Chemical and Physical Carcinogenesis: Advances and Perspectives for the 1990s

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Abstract

Carcinogenesis is a multistage process driven by carcinogen-induced genetic and epigenetic damage in susceptible cells that gain a selective growth advantage and undergo clonal expansion as the result of activation of protooncogenes and/or inactivation of tumor suppressor genes. Therefore, the mutational spectra of chemical and physical carcinogens in these critical genes are of interest to define endogenous and exogenous mutational mechanisms. The p53 tumor suppressor gene is ideally suited for analysis of the mutational spectrum. Such an analysis has revealed evidence for both exogenous and endogenous molecular mechanisms of carcinogenesis. For example, an informative p53 mutational spectrum of frequent G→T transversions in codon 249 is found in hepatocellular carcinomas from either Qidong, People’s Republic of China, or southern Africa. This observation links exposure to aflatoxin B1, a known cancer risk factor in these geographic regions, with a specific mutation in a cancer-related gene. Other studies indicate that abnormalities in genes controlling the cell cycle may cause genomic instability and increase the probability of neoplastic transformation. Finally, mechanistic understanding of carcinogenesis is leading to improved cancer risk assessment and to the identification of individuals at high cancer risk.

Introduction

Carcinogenesis is a mature multidisciplinary field of cancer research that has a rich history of scientific accomplishments achieved by epidemiological observations (1–3); development and exploitation of both animal models relevant to human cancer (4) and in vitro cell and tissue models including the use of human tissues (5, 6); and most recently, advances in molecular genetics (7–11). Examples of conceptual advances and seminal observations in the areas of chemical and physical carcinogenesis are listed in Table 1; advances in viral carcinogenesis are reviewed by Howley in this issue (12).

Since the research accomplishments of carcinogenesis have been extensively reviewed (13–25), this commentary will focus on selected topics that are especially relevant to current understanding of human carcinogenesis and, in particular, to the molecular epidemiology of human cancer, an emerging field in cancer research.

Multistage Carcinogenesis

Carcinogenesis is a multistage process driven by carcinogen-induced genetic and epigenetic damage in susceptible cells that gain a selective growth advantage and undergo clonal expansion as the result of activation of protooncogenes and/or inactivation of tumor suppressor genes. The traditional view of carcinogenesis (Fig. 1) is derived primarily from studies of animal models. The first stage of the carcinogenic process, tumor initiation, involves exposure of normal cells to chemical, physical, or microbial carcinogens that cause a genetic change(s) providing the initiated cells with both an altered responsiveness to their microenvironment and moreover exerts a selective clonal expansion advantage when compared to the surrounding normal cells (26–28). The initiated cells may have decreased responsiveness to the inter- and intracellular signals that maintain normal tissue architecture and regulate the homeostatic growth and maturation of cells. For example, initiated cells may be less responsive to negative growth factors, inducers of terminal cell differentiation and/or programmed cell death (4, 26, 29–35).

Tumor promotion results in proliferation and/or survival of the initiated cells to a greater extent than normal cells and enhances the probability of additional genetic damage including endogenous mutations accumulating in the expanding population of these cells. The probability of a subpopulation of initiated cells converting to malignancy can be substantially increased by their further exposure to DNA-damaging agents (38–40) that may activate protooncogenes (41) and/or inactivate tumor suppressor genes. Malignant cells continue to exhibit progressive phenotypic changes during tumor progression (42) and may exhibit intrinsic genomic instability that is manifested by the abnormal number and structure of chromosomes, gene amplification, and altered gene expression (43–45).

This classical view of two stage carcinogenesis involving a mutation, tumor initiation, and an epigenetic change, tumor promotion, has been conceptually important but is also considered to be simplistic in that the number of independent genetic and epigenetic events may be 6 or more in certain types of cancer (46–48). Furthermore, chemical carcinogens may: (a) not be considered mutagens, termed nongenotoxic carcinogens (49); (b) have both genetic and epigenetic effects such as the ability to induce mutations and the property of decreasing DNA methylation, respectively (50) or increase cellular proliferation; and (c) have a linear or non-linear dose-response relationship with respect to tumor formation (51, 52). These and other observations have generated an active debate concerning both the contribution of endogenous mutagenic mechanisms, e.g., oxy-radical DNA damage, DNA depurination, DNA polymerase infidelity, and deamination of 5-methylcytosine, versus exogenous environmental mutagens and the value of animal biosays to identify carcinogens and to aid in human cancer risk assessment (53–63). This debate is not merely an academic one in that societal and regulatory decisions critical to public health are at issue.

Carcinogen Metabolism and DNA Damage

Many chemical carcinogens require metabolic activation generally to high energy electrophiles to exert their carcinogenic
### Table 1 Examples of advances in chemical and physical carcinogenesis

#### A. Cancer etiology

1. Life-style factors (tobacco snuff or smoke) can cause human cancer.  
   - Hill, 1759 (329)  
   - Soemmerring, 1795 (330)  
   - Doll and Hill, 1950 (331)  
   - Wynder and Graham, 1950 (332)

2. Occupational agents and factors can cause human cancer.  
   - a. Nun (childlessness)  
     - Ramazzini, 1713 (333)  
     - Pott, 1775 (334)  
     - Thiersch, 1875 (335)  
     - Rehn, 1895 (337)  
     - DeLore and Borgan, 1928 (340)  
     - Harting and Hesse, 1879 (336)  
     - Frieben, 1902 (338)  
     - Clunet, 1910 (339)  
     - Martland and Humphries, 1929 (341)  
     - Ministry of Labour and National Service, 1949 (342)  
     - Wagner et al., 1960 (343)
   - b. Coal soot  
     - Yamagiwa and Ichikawa, 1918 (344)
   - c. Sunlight  
   - d. Aromatic amines  
   - e. Benzene  
   - f. Ionizing irradiation  
   - g. Asbestos

3. Mixtures of chemicals (coal tar) can cause cancer in experimental animals.  
   - Turner, 1941 (347)

4. Specific chemicals, e.g., dibenzanthracene or 2-naphthylamine, are carcinogens in experimental animals.  
   - Berenblum, 1941 (348)  
   - Fujiki et al., 1979 (349)

5. Naturally occurring compounds isolated from plants, e.g., croton oil, or marine organisms, e.g., teleocidin B and okadaic acid, have cocarcinogenic and/or tumor-promoting activities in experimental animals.

6. Naturally occurring chemicals such as alkaloids from plants, e.g., bracken fern (Pteridium aquilinum), cycad, and Senecio, and fungal toxins, e.g., aflatoxin B₁, can be carcinogens in experimental animals.

7. Naturally occurring chemicals such as N-nitrosamines can be carcinogenic in laboratory animals.  
   - Magee and Barnes, 1956 (362)

8. N-Nitrosamines can be carcinogenic in laboratory animals.

9. Cooking of red meat, fish, or specific amino acids can generate polynuclear aromatic hydrocarbons and, in some cases, heterocyclic amines that are carcinogenic in animal models.

10. Tobacco smoking and alcoholic beverages are human cancer risk factors and have multiplicative effects.  
    - Wynder et al., 1957 (367)  
    - Keller, 1967 (368)  
    - Rothman and Keller, 1972 (369)

11. Exposure of the human fetus to ionizing radiation increases risk of leukemia.

12. Tobacco smoking and occupational exposure to asbestos increases risk of human bronchogenic carcinoma.


14. Animal bioassays can identify chemical carcinogens, e.g., chloromethyl ethers and vinyl chloride, prior to epidemiological evidence.

