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CHAPTER 11

Principles of Carcinogenesis: Chemical

Chemical carcinogenesis has its roots in the epidemiology of cancer.' In 1759, John Hill reported that tobacco snuff caused oral cavity cancers.[2] Soon after, Percival Pott published his findings that working as a chimney sweep, exposure to soot, and poor hygiene led to scrotal cancer.[3] Other associations followed, such as bladder cancer with aromatic amines,[4] benzene with leukemia,[5] and lung cancer with tobacco smoke.[6,7] Epidemiologic relations were also explored in the laboratory. As early as 1918 and in reaction to Pott's findings, Yamigawa and Ichikawa reported that coal tar could cause skin cancer in laboratory animals,[8] which was followed by similar findings for individual chemicals. This chapter presents some of the basic principles of chemical carcinogenesis, focusing on topics that are especially relevant to the understanding of human carcinogenesis. The molecular epidemiology of human cancer, an emerging field in cancer research, is explored. Reviews of the research accomplishments in the field of chemical carcinogenesis are available and contain more extensive bibliographies.[1,9,10]

MULTISTAGE CARCINOGENESIS

Carcinogenesis is a multistage process driven by genetic damage and epigenetic changes (Fig. 11–1). The traditional view of carcinogenesis is derived primarily from studies of animal models, but more recent studies rely on the molecular analysis of cancer-related genes in human cells.' Tumor initiation begins in cells through mutations from exposure to carcinogens. These mutated cells may have an altered rcsponsivcness to their microenvironment and a selective growth advantage when compared with the surrounding normal cells. The tumor-initiated cells also may have a decreased responsiveness to the intercellular and intracellular signals that maintain their architecture and regulate homeostatic growth. For example, initiated cells may be less responsive to negative growth factors, terminal cell differentiation. or programmed cell death. Selective clonal expansion of the initiated cells may also occur by physical perturbation of the normal microenvironment (*e.g.*, wounding of mouse skin or partial hepatectomy in rodents), chemical agents (*e.g.*, phorbol esters on mouse skin or rat liver and phenobarbital). microbial agents (*e.g.*, influenza virus enhancement of rodent lung carcinogenesis or hepatitis virus in human liver carcinogenesis), or other inflammatory processes. Tumor promotion results in further selective clonal expansion and proliferation of the initiated cells, thereby enhancing the probability of additional genetic damage through endogenous mutations or DNA-damaging agents. During tumor progression. malignant cells continue to exhibit progressive phenotypic changes" and genomic instability, including gene amplification, chromosomal aberrations, and altered gene expression.'?[13]

The classic view of two-stage carcinogenesis, in which tumor initiation (mutation) is followed by tumor promotion (epigenetic changes). has been conceptually important but is too simplistic. There may be six or more independent mutational events.'"[15] Furthermore, chemical carcinogens may be genotoxic, nongenotoxic. or cause epigenetic effects.[16,17] Dose-response relations may be linear or nonlinear."[18] Endogenous mutagenic mechanisms, such as DNA oxy-radical damage. depurination, polymerase infidelity, and deamination of 5-methylcytosine also contribute to carcinogenesis.[20-23] None-

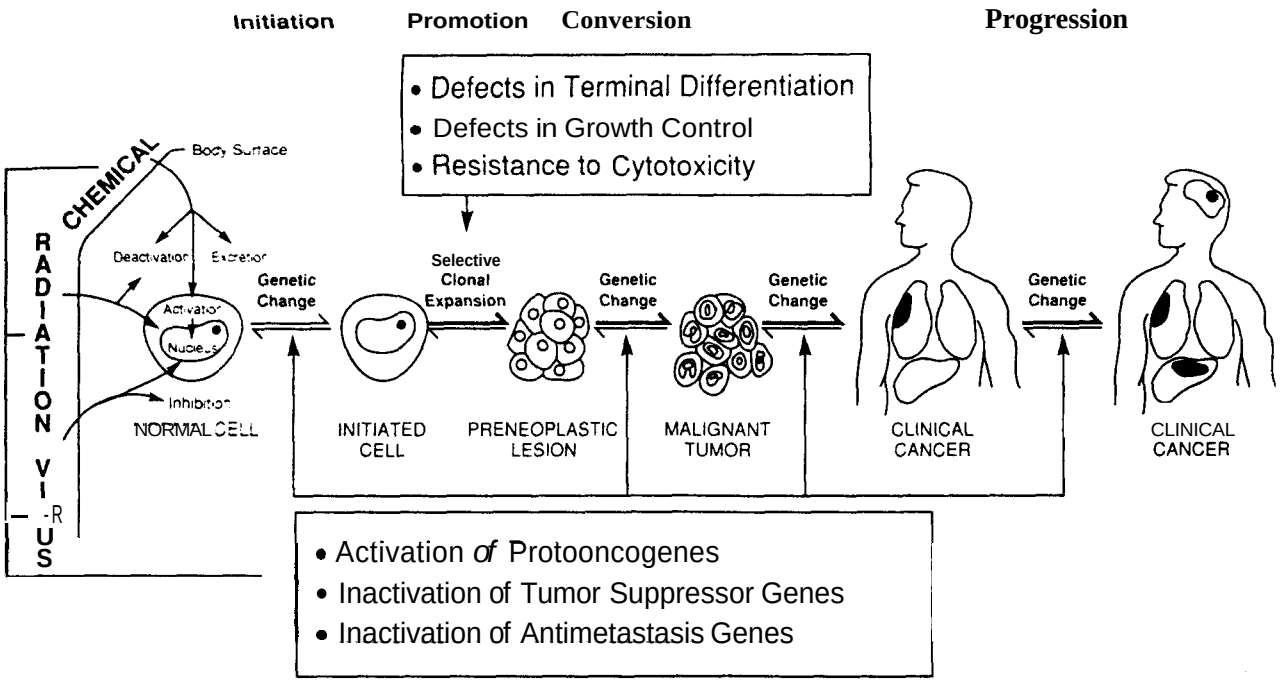

FIGURE 11–1. Multistage model of carcinogenesis.

theless, a debate continues regarding the relative importance of endogenous versus esogenous mutagenic events and the value of animal bioassays or short-term mutagenic assays for the assessment of human cancer risk.[23-26] Societal and regulatory decisions crucial to public health are at issue in this debate.

