA and protein microarray research.[41,42,153-155] During chemical carcinogenesis, the genome becomes altered and mutations accumulate. These mutations become evident in genes responsible for growth control and cellular homeostasis (including proto-oncogenes, tumor suppressor genes, and some DNA-repair genes), because corruption of these functions is part of carcinogenesis. In respect to chemical carcinogenesis, the most studied genes are Kirsten *ras* (*Kras*) and *TP53*. *Kras* is mutated in ~30% of lung adenocarcinomas, and may prove to be an indicator of prognosis or a guide to treatment.[108] The *TP53* tumor suppressor gene is mutated in most types of human cancers and it is the most commonly mutated gene yet known (eg, mutations in *TP53* are found in ~50% of lung cancers). Unlike *ras* gene mutations that are found in highly specific regions (codons 12, 13, 59, and 61), *TP53* mutations occur more widely. This is presumably because a positive growth advantage is conveyed only with specific *ras* mutations and the loss of *TP53* tumor suppressor function can occur with less specificity. However, for some malignancies, *TP53* mutations have provided clues to cancer etiology (see Table 15-4).[126,156] *TP53* is further distinguished from other genetic lesions in that several possible mutant phenotypes can exist. Mutations may simply lead to the absence of *TP53*, an inactive mutant protein may exist, or the mutant might convey a growth advantage. Several studies have investigated *TP53* expression, and even though its role in prognosis has not been clearly defined, it may be that it will provide a guide to treatment options.[157,158]

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 responses to tumor promoters have important implications in determining 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. 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 critical 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 an individual may be offset by specific concerns relating to that individual's responsibility to family members and psychosocial 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.[159]

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