15. Carcinogen-macromolecular adducts can be used as molecular dosimeters including exposure to life-style and occupational carcinogens.

16. Transplacental exposure to diethylstilbestrol increases risk of human vaginal carcinoma.

17. Fiber dimension and length is correlated with carcinogenicity in animal models

#### B. Multistage carcinogenesis

1. Chemicals can cause cancer at sites distant from the point of application in experimental animals.

2. Metabolites of chemical carcinogens are predicted to be the active carcinogenic agent.

3. Carcinogenesis is a multifactorial process involving tumor initiation and promotion in animal models.
4. Metabolic activation of some chemical carcinogens is essential for their carcinogenic activity.  

5. Tumor promoters can inhibit cell-cell communication of mammalian cells in vitro.  

6. A tumor promoter in experimental animals can specifically bind and activate a specific cellular receptor.  

7. Oncogenes cooperate in neoplastic transformation of primary rodent cells.  

8. Some tumor promoters act via the protein kinase C pathway for signal transduction.  

9. Cooperation between oncogenes is demonstrated in transgenic animal models.  

10. Overexpression of protein kinase C leads to morphological transformation of rodent cells.  

11. Multiple genetic events in protooncogenes and tumor suppressor genes are associated with human carcinogenesis.  

### C. Genetic and epigenetic mechanisms of carcinogenesis  

| 1. Human cancer can be inherited. | Watkins, 1904 (396)  
| 2. Chromosomal abnormalities are predicted to be involved in cancer. | Boveri, 1914 (397)  
| 3. Carcinogenic X-rays are also mutagens. | Muller, 1927 (398)  
| 4. Inbred mouse strains differ in their cancer incidence. | Furth et al., 1933 (399)  
| 5. Cancer may arise by inactivation of both alleles of a tumor suppressor gene. | Strong, 1935 (400)  
| 6. Chemical carcinogens can be mutagens. | Auerbach et al., 1947 (403)  
| 7. Chemical carcinogens may bind to DNA. | Boyland and Horning, 1949 (404)  
| 8. A specific chromosomal abnormality can be associated with human leukemia. | Boyland, 1952 (405)  
| 9. UV light causes a specific type of DNA damage, i.e., thymidine dimers (cyclobutane). | Wheeler and Skipper, 1957 (406)  
| 10. Carcinogenic potency of some chemical carcinogens correlates with their binding level to DNA in experimental animals. | Brooks and Lawley, 1960 (407)  
| 11. Defective DNA repair may predispose to UV-induced human skin cancer. | Farber and Magee, 1960 (408)  
| 12. Interindividual variation is observed in carcinogen metabolism and/or formation of carcinogen-DNA adducts in human cells and tissues. | Nowell and Hungerford, 1960 (409)  
| 13. Normal cells are dominant in normal-tumor murine cell hybrids. | Beukers and Berends, 1960 (410)  
| 14. In vitro bacterial mutagenesis can be used to screen putative carcinogens. | Brooks and Lawley, 1964 (411)  
| 15. RNA viral oncogenes have a normal cellular counterpart (protooncogene). | Cleaver, 1968 (412)  
| 16. Data consistent with the autocrine growth factor theory are presented. | Kunztman et al., 1968 (413)  
| 17. DNA from chemically transformed rodent cells contain an oncogene(s). | Welch et al., 1968 (414)  
| 18. Specific chromosomal loss is associated with tumorigenic revertants of normal-carcinoma human cell hybrids. | Huberman and Sachs, 1973 (71)  
| 19. Chromosomal translocation can activate protooncogenes in human cells. | Harris et al., 1969 (415)  
| 20. Oncogenes are cloned from human cancers. | McCann et al., 1973 (417)  
| 21. ras oncogene contains a base substitution when compared to protooncogene. | deKlein, 1975 (418)  

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22. Chemical carcinogens can activate ras protooncogenes in animal models.

23. Some mutant growth factor receptors are oncogenes in rodent cells.


25. Transferred oncogenes can cause neoplastic transformation of human epithelial cells.

26. The human retinoblastoma susceptibility (Rb) gene is cloned.

27. Nuclear oncogenes can act as transcription factors.


29. p53 functions as a tumor suppressor gene in rodent and human cells.

30. p53 functions as a tumor suppressor gene in rodent and human cells.

31. A candidate tumor suppressor gene on chromosome 18q may be involved in human colon carcinogenesis.

32. NF1, a GTPase-activating protein, may be a negative regulator of ras p21 and a tumor suppressor gene.

33. A candidate Wilms' tumor suppressor gene is cloned.

34. Germ-line p53 mutations are found in certain cancer-prone families.

35. Candidate tumor suppressor genes (MCC and APC) may be involved in familial adenomatous polyposis and colorectal carcinoma.

36. An environmental chemical carcinogen (aflatoxin B1) is linked with a specific mutation in the p53 tumor suppressor gene in human hepatocellular carcinogenesis.

37. A candidate tumor suppressor gene (protein tyrosine phosphatase γ) on chromosome 3p may be involved in human lung and renal carcinogenesis.

* These historical reviews have been useful references and are a source of additional examples of advances in carcinogenesis (350-354).

**Fig. 1. Carcinogenesis is a multistage process involving multiple genetic and epigenetic events in protooncogenes, tumor suppressor genes, and anti-metastasis genes.**

Effects (64). Although interspecies differences have long been known in the metabolism of xenobiotics (65-69), the metabolic pathways of activation and the resultant carcinogen-DNA adducts are generally qualitatively similar among various animal species, including humans (6). These observations support the qualitative extrapolation of carcinogenesis data from laboratory animals to the human situation. Quantitative and qualitative differences in cytochrome P-450 enzymes in various tissues (70) may be partly responsible for the divergent sites of cancer induction among animal species; e.g., 4-aminobiphenyl causes
liver cancer in rodents and bladder cancer in dogs and humans (65) and may confound tissue site-specific extrapolation of carcinogenesis data among animal species. Quantitative differences in carcinogen metabolism and in the formation of carcinogen-DNA adducts are, however, found among different cell types (71, 72), tissue types (6, 70, 73), and outbred individuals of the same species (74, 75). As discussed (see below) in the section “Molecular Epidemiology of Human Cancer,” these quantitative differences may influence tissue site of cancer and an individual’s cancer risk.

The influence of carcinogen metabolism on interspecies extrapolation of carcinogenesis data is becoming more well defined by the cloning of genes encoding enzymes involved in activation and detoxification of chemical carcinogens. Investigators have recently cloned more than 150 CYP4 genes (76) and other genes involved in carcinogen metabolism, such as human N-acetyltransferase (77), human epoxide hydrolase (78, 79), human NAD(P)H:menadione oxidoreductase (80), human glutathione S-transferases (81), and pig flavin-containing monooxygenase (82). As discussed by Gonzalez et al. (70), interspecies differences in carcinogen metabolism may, in part, be due to: (a) qualitative differences in CYP genes due to an absence of a CYP expression in an animal species; (b) quantitative and qualitative variations in regulation of CYP genes among animal species; and (c) differences in CYP substrate or product specificity. Since examples of interspecies differences in each of the 3 types noted above have been described, in vitro model systems including those using human cells and tissues (6) or genetically engineered cells or rodents containing single or combinations of human genes directing the expression of specific enzymes responsible for carcinogen metabolism (70, 83) will serve as a bridge between in vivo studies in animal models and the human situation (Fig. 2).