CARCINOGEN METABOLISM AND DNA DAMAGE

Most chemical carcinogens require metabolic activation through the generation of highly reactive electrophiles. These form DNA adducts by covalently binding to nucleic acids, exerting a promutagenic effect Metabolic activation is generally catalyzed by cytochrome P450 enzymes through oxidation Cytochrome P450 genes are continually being identified; organisms can have over 200 distinct P450 enzymes." A proposed nomenclature for individual genes uses the prefix *CYP* followed by an arabic numeral, letter, and numeral that indicate the family, subfamily, and gene number, respectively.[28] The families designated *CYP1, CYP2, CYP3,* and *CYP4* are primarily responsible for metabolism of foreign chemicals.[29] Family and subfamilies are determined by the percentage of gene sequence homolog). The ability to isolate and clone human cDNA has allowed the determination of substrate specificity for individual P430 enzymes, in many cases confirming earlier work using purified rodent proteins.[27]

The ability to metabolize carcinogens varies widely among animal species.[27] Cytochrome P450 enzymes also vary among different tissues within and among species.[30,31] Although interspecies differences have long been known to be quantitative, the metabolic activation pathways are qualitatively similar.[31] These observations support the qualitative extrapolation of carcinogenesis data from laboratory animals to humans, whereas quantitative differences are partly responsible for interspecies, interindividual, and intertissue responses to carcinogens.[32]

The metabolism of any individual carcinogen can be complex because it can be a substrate for several enzymes. For example, significant progress has been achieved in elucidating the metabolic activation and detoxification pathways for polycyclic aromatic hydrocarbons (PAH), such as benzo[*a*]pyrene (BP).[33] These compounds are composed of fused benzene rings that are essentially water insoluble but are readily absorbed through the lungs and gastrointestinal tract. They are commonly found as combustion products of fossil fuels (*e.g.*, coal, wood, diesel exhaust) and vegetable matter. Consequently, PAHs are common environmental pollutants. BP is metabolically activated by phase 1 enzymes forming a reactive diolepoxide (Fig. 11–2). Initially, CYP1A1 and epoxide hydroxylase catalyze the conversion of BP to a dihydrodiol. Then, cytochrome CYP3A4 converts this product to the diolepoxide (BP-7,8-diol 9,10-epoxide). Along this pathway, intermediates may be detoxified by conjugation, oxidation, or reduction and then excreted in urine or feces.

CRITICAL DNA TARGETS: PROTOONCOGENES AND TUMOR SUPPRESSOR GENES

Protooncogenes are normal cellular genes that can be inappropriately *activated* to cause dysregulation of cell growth and differentiation, which increases the *probability* of neoplastic transformation. They can be activated by carcinogens through nucleotide base substitutions, chromosomal translocations, and gene amplification. Among the best-studied protooncogenes is the ***RAS*** family. ***RAS*** protein products are involved in signal transduction pathways initiated by growth factors

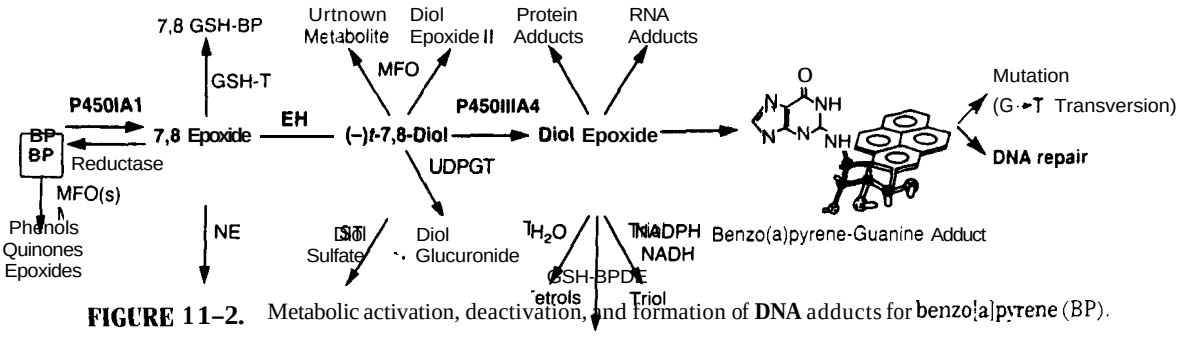

FIGURE 11–2. Metabolic activation, deactivation, and formation of **DNA** adducts for benzo[a]pyrene (BP).

and hormones at cell membrane receptors. In several experimental systems, activation is associated with tumor formation,[34] angiogenesis,[35] and metastasis.[36] Mutation of *RAS* protooncogenes have been observed in several types of human cancers.[37] Base substitutions occur nonrandomly at codons 12, 13, and 61 in vitro and in vivo on exposure to such agents as PAHs and radiation.[34,38–40] Mutation of the Ha-RAS protooncogene is an early event in rodent models of skin and mammary carcinogenesis.[38,41] In addition, the v-Ha-RAS transgene can substitute for the initiation step in mouse skin carcinogenesis in transgenic mice.[42,43]