The molecular and atomic analyses of the physical interactions between chemical carcinogens and DNA represent a major achievement of cancer researchers in the last two decades (84). The information gleaned from these studies of carcinogen-DNA adducts has provided a mechanistic explanation of the mutagenicity of many carcinogens, including their mutational spectra, and a rationale for improved carcinogen exposure assessment in molecular epidemiological studies of human cancer.

Critical DNA Targets: Protooncogenes and TumorSuppressor Genes

Protooncogenes are normal cellular genes that, when inappropriately activated as oncogenes, cause dysregulation of growth and differentiation pathways and enhance the probability of neoplastic transformation. Carcinogens can cause the genetic changes that can lead to activation of protooncogenes, including base substitution mutations (84), chromosomal translocations (85), and gene amplification (45, 86). For example, ras protooncogenes are activated primarily at codons 12, 13, and 61 by base substitution mutations caused by chemical and physical carcinogens in both cultured mammalian cells and animal models (87–90) including transplacental exposure to chemical carcinogens (91). Mutation of the Ha-ras protooncogene is an early event in rodent models of skin and mammary carcinogenesis (87, 92). In addition, the v-Ha-ras transgene can substitute for the initiation step in mouse skin carcinogenesis in transgenic mice (93, 94). This animal model should be useful for investigating the molecular mechanisms of tumor promoters and antipromoters.

In contrast to protooncogenes, tumor suppressor genes are normal cellular genes that, when inappropriately inactivated, cause dysregulation of growth and differentiation pathways and enhance the probability of neoplastic transformation. Based on

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<th>Table 2 Examples of functions of putative tumor suppressor genes</th>
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<td>Induce terminal differentiation</td>
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<td>Trigger senescence</td>
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<tr>
<td>Regulate cell growth</td>
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<tr>
<td>Inhibit proteases</td>
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<tr>
<td>Alter DNA methylase activity</td>
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<td>Facilitate cell-cell communication</td>
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* The abbreviations used are: CYP, cytochrome P-450; CHO, Chinese hamster ovary; BPDE, benzo(a)pyrene diol-epoxide; PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate; AHH, aryl hydrocarbon hydroxylase; GST, glutathione S-transferase; PAH, polycyclic aromatic hydrocarbon; HPLC, high performance liquid chromatography; PTPase γ, protein-tyrosine phosphatase γ.
the multifunctional classes of tumor suppressor genes (Table 2) their number may equal that of the oncogenes. Loss of function of the products of both alleles is the basis of the 2-hit hypothesis and the recessive nature of the genetic process (95). In addition to inactivation of both alleles by mutational mechanisms (base substitution mutations, deletions, chromosomal nondisjunction, and recombination) (96), inactivation of a normal allele by genomic imprinting (97) and its gene product by an increased rate of proteolytic digestion (98) or dominant negative mechanisms (99) have been proposed. Based on target size theory, the inactivation of a single allele of a tumor suppressor gene should have an intrinsically higher probability than activation of a protooncogene, e.g., one of the ras protooncogenes in which mutations in only a few specific codons will lead to 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 or the mechanism of inactivation is via a dominant negative mechanism, as proposed for the p53 tumor suppressor gene (99, 100).

Irradiology, carcinogenic hormones, metals, aldehydes, or fibers such as asbestos are generally inefficient in the production of point mutations at the gene level but are more efficient at causing chromosomal abnormalities and losses (62). Therefore, one would speculate that tumor suppressor genes would be targeted by these carcinogens more readily than protooncogenes. Whereas many electrophilic metabolites of chemical carcinogens form carcinogen-DNA adducts and cause point mutations, chemical carcinogens also cause chromosomal abnormalities and micronuclei. Thus, both cytogenetic and mutagenesis assays have been developed to assess these mutational changes (44, 101–103).

Mutational Spectrum

Since mutations are largely responsible for activating protooncogenes and inactivating tumor suppressor genes, the mutational spectra of chemical and physical carcinogens are of interest to define endogenous and exogenous mutational mechanisms. Studies using prokaryotic and simple eukaryotic assays and site-specific mutagenesis assays (104, 105) have shown that each carcinogenic agent produces a "fingerprint" (106) linking cause other genes essential for cell survival may be adjacent to chromosomal nondisjunction, and frame-shift mutations be underestimated the mutational frequency due to deletions, and endogenous genes (106) such as adenine phosphoribosyltransferase (aprt), dihydrofolate reductase (dhfr), and hypoxanthine-guanine phosphoribosyl transferase (hprt) have been utilized in the eukaryotic studies. These assays will underestimate the mutational frequency due to deletions, chromosomal nondisjunction, and frame-shift mutations because other genes essential for cell survival may be adjacent to the hemizygous nonessential detector gene. In addition, certain mutations may cause either clonal expansion or inhibition of the mutant cell and further complicate the interpretation of the mutational spectrum.

In both exogenous and endogenous types of target genes, the carcinogen-induced mutational spectra can be compared to the spontaneous mutational spectrum. The majority of spontaneous mutations analyzed in these assays are single base substitutions. However, interesting differences have been observed in the spontaneous mutational spectrum in the endogenous aprt of CHO cells compared to bacterial cells and exogenous genes carried by shuttle vectors in mammalian cells (111). Transitions of G:C→A:T are frequent in CHO cells suggesting deamination of 5-methylcytosine and/or DNA replication errors, whereas deletions and insertions are more frequent in the other assay systems. A comparison of the spontaneous mutational spectrum at the aprt locus in CHO cells with the spectra induced by ionizing radiation, UV radiation or benzo(a)pyrene diol epoxide is shown in Table 3. The mutational spectrum of UV light is consistent with the mutagenesis models of the promutagenic cyclobutanone and pyrimidine:pyrimidone (6:4) photoproducts induced by UV light. As noted previously, ionizing radiation is most efficient in causing deletions. BPDE, which binds to the 2-aminogroup of G, produces the predicted G:C→T:A transversions; a similar mutational spectrum for BPDE has been observed in human cell assays (112). Polycyclic aromatic hydrocarbons can also cause mutations at regions of the genes encoding pre-mRNA consensus splice sites, resulting in mRNA splicing defects. For example, mutations affecting splicing in the CHO dhfr gene were preferentially induced by 3a,4ß-dihydroxy-1a,2ß-dihydroxy-1,2,3,4-tetrahydrobenzo(c)phenanthrene (113). The hprt locus is of particular interest because of the potential to compare mutational spectra obtained from studies using cultured cells experimentally exposed to carcinogens with those observed in human lymphocytes obtained from donors exposed in vivo to environmental carcinogens (114).

The molecular analysis of mutationally activated ras protooncogenes in animal models of carcinogenesis generally supports the concept of mutational spectrum reflecting the DNA adduct formation and mutagenic activities elicited by specific chemical carcinogens. For example, the mutations found in activated ras protooncogenes associated with tumors of rodents exposed to the methylating N-nitroso compounds are predominantly G:C→A:T base substitutions (Table 4); these are likely due to methylation of deoxyguanine at the O6 position followed by mispairing with thymine during DNA synthesis (92, 110, 115–117). Although there are several deoxyguanine 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 one of the "possible" mutation sites. However, preferential mutation site are within the admittedly narrow range of possibilities for activating ras genes. Unexplained mutational specificities have been observed in other experimental systems (118–120) and may reflect the differences in the spectrum of promutagenic carcinogen-DNA adducts, specificity of DNA repair enzymes (121), and/or the resultant amino acid changes are either silent or lethal. Although the mutational spectrum of ras protooncogenes in rodent tumors caused by methylating carcinogens is consistent

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<th>Mutagen</th>
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<td>Spontaneous</td>
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<td>G:C→C:G</td>
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<td>A:T→T:A</td>
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<td>Benzo(a)pyrene diol epoxide</td>
<td>G:C→A:T</td>
<td>A:T→G:C</td>
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* Adapted from Ref. 106.
Table 4  Examples of predominant base substitution mutations in ras protooncogenes in rodent tumors

|--------------------------------|--------------|---------------|--------------|----------------|--------------|---------------|--------------|----------------|----------------|----------------|

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of mutational spectrum: (a) *p53* is well conserved in evolution (126) and 5 domains are >90% homologous in DNA sequence among humans and rodents that are frequently used in animal models of carcinogenesis; (b) *p53* is mutated in diverse types of human cancer (127–131) and, where examined, cancers in laboratory animals5–7; (c) a wide spectrum of mutational types and codon sites have been observed that presumably define regions of the *p53* protein likely to be essential for its biological activity in cell cycle control, in tumor suppression, and for its interaction with other cellular and viral proteins. A recent review (132) has concluded that the majority of the mutations in human tumors occur in the evolutionarily highly conserved domains in exons 5–8 of the *p53* gene, and the missense mutations are predominantly transitions at G:C base pairs and are almost all (>95%) at amino acids that are entirely conserved in mouse, rat, monkey, and human. Mutational spectra also vary among cancer types (Table 5). The G:C→A:T transitions are the most frequent in colon tumors and 68% of the mutations occur at CpG dinucleotides. These findings are consistent with the endogenous mutational mechanism due to deamination of 5-methylcytosine residues found at CpG dinucleotides in the mammalian genome. More than one-half of the *p53* mutations in colon tumors are at “hot spots,” codons 175, 248, 273, or 282, each of which is a CpG dinucleotide.

In contrast to colon tumors, CpG dinucleotides are less frequently sites of mutations in most other types of human cancers. Whereas G:C→T:A mutations are rare in colon tumors, these transversions are significantly more common in breast and lung cancers. The G:C→T:A mutations in *Ki-ras* protooncogene are also found in human lung adenocarcinomas (133–135) and in rodent lung cancers caused by benzo(a)pyrene (Table 4). Interestingly, the *p53* mutational spectra differ in small cell lung cancer and non-small cell lung cancer; G:C→T:A transversions are common in the latter group of cancers (136). Whereas CpG→TpG mutations occur with intermediate frequency in esophageal cancers, 36% of the mutations are at A:T pairs which may be due in part to DNA depurination and/or exposure to chemical carcinogens such as urethan (Table 4), a contaminant of certain alcoholic beverages.

The most striking *p53* mutational spectrum is found in hepatocellular carcinomas from either Qidong, People’s Republic of China (137) or southern Africa (138). Eleven of 12 base substitution mutations in 26 tumors were at a single site, the 3rd base position of codon 249, and all but 1 were G:C→T:A transversions. One additional mutation at codon 157 was also a G:C→T:A transversion. These tumors were from patients who live in geographic areas where aflatoxin B1 and hepatitis B virus are major risk factors. Aflatoxin B1 is a naturally occurring hepatocarcinogen that forms N7-deoxyguanosine pro-mutagenic adducts and induces primarily G:C→T:A transversions and G:C→A:T transitions in experimental systems (139) (Table 4). Analysis of liver tumors from geographical areas where aflatoxin B1 is considered not to be a significant risk factor will be instructive in determining whether exposure to this fungal carcinogen may be responsible for these *p53* mutations.

An analysis of mutational spectra in human tumors from populations with differing risk factors may provide insight in a manner similar to those of the studies in experimental animals described above (Table 4). In addition to further studies of liver cancer, the mutational spectra of *Ki-ras* and/or the *p53* gene could be compared to bronchogenic cancers, including the use of paraffin-embedded, fixed archival tissue, from cigarette smokers versus nonsmokers, and from workers in high risk occupations, e.g., uranium mining, mustard gas production, asbestos mining, and chloromethyl ether production.

Selective Clonal Expansion

The majority of human cancers and those induced by chemical and physical carcinogens in animal models are unicellular in origin (43, 140). The clonal nature of neoplasia is the cornerstone in the concept of multistage carcinogenesis and provides the basis for current strategies designated to elucidate the component genetic changes. If all of the clonally derived cells in a tumor, although heterogeneous in other aspects (discussed below), have in common the critical genetic lesion(s) that triggers and/or drives their selective clonal growth then the identification of this lesion(s) is a realistic goal.

Recent data suggest that alterations in the *p53* and *Rb* tumor suppressor genes represent such critical genetic lesions. For example, germline mutations in the *Rb* gene predispose to retinoblastoma (95, 96) and similar mutations in the *p53* gene confer an inherited susceptibility to cancers of the breast, soft tissues, brain, and other tissues (141, 142). *In vitro* transfection of wild type *p53* into neoplastic colon (143), bladder (144), brain (145), and bone (146) cell lines containing an endogenous mutant *p53* gene suppresses cellular growth and/or tumorigenicity. Similar transfection studies using putative wild type *Rb* have also suppressed, to varying degrees, cell growth (147, 148). Although the mechanism(s) of growth retardation of these cell lines by *p53* or *Rb* is uncertain, the inhibition of the cell cycle progression into S phase following induction of wild type *p53* is accompanied by selective down-regulation of mRNA and protein expression of proliferating cell nuclear antigen, a cofactor of DNA polymerase δ, an important component of DNA replication (149).

Studies of animal models suggest that certain activated protooncogenes may also be candidate critical genes. Mutations in the Ha-*ras* protooncogene are early genetic events in mouse skin carcinogenesis (150) and rat mammary carcinogenesis (151, 152). Certain oncogenes impart, as transgenes, an “initiated” state (93, 94), and cooperation among oncogenes has been shown to increase cancer risk in transgenic mouse models (153). Whereas germline mutations occur in tumor suppressor genes that increase cancer risk in the affected individuals (141, 142), germline mutations that activate protooncogenes in humans have yet to be described. The success of transgenic mouse studies argues against the possibility that such mutations would lead to fetal death and thus not be observed in the human population.