In contrast to protooncogenes, tumor suppressor genes are normal cellular genes that can be inappropriately inactivated to cause dysregulation of growth and differentiation pathways. This also increases the probability of neoplastic transformation. Tumor suppressor genes perform different functions (Table 11–1). Although the loss of suppressor gene function is dominant in carcinogenesis, the inheritable trait is recessive; loss of function for both alleles is the basis of the two-hit hypothesis for carcinogenesis.[44,45] Alternatively, suppressor genes can be inactivated by genomic imprinting,[46] increased proteolytic digestion of gene products,[47] or other dominant negative mechanisms.[48] Based on target size theory, the inactivation of a single allele of a tumor suppressor gene (in which mutations at multiple sites may cause inactivation) should have an intrinsically higher probability than activation of a protooncogene (in which only a few mutations at specific codons can cause activation). The requirement for inactivation of both alleles of a tumor suppressor gene counterbalances this probability except in those cases in which an inactivated allele is inherited (*e.g.,* familial polyposis coli, Li-Fraumeni syndrome, Wilms' tumor, or retinoblastoma) or the process of inactivation is by a dominant negative mechanism as proposed for the p53 tumor suppressor gene.[48,49] Ionizing radiation, carcinogenic hormones, metals, aldehydes, and fibers such as asbestos tend to cause gross chromosomal abnormalities.[50] Therefore, tumor suppressor genes may be targeted by these carcinogens more readily than protooncogenes. Chemical carcinogens frequently cause promutagenic DNA adducts and chromosomal abnormalities.

TABLE 11–1. Examples of Functions of Putative Tumor Suppressor Genes

- Induce terminal differentiation
- hlaintain genomic stability
- Trigger senescence
- Regulate cell growth
  - Signal transducers of negative growth factors
  - Regulators, *e.g.,* PTPase-γ, of tyrosine kinases
- Induce proteases
- Induce programmed cell death
- Alter DNA methylase activity
- Modulate histocompatibility antigens
- Regulate angiogenesis
- Facilitate cell–cell communication

MUTATIONAL SPECTRUM

The mutational spectra of endogenous and exogenous carcinogens are largely responsible for activating protooncogenes and inactivating tumor suppressor genes. Studies using prokaryotic, simple eukaryotic, and site-specific mutagenesis assays have suggested that carcinogenic agents produce a fingerprint of DNA adducts and mutations. Similar evidence exists in eukaryotic studies such as esogenous gene insertion by shuttle vector" [52] or endogenous gene analysis.[53] These can become mutated at specific loci to produce detectable phenotypic changes (adenine phosphoribosyl transferase [APRT].dihydrofolate reductase [DHFR].and hypoxanthine-guanine phosphoribosyl transferase [HPRT]) However, these assays may underestimate mutational frequency because gene deletions, chromosomal nondisjunction. and frameshift mutations cause loss of other genes essential for cell survival. In addition, certain mutations mal cause clonal expansion or inhibition of the mutant cell. further complicating the interpretation of the mutational spectrum.

A comparison of the spontaneously occumng mutational spectrum at the *APRT* locus in Chinese hamster ovary (CHO) cells with spectra induced by ionizing radiation. ultraviolet radiation. or benzo[a]pyrene diolepoxide is shown in Table **11–2.** The mutational spectrum of ultraviolet light is consistent with mutagenesis models of promutagenic cyclobutane and pyrimidine-pyrimidone photoproducts. Ionizing radiation, in contrast, most frequently causes deletions. although specific point mutations are also observed. Benzo[a]pyrene diolepoxide, which binds to the 2-amino group of deoxyguanosine, produces predominantly G:C→T:A transversions, a similar mutational spectrum for benzo[a]pyrene diolepoxide has been observed in human cell assays.[54] The *HPRT* locus is of particular intcrest because of the potential to compare mutational spectra in cultured cells with those in human lymphocytes of persons esposed in vivo to environmental carcinogens.[55]

The molccular analysis of mutationally activated *RAS* protooncogenes in animal models suggests that mutational spectra

TABLE 11–2. Percent Distribution of Mutations in the Endogenous Adenine Phosphoribosyl Transferase Locus in Chinese Hamster Ovary Cells

	Transitions		*Transversions*					
Mutagen	*G:C → A:T*	*A:T → G:C*	*G:C → T:A*	*G:C → C:G*	*A:T → C:G*	*A:T → T:A*	*Deletions*	*Insertions*
Spontaneous	71	0	13	3	0	7	6	0
Ultraviolet radiation	61	2	5	10	5	12	2	2
Ionizing radiation	19	6	6	6	19	13	31	0
Benzo[*a*]pyrene diolepoxide	5	0	62	14	0	9	5	5

reflect DNA-adduct formation [1] For example, mutations found in activated *RAS* protooncogenes of rodent tumors after *N*-nitroso compound exposure are predominantly G:C→A T base substitutions; these are likely due to methylation of deoxyguanosine at the O^6 position followed by mispairing with thymine during DNA synthesis.[56] Although there are several guanine residues in *RAS* codons that would generate a transforming protein if substituted with adenine, the animal experiments have revealed that the mutations occur overwhelmingly at only certain mutation sites (codons 12, 13, and 61). Unexplained mutational specificities have been observed in other experimental systems and may reflect the differences in the spectrum of promutagenic carcinogen-DNA adducts, specificity of DNA repair enzymes, or resultant amino acid changes that are silent or nonlethal [57-59] The mutational spectra of activated *RAS* protooncogenes in tumors and preneoplastic lesions of laboratory animals may be instructive in the interpretation of mutations in human cancers. The spectra of Ki-*RAS* protooncogene mutations in human adenocarcinomas vary according to tissue site, although the base substitution is not always specific.-For example, G:C→T:A transversions occur in lung tumors, which can be caused by PAH exposure, endogenous mutagens (8-OH-deoxyguanosine caused by oxy-radical damage), DNA depurination, or polymerase infidelity.[60 61]