Activated protooncogenes can modulate the responsiveness of mammalian cells to their microenvironment and their cellular neighbors. For example, rodent cells transformed by certain transfected oncogenes have defective intercellular communication (154) and the *EIA* oncogene induces resistance to the growth-inhibitory effects of transforming growth factor β1 (155). When Ha-*ras*, Ki-*ras*, or the combination of c-myc and c-raf are transferred into SV40 T “immortalized” human bronchial epithelial cells (BEAS-2B), the cells had a reduced responsiveness to growth inhibition by either transforming growth factor type β or serum (156–158) and thus have potentially
gained a selective clonal expansion advantage. Antisense oligonucleotides or expression vectors producing antisense DNA fragments of the mRNAs of these oncogenes should be useful in assessing the direct role of ras, myc, or raf in the modulation of growth and differentiation in this in vitro model system of human bronchial epithelial carcinogenesis.

The molecular mechanisms responsible for selective growth advantage of initiated cells are likely to be numerous and involve cell-cell interactions (154), cell-matrix interactions (159), cell-growth factor interactions (34, 160) and intrinsic aberrations of initiated cells are likely to be numerous and involve human bronchial epithelial carcinogenesis.

Protein kinase and protein phosphatases are responsible for the reversible phosphorylation of cellular proteins that control growth and differentiation of mammalian cells (173, 456). PKC is a multigene family with at least 8 members that have differential expression in specific tissue types and functions in signal transduction (28, 174). Exposure of certain cells to the tumor promoter TPA causes PKC to translocate to cellular membranes and the resultant activation of PKC isoforms is associated with rapid induction of expression of nuclear protooncogenes c-jun and c-fos (175). Since exposure to TPA produces many phenotypic responses that are cell type dependent (176), and activated PKC modulates cytosolic calcium concentration and phosphorylates multiple protein substrates, the signal transduction pathways involving PKC are difficult to delineate (28). One hypothesis of the mechanistic role of PKC in tumor promotion in mouse skin carcinogenesis is that certain proteins, when phosphorylated at specific sites directly or indirectly by PKC, activate signal transduction pathway(s) positive for cell growth. Assuming that the phosphorylated protein(s) is in the hypothetical active state, dephosphorylation at specific sites would lead to inactivation. Therefore, inhibitors of the responsible protein phosphatase(s) would increase the steady state levels of the critical phosphoprotein(s) in the activated state and would be candidate tumor promoters. Although the phosphoprotein(s) critical in tumor promotion has not as yet been identified, this latter hypothesis is supported by the recent discovery of non-TPA-like tumor promoters (177) such as okadaic acid, a potent inhibitor of protein phosphatases I and IIa (178, 179). The interpretation of the above studies is restricted because of the intrinsic limitations of experimental designs using agonists and/or inhibitors that frequently have multiple nonspecific effects. Since members of the PKC and the protein phosphatase multigene families are being cloned, more powerful genetic strategies are becoming available.

Genomic Instability

Normal human somatic cells maintain a diploid karyotype and have a mutation frequency of about $10^{-8}$ for hemoglobin variants (point mutations), about $10^{-7}$ for hprt variants (measures many classes of mutations, but the assay does not detect mitotic recombination), and about $10^{-5}$ for glyco phosphoryl A or HLA variants (measures most classes of mutations including somatic recombination, gene conversion, or chromosomal non-disjunction) (114, 180, 181).

The potential sources for these observed mutation frequencies include exposure to “background” levels of environmental chemical and physical agents and endogenous mechanisms (55) such as: (a) the chemical instability of DNA, e.g., depurination [one can calculate that each cell undergoes $10^5$ depurinations/day (125)] or deamination of 5-methylcytosine to yield thymidine (182, 183); (b) free radicals of oxygen, e.g., hydroxyl ions [from measurements of 8-hydroxyguanine and thymine glycol in urine, about $10^4$ oxy radical-induced DNA lesions occur in each human cell per day (54)]; and (c) DNA replication errors. Imbalances in deoxynucleoside triphosphate pools (184) and mutations in DNA polymerase $\alpha$ (185) are examples of mechanisms leading to infidelity of DNA synthesis. Inactivation of RAD18, a putative DNA repair gene in Saccharomyces cerevisiae, leads to an increase in the frequency of G:C$\rightarrow$T:A transversions (186). Considering the multiplicity of proteins involved in DNA synthesis, DNA repair, mitosis, and the cell cycle, the number of potential gene targets in somatic cells is large and could result in the mutator phenotype (43, 55, 187) (Table 6) that could increase the probability of both neoplastic transformation and the generation of more malignant subclones during tumor progression (42, 188). If such mutations occur in germ cells and are nonlethal, they could lead to congenital abnormalities and increased cancer risk in an inherited manner. The hypothesis that genomic instability may have an inherited basis and be associated with increased cancer risk is supported by studies of Bloom’s syndrome, where an increase in cytogenetic end points is observed (189), and of colonie carcinomas, where a significant increase in allelic deletions are found in tumors from patients who have first degree relatives with cancer (190).

Cytogenetic studies (191, 192) have provided the bulk of the indirect evidence supporting the hypothesis that genomic instability is a potential mechanism driving tumor progression. The evidence is less compelling (193) when the frequencies of either spontaneous point mutations (194–201) or drug-induced gene amplification (202–206) are compared in normal and neoplastic cells.

Recombinase infidelity (207), telomere reduction (208), and DNA hypomethylation (47, 209) are examples of additional mechanisms of genomic instability. The frequency of somatic recombinational events at certain loci, e.g., immunoglobulin genes, is relatively high, but specific DNA sequences at these loci are recognized by the responsible V(D)(J) recombinases. Although the degree of infidelity of wild type and mutant recombinases is not known, a possible example of “illegitimate”

5031s
The table below lists examples of gene abnormalities associated with genomic instability.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Activation</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD 9</td>
<td>S. cerevisiae</td>
<td>Mutation</td>
<td>Chromosomal loss</td>
</tr>
<tr>
<td>RAD 18</td>
<td>S. cerevisiae</td>
<td>Mutation</td>
<td>Base substitution (G:C—T:A transversion)</td>
</tr>
<tr>
<td>p34&lt;sup&gt;cyc&lt;/sup&gt;</td>
<td>S. pombe</td>
<td>Mutation</td>
<td>Aneuploidy</td>
</tr>
<tr>
<td>Topoisomerase II</td>
<td>S. pombe</td>
<td>Mutation</td>
<td>Abnormal chromosomal segregation</td>
</tr>
<tr>
<td>nod</td>
<td>Drosophila</td>
<td>Mutation</td>
<td>Abnormal chromosomal segregation</td>
</tr>
<tr>
<td>DNA polymerase α</td>
<td>Human</td>
<td>Mutation</td>
<td>Base substitution</td>
</tr>
<tr>
<td>p53</td>
<td>Human (Li-Fraumeni syndrome)</td>
<td>Mutation</td>
<td>Aneuploidy</td>
</tr>
<tr>
<td>RAG-1</td>
<td>Human</td>
<td>Mutation</td>
<td>Chromosomal rearrangements and deletions</td>
</tr>
<tr>
<td>scid</td>
<td>Murine</td>
<td>Mutation</td>
<td>Aberrant V(D)J recombination and double-strand break DNA repair</td>
</tr>
<tr>
<td>Unknown</td>
<td>Human (Bloom’s syndrome)</td>
<td>Unknown</td>
<td>Hyperrecombination</td>
</tr>
</tbody>
</table>

*In vitro experimental studies.

**V-(D)-J recombinase activity leading to interstitial deletion and rearrangements in a growth-regulatory gene has been described** (210). In addition, the scid (severe combined immunodeficiency) mutation in mice is due to a defect in the site-specific V(D)J recombination pathway and has been recently linked to a deficiency in double-strand break DNA repair. This linkage suggests that the scid gene product performs a similar function in both pathways.

Hastie et al. (208) have shown that telomere reduction is common in both colorectal adenomas and carcinomas and have speculated that telomere reduction may be involved as an early event in carcinogenesis. Interestingly, studies of yeast (211) and maize (212) have shown that chromosomes without terminal repeats or telomeres are prone to rearrangements or loss. Studies in yeast have also identified genes involved in cell cycle control and mitosis, e.g., RAD9 and cdc-2, that, when mutated, influence the fidelity of mitosis (213, 214) (Table 6). For example, certain mutations in RAD9 of S. cerevisiae increase the rate of chromosomal loss by 7–21-fold (215) and topoisomerase II mutants in *Saccharomyces pombe* have abnormal segregation of the intertwined daughter chromosomes at the end of DNA replication (216) and abnormal chromosome condensation (217). Since human homologues of such genes are being identified, similar investigations could be conducted using cultured human cells, and human tumors could be examined for mutations in these genes.

Analogous to the yeast paradigm of a mutator phenotype associated with dysregulation of cell cycle control (213), certain activated protooncogenes or inactivated tumor suppressor genes could lead to genomic instability. The rapid appearance of aneuploidy and “spontaneous” immortalization of cultured human fibroblasts from donors with the Li-Fraumeni syndrome, who have an inherited mutant *p53* allele (218), is consistent with this hypothesis. The genomic instability caused by certain DNA viruses such as SV40, adenovirus, and papillomavirus could also be due to the inactivation of *p53* and/or *Rb* by complexing with the viral proteins.

As argued by Loeb (55), the impression of a high degree of genomic stability may be misleading when one considers that the human body contains about 10<sup>14</sup> cells and that, within the average human life span, the cells undergo a total of 10<sup>14</sup> divisions. Therefore, spontaneous mutagenesis may significantly contribute to the prevalence of certain cancer types that have no apparent risk factors and minimal variations in incidence occur among different geographic areas. However, epidemiological studies have: (a) identified significant cancer risk factors, e.g., tobacco smoke and hepatitis B virus; (b) found substantial geographic variation in the incidence of the most common types of human cancer; and (c) found that migrant populations frequently acquire the risk of cancer prevailing in their new geographic location of residence (1, 2, 219). Based on the above and other considerations (1), it has been estimated that about 80% of all cancer in the United States is due to environmental factors. Finally, one can speculate that the rates of spontaneous mutations, e.g., deamination of 5-methylcytosine, may be altered by environmental chemicals directly and/or indirectly via generation of endogenous agents such as oxy radicals and the intracellular signal transducer, nitric oxide.

**Molecular Epidemiology of Human Cancer**

Combined laboratory-epidemiological studies of human cancer have a long history and have been named metabolic (220), biochemical (221), and molecular epidemiology (222). Whereas classical epidemiology has been successful in identifying high cancer risk populations, e.g., cigarette smokers, the primary goal of molecular epidemiology is to identify individuals in these populations who are at the highest cancer risk. The current strategy involves improved exposure assessment by measuring biologically effective doses of carcinogens and secondly, analysis of host susceptibility factors within the framework of well-designed epidemiological studies (223).

**Exposure Assessment**

Carcinogen-macromolecular adducts, mutations, and cytogenetic changes can be measured in the target epithelial cell population and in surrogate blood cells as shown in Fig. 3. This paradigm is now feasible to explore experimentally because of recent advances in methods to measure carcinogen-macromolecular adducts (224), somatic gene mutations (181, 225), and cytogenetic endpoints (103). In addition, polymerase chain reaction technology and DNA sequencing allows a rapid assessment of mutations in protooncogenes, tumor suppressor genes, and antimetastasis genes in the epithelial cells. Since the ras protooncogene family is occasionally and the *p53* tumor suppressor gene is frequently mutated in human cancers, as discussed above, these genes are suitable candidates for assessing the genetic consequences of carcinogen-DNA adducts and endogenous mutagens. The mutational spectra in ras and *p53* genes can be measured by current technology due to the clonal expansion of the cells driven by these mutant genes. Although small clonal populations of a few hundred cells are sufficient for analysis of mutational spectra by polymerase chain reaction and direct DNA sequencing, additional advances in methodology will be required to determine the mutational spectra in single cells that have not clonally expanded and are diluted in a much larger population of normal cells.
Host Susceptibility Factors

Both inherited and acquired host factors that predispose an individual to cancer caused by environmental agents have been identified (37, 226). Inheritance of a germline mutation in the tumor suppressor genes, p53 (141, 142) or, perhaps, Rb (227), predisposes to the development of adult types of cancer (Table 7). Other inherited host susceptibility factors include certain DNA repair deficiencies (228), genetic polymorphism in certain xenobiotic metabolizing enzymes (70), and, perhaps, the prooxidant state in Bloom’s syndrome (229).

Interindividual Variation in Human Carcinogenesis

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... The Origin of Species
Charles Darwin, 1859

Scientists have repeatedly recognized person-to-person differences in behavior, morphology, and risk of disease. Such interindividual differences may be either inherited or acquired. For example, inherited differences in susceptibility to physical or chemical carcinogens have been observed among individuals, including an increased risk of sunlight-induced skin cancer in people with xeroderma pigmentosum (228), bladder cancer in dye stuff workers with a poor acetylator phenotype (230), and bronchogenic carcinoma in tobacco smokers who have an extensive debrisoquine hydroxylator phenotype (231, 232).

Because most chemical carcinogens require metabolic activation to exert their oncogenic effects and the amount of ultimate carcinogen produced results from the action of competing activation and detoxication pathways, interindividual variation in carcinogen metabolism is considered to be an important determinant of cancer susceptibility (75). DNA adducts are one form of genetic damage caused by chemical carcinogens and may lead to mutations that activate protooncogenes and inactivate tumor suppressor genes in replicating cells. The steady state levels of these adducts depend on both the amount of ultimate carcinogen available to bind and the rate of removal from DNA by enzymatic repair processes. The genomic distribution of adduct formation and repair is nonrandom and is influenced by both DNA sequence and chromatin structure (121, 233–235).

Carcinogen Metabolism and DNA Adduct Formation. Chemical carcinogens are metabolized by a wide variety of soluble and membrane-bound enzymes. Multiple forms of human cytochrome P-450 are involved in the oxidative metabolism of chemical carcinogens, e.g., polycyclic aromatic hydrocarbons (70). Several thousand-fold interindividual variation has been observed in placental AHH activity which is catalyzed by the CYP1A1; some of this variability is under direct genetic control, but variations are also the result of an enzyme induction process due to maternal exposure to environmental carcinogens, e.g., tobacco smoke, or dietary factors (236–238). However, the induction process itself may have a genetic component (239) and inducible AHH activity is higher in cultured lymphocytes from lung cancer cases when compared to controls (240). Trell and coworkers (241) are currently conducting a prospective study to determine if high inducibility of AHH is a risk factor for the development of cancer. Increased binding of benzo(a)pyrene metabolites to DNA in lymphocytes of patients with lung cancer versus noncancerous controls has also been observed (242). A highly inducible allelic variant CYP1A1 has been identified in humans (243). This variant is associated with increased risk of smoking-associated lung cancer in a Japanese population (244). This association has not been found in a U.S. population (245).