The p53 tumor suppressor gene is ideally suited for analysis of mutational spectra. First, p53 is well conserved in evolution and the DNA sequence of 5 domains are more than 90% homologous among humans and rodents.[62] Second, p53 is mutated in diverse types of human cancer.[63] Third, a wide spectrum of mutational types and codon sites has been observed that presumably define regions of the p53 protein likely to be essential for tumor suppression, cell-cycle control, and interactions with cellular and viral proteins. Most mutations in human tumors occur in the evolutionarily highly conserved domains in exons 5 to 8 of the p53 gene.[63] Moreover, the missense mutations are predominantly transitions at G:C base pairs, and 95% of the amino acids are entirely conserved in mouse, rat, monkey, and human. Mutational spectra also vary among cancer types (Table 11–3). The G:C→A:T transitions are most frequent in colon tumors and 68% occur at CpG dinucleotides. These findings are consistent with endogenous mutational mechanisms due to deamination of 5-methylcytosine residues. More than half of the p53 mutations in colon tumors are at hotspot codons 175, 248, 273, or 282, each of which is a CpG dinucleotide. In contrast to colon tumors, CpG dinucleotides are less frequently found at mutation sites of other human cancers. For example, G:C→T:A transversions are seen commonly in breast and lung cancers but not in colon tumors. CpG→TpG mutations occur with intermediate frequency in esophageal cancers, but 36% of these mutations are at A:T pairs, which may be due in part to DNA depurination or exposure to chemical carcinogens such as urethane (a contaminant of certain alcoholic beverages) or acetyladehyde (a metabolite of ethanol).

The most striking p53 mutational spectrum is found in hepatocellular carcinomas from Qidong, People's Republic of China[64] and southern Africa.[65] Eleven out of 12 base substitution mutations in 26 tumors were at the third base position of codon 249, and all but one were T:A transversions. One additional mutation at codon 249 was also a G:C→T:A transversion. These tumors were from patients who live in geographic areas where aflatoxin B_1 and hepatitis B virus are major risk factors for liver cancer. Aflatoxin B_1, a product of mold that grows in crops, forms promutagenic adducts on deoxyguanosine and induces primarily G:C→T:A transversions and G:C→A:T transitions in experimental systems.[66] Analysis of liver tumors from geographical areas where aflatoxin B_1 is considered not to be a significant risk factor will help determine whether exposure to this carcinogen or another coincident carcinogen may be responsible for these p53 mutations.

CHEMICAL-VIRAL INTERACTIVE EFFECTS

Interactive effects of chemicals, viruses, physical agents, and host factors have been observed (Table 11–4). One example is the relation of aflatoxin B_1 and hepatitis virus to the development of hepatocellular carcinoma. Hepatitis B virus (HBV) and more recently hepatitis C virus have been linked to primary hepatocellular carcinoma.[67] Geographic location of HBV carriers indicates that factors such as aflatoxin B_1 and alcoholic beverages are also important. Another example is in uranium miners, in whom the coexposure of tobacco smoke and radon significantly acts to increase risk of lung cancer.[68] Occupational asbestos exposure and tobacco consumption also act synergistically to increase the incidence of bronchogenic carcinoma.[69] In the laboratory, in vitro DNA strand breaks increase with the combined exposure.[70] Moreover, tobacco smoke and asbestos, through their ability to induce inflammation and lipid peroxidation, cause oxidative DNA damage.[71,72] Another consideration for the interactive effect is the ability of asbestos to adsorb PAHs and act as a carrier for the carcinogen.[73]

TABLE 11–3. Examples of Human Cancers With Base Substitution Mutations in the p53 Gene

Worldwide Cancer Burden (Rank)[182]	*Cancer*	*Number of Mutations Detected in Tumor Cell Lines and Tumors'*	*Mutational Hotspots (Codon)*	*Mutations*					
				A:T	*G:C*	*CpG → TpG*	*G:C → A:T*	*G:C → T:A*	*G:C → CG*
1	Stomach	6		2	4	2	3	1	0
2	Lung	70	273	7	63	14	22	32	9
3	Breast	63		15	48	14	27	12	9
4	Colon	48	175, 248, 272,282	10	38	30	36	0	2
7	Esophagus	38		12	26	7	16	10	0
8	Liver	22	249	2	20	0	3	16	1
11	Bladder	15		3	12	5	7	2	3
9, 12	Leukemia and lymphomas	84	175, 213, 248,272, 282	26	58	35	45	6	7
—	Skin	16		0	16	1	11 t	4	1
—	Sarcomas	12		0	12	7	8	2	2
—	Brain	24	273	4	20	9	17	2	1
—	Ovary	14		3	11	1	6	1	4

* Mutation screening by an RFLP analysis of one site is not included.
t Includes tandem double mutations.

ASSESSMENT OF CANCER RISK IN HUMANS

Our understanding of carcinogenesis and risk to human health comes from experimental models and methods including mutagenesis assays, mammalian cell-culture experiments, animal studies, classic epidemiology, and molecular epidemiology. The usefulness of each method can be contrasted with its limitations (Table 11–5). The evaluation of an individual patient must rely on an accurate history, physical examination, and research data. The latter is often beyond the scope of the practitioner, but methods of individual cancer risk assessment have been proposed and resources are available.[74] Some known or potential human carcinogens are indicated in Table 11–6.