Cytochrome P-450 (CYP2D6) activity is polymorphic and has also been linked to lung cancer risk (231, 232). CYP2D6 hydroxylates xenobiotics such as debrisoquine, an antihypertensive drug, and a tobacco-specific N-nitrosamine (70), and 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, and lung, liver, or advanced bladder cancer patients are more likely to have the extensive hydroxylator phenotype when compared to noncancer controls (231, 245, 246). In a case-control study of lung cancer in the United States, the extensive hydroxylator phenotype had an 8-fold increased cancer risk when compared to the poor hydroxylator phenotype and the increased risk was primarily for histological types other than adenocarcinoma of the lung (232). In addition, analysis of data derived from a British population (231) has revealed that individuals with the extensive hydroxylator phenotype who are occupationally exposed...
posed to high amounts of either asbestos or polycyclic aromatic hydrocarbons have relative lung cancer risks of 18- and 35-fold, respectively (247). CYP2D6 may activate a chemical carcinogen(s) found in tobacco smoke, e.g., certain N-nitrosamines, and/or, a more speculative possibility, inactivate nicotine, the addictive pleasure component of tobacco smoke that would decrease its steadystate level and increase the drive to smoke more cigarettes. Thereby, the individual with the extensive metabolizer phenotype would be at greater cancer risk. Another hypothesis is that an allele of the CYP2D6 gene is in linkage disequilibrium with another gene that influences cancer susceptibility.

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. Acetylation of carcinogenic aromatic amines has been proposed as a cancer risk factor (248). The slow acetylator phenotype has been linked to occupationally induced bladder cancer in dye workers exposed to large amounts of N-substituted aryl compounds (230). Interestingly, the rapid acetylator phenotype is commonly found in colon cancer cases (249). Whether or not this association is due to metabolism of a carcinogenic aromatic amine in the colonic epithelium is not known.

Wide interindividual differences in detoxifying enzymes of carcinogens are also found. For example, at each step in the metabolic pathway of benzo(a)pyrene activation to electrophilic diol epoxides, competing detoxifying enzymes are found (250). Confirming previous observations (251), a recent study of several of the enzymes involved in benzo(a)pyrene metabolism showed a more than 10-fold person-to-person variation in their activities (252) and presented indirect evidence that tobacco smoke induced many of these enzymes. Genetic control of the presumed detoxification of benzo(a)pyrene by conversion to water-soluble metabolites has been reported (239).

GSTs are multifunctional proteins (253) that catalyze the conjugation of glutathione to electrophiles, including the ultimate carcinogenic metabolite of benzo(a)pyrene (254), and are considered to be a detoxification pathway of carcinogenic polycyclic aromatic hydrocarbons (255). The three isoenzymes of GST (α, γ, and π) vary in their substrate specificity, tissue distribution, and activities among individuals (256). Expression of GST-γ is inherited as an autosomal dominant trait (257) and individuals with low GST-γ activity may be at greater risk of lung cancer caused by cigarette smoking (258, 259). Further functional and genetic studies of acquired and inherited deficiencies in the activities of enzymes involved in the detoxification of chemical carcinogens are warranted.

Metabolism of carcinogens from several chemical classes, including N-nitrosamines, polycyclic aromatic hydrocarbons, hydrazines, mycotoxins, and aromatic amines, has been studied using human tissues, cells, and microsomes (73, 260–267). The enzymes responsible for the activation and deactivation of procarcinogens, the metabolites produced, and the carcinogen-DNA adducts formed by cultured human tissues and cells, in general, are qualitatively similar among donors and tissue types. The DNA adducts and carcinogen metabolites are also similar to those found in most laboratory animals, an observation that generally supports the qualitative extrapolation of carcinogenesis data from the laboratory animal to the human (73). However, some notable differences among animal species have been reported, including metabolism of aromatic amines in the guinea pig (65), benzo(a)pyrene in the rat and rabbit (66, 268), and aflatoxin B1 in the Syrian golden hamster (67).

Although the major DNA adducts are qualitatively similar for the chemical carcinogens thus far studied in these in vitro models, quantitative differences have been found among individuals and their various tissue types (73, 260, 269–271). The differences in formation of DNA adducts range from approximately 10-150-fold among humans, the interindividual distribution is generally unimodal, and the variation is similar in magnitude to that found in pharmacogenetic studies of drug metabolism (75).

DNA Repair Rates. DNA repair enzymes modify DNA damage caused by carcinogens in reactions that generally result in the removal of DNA adducts. Studies of cells from donors with xeroderma pigmentosum have been particularly important in expanding our understanding of DNA excision repair and its possible relationship to risk of cancer (228). The rate but not the fidelity of DNA repair can be determined by measuring unscheduled DNA synthesis and removal of DNA adducts, and substantial interindividual variations in DNA repair rates have been observed (272). As discussed above, the fidelity of DNA repair could also vary among individuals, and recent advances in the identification of mammalian DNA repair genes and their molecular mechanisms (273, 274) should provide an opportunity to investigate the fidelity of excision DNA repair in the near future. In addition to finding excision repair rates severely depressed in xeroderma pigmentosum cells (e.g., complementation group A), an approximately 5-fold variation among individuals in unscheduled DNA synthesis induced by UV exposure of lymphocytes in vitro has been found in the general population (272). 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 when compared to those without a family history (275, 276).

Interindividual variation has been noted in the activity of O6-alkyldeoxyguanine-DNA alkyltransferase; this enzyme repairs alkylation damage to O6-deoxyguanine (277–280). In addition to these person-to-person differences of about 40-fold, wide variations in this DNA repair activity have been observed in different types of tissues (277, 278, 281, 282), fetal tissues exhibit 2- to 5-fold less activity than the corresponding adult tissues (283), and cells may have lower repair rates after terminal differentiation (284). The activity of this DNA repair enzyme is inhibited by certain aldehydes (281) and alkylating cancer chemotherapeutic agents (285). A decrease in this DNA repair activity has been observed in fibroblasts from patients with lung cancer when compared to donors with either melanoma or noncancer controls (286). Therefore, acquired and/or inherited deficiency in O6-alkyldeoxyguanine-DNA alkyltransferase may be a cancer risk factor in tobacco smokers.

A unimodal distribution of repair rates of benzo(a)pyrene diol-epoxide-DNA adducts has been observed using human lymphocytes in vitro (287). The interindividual variation was substantially greater than the intraindividual variation which suggests a role of inherited factors. The influence of these variations in DNA repair rates in determining tissue site and risk of cancer in the general population remains to be determined.

Tumor Promotion. Because cancer is the result of complex interaction between multiple environmental factors and both acquired and inherited host factors, one should consider carcinogen-DNA adducts as only one piece in the puzzle. Other pieces include determinants of tumor promotion. In the skin carcinogenesis studies, wide variations in susceptibility to tumor-promo-
motting agents have been observed among animal species and among different inbred strains of a single species (288–291). Epidemiological studies suggest that tumor promotion may influence both tumor probability and latency period in humans (1), and mathematical models of carcinogenesis indicate that cell proliferation is an important determinant of cancer risk (292). Methods to identify human tumor promoters and to predict responses to tumor promoters among different humans need to be developed.