Short-term assays for mutagenesis provide quick and inexpensive screens for potential carcinogens.[75,76] Among the most widely used and sensitive is the Ames' assay. in which frameshift mutations or base-pair substitutions are measured in *Salmonella typhimurium* bacteria.[77] The Ames' assay also

TABLE 11–4. Interactive Effects of Carcinogens, Viruses, and Host Factors

Type	*Example*	*Associated Tumor*
Chemical-chemical	Tobacco smoke and alcoholic beverages	Otolaryngeal. esophageal
Viral-chemical	HPV and tobacco smoke	Cervical
	EBV and N-nitrosamine	Nasopharyngeal
	HBV and aflatoxin B_1	Liver
Physical-chemical	Asbestos and tobacco smoke	Lung
	Radon and tobacco smoke	Lung
Chemical-host	PAH and CYP2D6	Lung
	Tobacco smoke and CYP2D6	Lung
Physical-host	Asbestos and CYP2D6	Lung
	Sunlight and xeroderma pigmentosum	Skin
	Radiation and RB-deficient genotype	Osteosarcoma
Viral-host	EBV and X-linked immunodeficiency syndrome	Lymphoma

HPV. human papilloma virus; ERV. Epstein-Barr virus: HBV, hepatitis B virus; CYP2D6. cytochrome P-450 CYP2D6 metabolic phenotype determined by debrisoquine sulfate administration and measurement of urinary metabolites; PAH, polycyclic aromatic hydrocarbon; RB, retinoblastoma susceptibility gene.

TABLE 11–5. Testing for Carcinogenicity

Method	*Advantages*	*Disadvantages*
In vitro testing	Economical Rapid results Human cells can be used	Uncertain in vitro to in vivo extrapolations Frequent false-positives and false-negatives Mutagenicity is not carcinogenicity Substantial interlaboratory variation
Animal bioassay	More predictive of human experience than short term tests Elucidates species differences	Expensive Doses are higher than those experienced by humans Uncertain animal to human extrapolation
Classic epidermiology	Direct measurement of human experience Covariables examined Dose-response data	Insensitive Does not prove causation Unknown confounding variables
Molecular epidemiology	Measures internal dosimeter of exposure and genetic predisposition Identifies risk in an individual	Early stage of development and validation

TABLE 11–6. Selected Known or Potential **Human** Carcinogens[183]

*Known or Potential Carcinogen**	*Target Organs*	*Known or Potential Carcinogen**	*Target Organs*
Chemical		**Industry†**	
Aflatoxin	Liver	Aluminum production	Lung, bladder
4-Aminobiphen! 1	Bladder	Auramine manufacture	Bladder
Arsenic	Skin, lung	Boot and shoe manufacture	Bone marrow, nasal sinus
Asbestos	Lung, pleura	Coal gasification	Skin, lung, bladder
Benzene	Bone marrow	Coke production	Skin, lung, kidney
Benzidine	Bladder	Furniture and cabinet manufacture	Nasal
Benzo[*a*]pyrene	Skin, lung	Iron and steel founding	Lung
Beryllium	Lung	Hematite mining (radon)	Lung
bis(chloromethyl)ether	Lung	Isopropyl alcohol manufacture	Nasal sinus
Cadmium	Prostate, lung	Magenta manufacture	Bladder
Chromium	Lung	Paints	Lung
Coal tar and pitch	Skin, lung, bladder	Rubber industry	Bone marrow, bladder
Nickel compounds	Lung, nasal cavity	**Viral**	
Vinyl chloride	Liver, lung, brain	Epstein-Barr virus	Nasopharynx
Mineral oils	Skin	Hepatitis B	Liver
Mustard gas	Lung	Hepatitis C	Liver
2-Napthylamine	Bladder	Human immunodeficiency	Lymphatic
Shale oils	Skin	Human T-lymphocyte	Lymphatic, bone marrow
Soots	Skin, lung	Human papilloma	Cervical
Lifestyle and Diet			
Betel quid with tobacco	Oral		
Tobacco smoke	Multiple sites		
Alcoholic beverage	Oral, esophageal, pancreas, liver		
Smoked, salted, pickled foods	Gastrointestinal		
High fat diet	Colon, mammary		

* Carcinogenic agents or activities in this table are classified by the International Agency For Research on Cancer as recognized human carcinogens. The list is not all inclusive.
† Refers to only some occupations within an industry.
(Tomatis L, Aitio A, Wilbourn J, Shuker L., Human carcinogens so far identified. Jpn J Cancer Res 1989;80:795–807)

has been used as a biomonitor in hurnans. Urine from cigarette smokers, for example, is mutagenic:.[78] Other short-term assays use Chinese hamster ovary cells, mouse lymphoma cells, V79 cells, or rat hepatocytes to measure forward mutations, sister chromatid exchanges, and unscheduled DNA synthesis.[79] Although short-term assays are useful in identifying potentially carcinogenic compounds in the respective cell system, the same sensitivity makes the results difficult to extrapolate to humans; positive results might be unique to the strain. Factors such as metabolism, repair, and exposure cannot be assessed.

Laboratory animal studies provide an important source for the identification of potential carcinogens in humans, mostly because few better alternatives exist. These animal bioassays are also expensive and time-consuming, preventing the testing of a large number of chemicals.[76,80] As recommended by the National Toxicology Program, carcinogenicity bioassay studies should use lifetime exposures in rats and mice with maximally tolerated doses (MTD), that is, those not producing clinically evident toxic effects. To infer that a carcinogenic effect is present in laboratorv animals, dose-response relations, overall mortality rates, and consistency with data from other species must be examined. The limitations in these experiments include the routine use of the MTD that potentially increases cell replication and endogenous mutations; interspecies and interstrain differences; use of rodents known to have high spontaneous rates of cancer; an inability to account for metabolic differences between high- and low-dose exposure; and difficulty in interpreting data from doses that commonly exceed those experienced by humans.[81–83]

Human investigations provide the most relevant data regarding human risk. Classic epidemiology measures the incidence or prevalence of disease in human populations. Epidemiologic studies have identified previously unknown risks such as asbestos-related pleural mesothelioma, benzene-induced leukemia, and bladder cancer in dye workers. Epidemiologic methods by themselves do not demonstrate causation. The assessment of causation can be aided by Sir Austin Bradford-Hill'sproposed criteria, which consider the strengths of an association, consistency, specificity, temporality, biologic gradients, biologic plausibility, coherence, and analogies.[84] Study design and controlling for confounding variables are important determinants of a true association. For example, assessing cancer mortality rates from death certificates can be unreliable because the certificate diagnosis frequently is not accurate. Loss of persons in follow-up, inappropriate choice of control populations, ascertainment bias secondary to an unrelated cancer cluster, and "healthy worker effects" may also influence outcomes. The latter effect can be important if an occupation impacts on risk factors, for example, by not allowing tobacco consumption at the workplace. It is also important to examine existing exposure data and dose-response relations. Finally, statistical methods, beyond the scope of this chapter, must be carefully chosen to determine whether a finding is attributable to chance.

The concordance between different methods for inferring human cancer risk is variable. In such cases as aflatoxin B,, mustard gas, radon, and vinyl chloride. animal testing revealed a carcinogenic risk before human epidemiologic evidence was available.[83] Comparing DNA reactivity based on chemical structure, mutagenicity, carcinogenicity in laboratory animals and epidemiology, most chemicals that have positive results in one method are generally positive with other methods, although 100%concordance does not exist. Sensitivity for short-term assays is high but specificity is low.[83,85] For example, chemicals that are predictive of reactivity by chemical structure are commonly mutagenic, but 84% of tested carcinogens and 66%of noncarcinogens are mutagenic.[83,86] Chemicals that are not predictive of reactivity and are nonmutagenic are carcinogenic less than 5% of the time. The concordance from animal to human experience is wide ranging (5% to 70%), although carcinogens that are more potent in one species tend to be more potent in others.[85,87]

The physician can look to various regulatory, governmental, or review organizations for extensive evaluation of the scientific literature. Some organizations generate documents reporting the findings of a panel of experts who critically review the scientific literature, whereas others simply summarize data from other organizations. Several lists of carcinogens have also been published, but the evaluations of the literature and the definitions can vary greatly among organizations.[74] The physician should be aware of the purposes and goals of an organization when requesting its information. Quantitative risk assessment methods are used by regulatory agencies to estimate the risk to a population exposed to a particular carcinogen at a specific dose. Risk assessments serve public health interests as they attempt to predict the frequency of cancer in a population before epidemiologic investigations can be performed and adverse outcomes occur. Several mathematical models rank relative risks and suggest regulatory exposures limits. Risk assessments include four general steps."

1. Hazard assessment, which qualitatively reviews scientific literature to determine whether a hazard might exist
2. Dose-response assessment. which evaluates the doses used in scientific studies and relates them to human exposures
3. Exposure assessment. which examines a population thought to be at risk with regard to the quantity, duration, and routes of exposure. Necessary information includes quantitative measurements of relevant media such as soil, water, air, or body fluids.
4. Risk characterization. which incorporates the above information and evaluates the assumptions and the uncertainties to estimate risk. At the conclusion. an incidence of cancer will be predicted. such as 1 additional person in 1 million persons.

Each of these steps requires assumptions that are open to debate, such as subjective evaluations of the literature, extrapolations from laboratory animals to humans. and mathematical methods. It is tempting to extrapolate conclusions of a formal risk assessment to risk for a given individual. especially if that person is a cancer patient. Such an exercise is inherently flawed because risk assessments calculate risk in a group of people and do not assess coexposures or inheritable variables that may increase or decrease an individual's risk of cancer. Separately. risk assessments must be reevaluated when experimental or epidemiologic data become available. One example is dioxin exposure. whose laboratory animal carcinogenicity data led to strict regulation in humans and with considerable cost. However, recent data have led the Environmental Protection Agency to review its policy.[89]

MOLECULAR EPIDEMIOLOGY OF HUMAN CANCER

Molecular epidemiology is a multidisciplinary field that seeks to explore cancer risk through molecular genetics and biochemical methods.[90] In contrast to classic epidemiology, which identifies cancer risk in populations, molecular epidemiology explores cancer risk in individuals. This strategy includes exposure assessments by measuring biologically effective doses of carcinogens in target tissues (or surrogate tissues) and an analysis of host susceptibility factors, within the framework of well-designed epidemiologic studies.

EXPOSURE ASSESSMENT

Carcinogen-DNA adducts, somatic gene mutations, and cytogenetic changes can be measured in the DNA of target cells or in surrogate blood cells.[90-94] The observation that carcinogen-DNA adducts formed in cultured human tissues are generally the same as those found in experimental animal cancer models has encouraged investigators to search for DNA adducts in humans. Carcinogen-DNA adducts are the result of exposure, absorption, metabolism, and DNA repair. The amount of a carcinogen that reaches the target DNA reflects the biologically effective dose and can be distinguished from the relevance of ambient air or other environmental measurement of exposure. A variety of assays are available to identify carcinogen-DNA adducts in human tissues.[90,91] Studies using laboratory animals generally demonstrate a relation between dose, adduct level, and carcinogenicity.[91,95] It is expected that human epidemiologic studies will bear a similar relation.

The most important lifestyle risk factor in carcinogenesis is tobacco smoke exposure.[96] Due to aggressive advertising and the addictive nature of cigarettes, tobacco smoke has become the major cause of cancer. Of growing concern is the documentation that passive exposure to tobacco smoke also increases the risk of lung cancer.[97,98] Putative adducts have been correlated with consumption by the ^{32}P-postlabeling assay in human lung,[99-101] alveolar lavage cells,[102] and placenta,[103] but not in lymphocytes[104] or oral mucosa.[105] Tobacco-specific N-nitrosamines are potent carcinogens in laboratory animals.[96] Levels are higher in secondary rather than mainstream smoke, highlighting the role of passive smoke exposure. Hemoglobin adducts are increased in smokers over nonsmokers, whereas even higher levels are reported in snuff dippers.[106] Nitrosamine-related adducts have also been found in the human lung.[107,106] Urine also can be used as a biomarker for tobacco smoke exposure because it is mutagenic in smokers. However, this cannot be solely attributed to tobacco because dietary heterocyclic amines also are found in the urine.[109]

PAHs, associated with an increased risk of lung and skin cancer, can cause adducts found in the white blood cells of persons exposed to coke oven emissions, tobacco smoke, and urban areas (*e.g.*, from industrial pollution).[110-116] Dietary exposure resulting from the overcooking of meats and fish also results in elevated adduct levels.[117] Adducts also have been found in human lung and placental samples.[118] Wide interindividual variations in levels have been noted, presumably reflecting variable cancer risk in individuals.