**Interindividual Variation in Carcinogen-Macromolecular Adducts in People Exposed to Chemical Carcinogens.** Results from in vivo and in vitro studies discussed above serve as a basis for investigations in biochemical and molecular epidemiology. For example, the observation that the carcinogen-DNA adducts formed in cultured human tissues are generally the same as those found in experimental animals in which these chemicals induce cancer has encouraged investigators to search for DNA adducts in biological specimens obtained from people exposed to either specific carcinogens such as benzo(a)pyrene or chemical-therapeutic agents.

The development of highly sensitive methods to detect carcinogen-macromolecular adducts has made it possible to measure adducts in blood and tissue samples (293–295). Although hemoglobin and albumin are not considered targets for the pathobiological effects of carcinogens, these macromolecules have certain advantages for the molecular dosimetry of exposure to carcinogens. For example, RBC have a lifetime of about 120 days in humans, so the levels of carcinogen-hemoglobin adducts may be an integrative measure of exposure during a period of nearly 4 months. In addition, these protein adducts, unlike DNA adducts, are not considered to be repaired in the circulating RBC, and mg quantities of hemoglobin can be obtained from a few ml of blood. Ehrenberg et al. (296) pioneered this area of research by measuring alkylating agents such as ethylene oxide bound to hemoglobin. The amounts of ethylene oxide-hemoglobin and 4-aminobiphenyl-hemoglobin adducts are substantially increased in tobacco smokers compared to nonsmokers (297, 298), and the 4-aminobiphenyl-hemoglobin adducts levels decrease upon smoking cessation (299). The kinetics of adduct loss mirrors the 120-day half-life of the erythrocytes. Occupational exposure to ethylene oxide has also been monitored (300). Dietary exposure to chemical carcinogens can be monitored by measuring carcinogen-albumin adducts. Wild et al. (301) have found that exposure to aflatoxin B1, in contaminated grain products is widespread in The Gambia and Senegal, >95% of the subjects had detectable aflatoxin-albumin adducts, and the interindividual variation even within a single village can be 100-fold.

Carcinogen-DNA adducts have also been measured in workers exposed to chemical carcinogens usually in complex mixtures such as coke oven exhaust. For example, PAH-DNA adducts in peripheral blood lymphocytes have been measured by enzyme immunoassays in coke oven workers, foundry workers, roofer, and fire fighters (302–307). In the occupationally exposed individuals, enzyme immunoassays detected 1 to 1200 adducts (BPDE-DNA antigenic equivalents) per 10^8 unmodified nucleotides in 25 to 100% of the subjects (303–305, 307–309). These adducts have also been detected by complementary methods in placental DNA samples in levels that ranged between 10 and 500 in 10^8 nucleotides. In this case, synchronous scanning fluorimetry and gas chromatography-mass spectrometry have given consistent results (310–315).

A comparison between detection methods and end points has also been performed for coke oven workers in the United States and Scandinavian countries (308, 316). However, levels of PAH-DNA adducts reported using the 32P-postlabeling assay for detection are lower than those reported using enzyme immunoassay for detection. These data can in part be explained by the relative substrate specificities of each assay which have not yet been fully standardized or rigorously compared. Anti-PAH-DNA antibodies that presumably indicate genotoxic exposure to metabolically activated PAHs have also been detected in human serum of both occupationally exposed and nonexposed populations (coke oven workers; smokers and nonsmokers) (303, 304, 309).

Alkyl-DNA adducts have also been detected in human samples. The recent use of HPLC or immunoaffinity chromatography in combination with the 32P-postlabeling assay has revealed the presence of O-containing-deoxyguanosine, O-ethyldeoxyguanosine, O-ethylthymidine, and N^7-methyldeoxyguanosine as well as other types of alkyl adducts in the human population, including cancer patients (317–322). The combination of HPLC and the 32P-postlabeling assay has been used to detect O-methylG in lung cancer cases (320, 323) and immunoaffinity chromatography and HPLC have been used to detect these alkyl adducts in placental DNA (321, 324). The power of HPLC combined with the 32P-postlabeling assay is evident because O-ethyldeoxyguanosine and N^7-methyldeoxyguanosine adducts can be detected with a high degree of sensitivity and specificity in lung tissues at the level of 1 adduct in 10^7 nucleotides (325, 326).

The wide range in the amounts of carcinogen-macromolecular adducts measured in the studies discussed above is in part a reflection of occupational, environmental, or lifestyle exposure to chemical carcinogens. Interindividual differences in the balance between metabolic activation and deactivation of chemical carcinogens and in DNA repair rates are also likely to contribute to the variation.

Although methods to measure carcinogen-macromolecular adducts are an important contribution to improved carcinogen exposure assessment, carcinogen-macromolecular adducts will not be a precise quantitative predictor of cancer risk because of the multistage and complex nature of human carcinogenesis.

In biology, one rarely deals with classes of identical entities, but nearly always studies populations consisting of unique individuals. This is true for every level of hierarchy, from cells to ecosystems.

*Cancer Risk Assessment Perspective*  
Quantitative risk assessment is currently more mathematical extrapolation and scientific intuition than a rigorous science. However, the status of cancer risk assessment should improve during the 1990s. This optimism is based largely on the recent advances that have increased our understanding of the molecular mechanisms of human disease pathogenesis. The discovery of multiple genetic and epigenetic changes during carcinogenesis is an example that provides opportunities for both early diagnosis of preneoplastic lesions and novel interventive methods to inhibit or reverse carcinogenesis. These advances are driven in part by improved biotechnology. For example, polymerase chain reaction coupled with direct DNA sequencing allows the detection of mutations in formalin-fixed, paraffin-embedded tissue sections and the "retrospective epidemiologi-
cal” study of archival material (327).

Quantitative methods to measure human exposure to biologically effective doses of chemical and physical carcinogens continue to improve and their current limitations and advantages have been extensively discussed (224, 293). If development and validation continue at the present pace, methodology for carcinogen exposure assessment should be satisfactory by the end of this decade.

The identification of inherited or acquired host susceptibility factors is also becoming an important factor in the risk assessment equation (223) (Table 7). Analogous to the confounding of exposure assessment by complex mixtures, time course variables, etc., generic problems concerning the use of data on host susceptibility factors in risk assessment are evident: (a) rank order of “potency or weight” of various host susceptibility factors influencing the cancer risk in individuals exposed to carcinogens; (b) “potency” impact of the degree of the interindividual variation in a specific host susceptibility factor; and (c) interactive effects among various host susceptibility factors. In addition, societal and ethical issues come to the forefront whenever individuals at high disease risk are identified. These issues are already being actively discussed (328). Finally, the conduct of well-designed and multifaceted molecular epidemiological studies will require the development of a new cadre of scientists who have knowledge and skills in both laboratory science and analytical epidemiology.

By the end of the 1990s, molecular epidemiology of human cancer risk is predicted to have a substantial impact on the quality of quantitative risk assessment and should contribute to the identification of individuals who will most benefit by cancer prevention strategies including intervention by chemopreventive therapy during the multistage process of carcinogenesis.

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Carcinogenesis


Carcinogenesis


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