Among the best-studied dietary carcinogens are the aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*. These molds are contaminants of corn, peanuts, sorghum, and rice. The risk of hepatocellular carcinoma correlates with the degree of contamination by geographic region.[119] Adduct levels also correlate with regional exposure and are inversely correlated with residence in industrialized countries.[""."" N-nitrosamines also are dietary (and tobacco smoke) carcinogens whose adducts can be measured in DNA.[121-126] In this case, adduct levels are elevated in Chinese persons with esophageal cancer[121] and in Japanese persons with liver cancer,[" confirming the risk associated with these adducts and their parent N-nitroso compounds.

INTERINDIVIDUAL VARIATION AND HOST SUSCEPTIBILITY FACTORS

> No one supposes that all the individuals of the same species are cast in the very same mould. These individual differences are highly important for us. . . .
>
> Charles Darwin, *The Origin of Species*, 1859

Metabolic *Activation and* Deactivation

Physicians and scientists have repeatedly recognized person-to-person differences in behavior, morphology, and risk of disease. Such interindividual differences reflect inherited and acquired factors. For example, inherited differences in susceptibility to physical or chemical carcinogens have been observed, including an increased risk of sunlight-induced skin cancer in people with xeroderma pigmentosum,[127] bladder cancer in dye stuff workers with a poor acetylator phenotype,[128] and bronchogenic carcinoma in tobacco smokers who have an extensive debrisoquine hydroxylator phenotype.[129,130] Because most chemical carcinogens require metabolic activation to exert their oncogenic effects, interindividual variation in metabolism is considered to be an important determinant of cancer susceptibility.[32]

PAH metabolism is probably the best example of the effects of interindividual variation and cancer risk. Several thousand-fold interindividual variation has been observed in lung.[32] Placental aryl hydrocarbon hydroxylase (AHH) activity, which is under direct genetic control, can be induced by maternal exposure to environmental carcinogens (*e.g.*, tobacco smoke or dietary factors).[131,132] Higher AHH activity is generally correlated with higher adduct level.[101] The induction process itself may have a genetic component,[133] and inducible activity is higher in cultured lymphocytes from lung cancer cases compared with controls.[134] The ability to form benzo[*a*]pyrene diolepoxide–DNA adducts was higher in lung cancer patients than in controls.[135,136] A highly inducible allelic variant of CYP1A1 has been identified in humans.[137,138] A restriction fragment length polymorphism for CYP1A1 has also been described that is associated with increased risk of tobacco smoking associated lung cancer in a Japanese study.[139]

CYP2D6 activity, another P450 cytochrome, is polymorphic and has also been linked to lung cancer risk.[129,130] CYP2D6 hydroxylates xenobiotic antihypertensives (including debrisoquine), antidepressives, and a carcinogenic tobacco-specific *N*-nitrosamine.[140] An individual's polymorphic phenotype is inherited in an autosomal recessive manner. The rate of 4-hydroxylation of debrisoquine varies several thousand-fold

among people. Lung, liver, and advanced bladder cancer patients are more likely to have the extensive hydroxylator phenotype when compared with noncancer controls.[129,141,142] The increased risk in lung cancer is found primarily for histologic types other than adenocarcinoma of the lung and increases in persons who are occupationally exposed to high amounts of asbestos or PAHs.[130,143]

Acetylation of carcinogenic aromatic amines has been proposed as a cancer risk factor.[144] The *N*-acetylation polymorphism is controlled by two autosomal alleles at a single locus in which rapid acetylation is the dominant trait and slow acetylation is recessive. The slow acetylator phenotype has been linked to occupationally induced bladder cancer in dye workers exposed to large amounts of N-substituted aryl compounds.[128] In contrast, the rapid acetylator phenotype is more commonly found in cases from two studies of colon cancer[145,146] but not in another.[147] Whether this association is due to metabolism of a carcinogenic aromatic amine in the colonic epithelium is not known.

Glutathione S-transferases (GST) are multifunctional proteins that catalyze the conjugation of glutathione and electrophiles, including the ultimate carcinogenic metabolite of benzo[*a*]pyrene.[148,149] The three isoenzymes of GST (α, μ, and π) vary in their substrate specificity, tissue distribution, and activities among individuals.[150] Expression of GST-μ is inherited as an autosomal dominant trait[151] and individuals with low GST-μ activity may be at a greater risk for lung cancer caused by cigarette smoking.[152,153]

Protooncogenes and Tumor Suppressor Genes

Genetic differences in protooncogenes and tumor suppressor genes can also be predictive of cancer risk. Inheritance of a germline mutation in the tumor suppressor gene p53[154,155] and perhaps *RB*[156] predisposes to the development of cancer. It has been found that inheritance of rare alleles for H-*RAS*, detected by a restriction enzyme digest and Southern blot analysis, is found more frequently in lung cancer cases than controls.[157,158] These alleles may provide an unstable site where recombination *or* amplification occurs. The relation of this polymorphism to lung and other cancers has been extensively reviewed.[159] Analysis of the L-*MYC* protooncogene also demonstrates a polymorphic restriction enzyme site. The site present allele has been detected more frequently in persons with soft tissue sarcomas and gastric and lung cancer, although not consistently in lung cancer.[160–162] The association also suggests a worse prognosis at the time of diagnosis in renal cell cancer patients.

DNA Repair Rates

DNA repair enzymes modify carcinogenic damage by the removal of DNA adducts. Studies of cells from donors with xeroderma pigmentosum have been particularly important in expanding our understanding of DSA excision repair and its possible relation to risk of cancer.[163] DNA repair rates. but not fidelity. can be determined by measuring unscheduled DNA synthesis and removal of DNA adducts. Substantial interindividual variations in DNA repair rates have been observed.[163] The fidelity of DNA repair also may vary among individuals, and recent advances in the identification of mammalian DNA repair genes and their molecular mechanisms provide an opportunity for investigation.[164,165] In addition to finding severely depressed excision repair rates in xeroderma pigmentosum cells (*e.g.*, complementation group A), a fivefold variation among individuals in unscheduled DNA synthesis induced by UV exposure of lymphocytes in vitro has been found in the general population.[163] A significant reduction in unscheduled DNA synthesis induced in vitro by *N*-acetoxy-2-acetylaminofluorene has been observed in mononuclear leukocytes from individuals with a history of cancer in first-degree relatives compared with those without a family history.[166,167]

Interindividual variation has been noted in the activity of O^6-alkyldeoxyguanine-DNA alkyltransferase; this enzyme removes the adduct O^6-deoxyguanine caused by N-nitrosamine exposure. It is a suicide protein that transfers adducted alkyl groups to itself and becomes irreversibly inactivated. Cell cytotoxicity and tumor-cell resistance are negatively correlated with levels of this enzyme.[168] Wide variations in DNA repair activity have been observed in different types of tissues,[169,170] and cells may have lower repair rates after terminal differentiation.[171] The activity of this DNA repair enzyme is inhibited by certain aldehydes[169] and alkylating cancer chemotherapeutic agents.[171] A decrease in this DNA repair activity has been observed in fibroblasts from patients with lung cancer compared with donors with melanoma *or* noncancer controls.[172] Therefore, acquired *or* inherited deficiency in O^6-alkylguanine-DNA-alkyltransferase may be a cancer risk factor in tobacco smokers.

CARCINOGENICITY OF CHEMOTHERAPY

The carcinogenic effect of some chemotherapeutic agents in humans has been well documented and supported by laboratory animal studies (almost an 85% concordance), and reviews with extensive bibliographies are available.[174–176] The International Agency For Research on Cancer, through working groups of scientists and physicians, has identified about 20 single agents or combination chemotherapy regimens for which there is sufficient evidence of a carcinogenic effect in humans. and about 50 others in which a carcinogenic effect is suspected (Table 11–7).[174,177] The identification of a carcinogenic effect does not preclude its use for treatment in patients The decision process depends on the specific risks and benefits, considering the prognosis and life expectancy of the patient with and without treatment.

Carcinogenic chemotherapeutic agents generally exhibit target organ specificity. The bone marrow, lymphatic system, and urinary bladder are most commonly affected. As with other carcinogens, genotoxic agents generally act by forming promutagenic DNA adducts and crosslinking,[174] although chromosomal aberration also occurs.[178] P450 metabolic activation is not necessarily required. Nitrogen mustard–type compounds (including nitrogen mustard. chlorambucil, melphalan, and chloronaphazine) have highly reactive electrophilic centers that react with DNA to form adducts without metabolic activation. Cyclophosphamide, in contrast, is a nitrogen mustard that undergoes cytochrome P450 metabolism in the liver, where it is converted to acrolein and 4-hydroxycyclophosphamide. This latter compound breaks down spontaneously to phosphoramide mustard, which then reacts with DNA.

TABLE 11–7. Selected Pharmaceutical Agents That Are Known or Potential Human Carcinogens*

Agent	*Animal Target Organ*	*Human Target Organ*
Phenacetin	Urinary tract. nasal	Kidney
Chloramphenicol	—†	Bone marrow
Doxorubicin	Mammary, skin	—
Azacitidine	Bone marrow, lymph. lung, skin	—
Chloronaphazine	Skin, lung	Bladder
Bischloroethylnitrosourea	Lung. neurologic. peritoneum	Bone marrow
Busulphan	Thymus. ovary	Bone marrow
Chlorambucil	Lung. bone marrow, ovary	Bone marrow
Chlorzotocin	Peritoneum	Bone marrow
Cisplatin	Lung, bone marrow	—
Cyclophosphamide	Mammary, bone marrow, bladder. liver. testis, neurologic	—
Melphalan	Lymphatic. lung	Bladder, bone marrow
Nitrogen mustard	Skin. lung. lymphatic	Bone marrow
Procarbazine	Lung, bone marrow, kidney	Skin
Streptozocin	Liver, pancreas	—
Thiotepa	Lung, lymphatic, bone marrow, uterus. mammary	—
Arsenic salts	Lung, stomach	Bone marrow
Coal tars	Skin	Skin
8-Methosypsoralen & UVA	Skin	Skin
Testosterone	Cenix. uterus. prostate	Skin
Nonsteroid estrogen	Vagina. cervix, uterus. ovary. mammary	Liver Vagina, cervix
Estrogen replacement	Kidney	Endometrium
Azathioprine	Lymphatic	Lymphatic
Cyclosporine	Lymphatic	Lymphatic

* Classified by the Agency for Research on Cancer as known or possible human carcinogens. This list is not all inclusive.
† Cancer not observed.

Chemotherapy combinations can significantly raise the risk of secondary tumors, especially nonlymphocytic leukemias. The combination of lomustine, cyclophosphamide, and vincristine led to a leukemia incidence of **14%** over **4** years after treatment.[179] Nitrogen mustard, vincristine, prednisone, and procarbazine for the treatment of Hodgkin's disease yield leukemia rates up to 17%.[180] More than six cycles increased the relative risk from 9 to **14.** Radiation further increases the risk of leukemia.[181]

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