Environmental Health Perspectives
Vol. 62, pp. 185-191, 1985

Future Directions in the Use of DNA Adducts As Internal Dosimeters for Monitoring Human Exposure to Environmental Mutagens and Carcinogens

by Curtis C. Harris*

Scientific opportunities generally arise when two or more research areas converge and/or advances in methodology occur. This occurred at the turn of the 19th century in the field of infectious bacterial and fungal diseases. As we draw near to the 21st century, research in the laboratory is providing us with both critical information on mechanisms of carcinogenesis and new technological advancements, including those in immunology, biochemistry, and molecular biology. Investigations in the field of epidemiology have clearly demonstrated the importance of environmental exposure to carcinogens and have identified populations at high cancer risk. It is now practical to integrate laboratory determinations into classic epidemiological approaches. Several markers, e.g., carcinogen–DNA adducts, related to tumor initiation and perhaps to tumor conversion, are currently being evaluated. We also need to develop indicators of tumor promotion and progression. The potential of biochemical and molecular epidemiology to predict cancer risk in an individual prior to the onset of clinically evident cancer provides an exciting new opportunity in cancer research and prevention.

Introduction

The topic of this conference is a facet of an expanding area of cancer research—the biochemical and molecular epidemiology of cancer (*1,2*). This multidisciplinary area combines epidemiological and laboratory approaches. Its primary goal is to identify individuals at high cancer risk by obtaining evidence of high exposure to carcinogens leading to pathobiological lesions in target cells and/or increased oncogenic susceptibility due to either inherited or acquired host factors. Clinical and epidemiological studies have identified populations at high cancer risk, and in many cases also the etiological agents, e.g., tobacco smoke as the major cause of lung cancer and asbestos as the primary etiological agent for mesothelioma. Laboratory studies have extended these epidemiological findings by identifying specific carcinogens found in complex mixtures and have provided us with a better understanding of the pathogenesis of the multistage carcinogenic process.

The concepts of tumor initiation, promotion, conversion, and progression have developed from studies in experimental carcinogenesis and are schematically represented in Figure 1. This simplified diagram can be used as intellectual framework to consider the stages at which carcinogen–DNA adducts may play a role in the multistage process of carcinogenesis. Assuming that these adducts lead to genetic lesions, including mutations, tumor initiation and conversion are the stages where our attention should be focused. The earliest events in chemical carcinogenesis, i.e., tumor initiation, are considered to include exposure to the carcinogen, transport of the carcinogen to the target cell, activation to its ultimate carcinogenic metabolite if the agent is a procarcinogen, and DNA damage leading to an inherited change and the preneoplastic "initiated" cell. Tumor conversion is an updated version of an old concept, i.e., benign tumors can convert to malignant tumors. Indirect evidence for this view is based on the finding of microinvasive carcinoma in putative preneoplastic lesions such as squamous metaplasia in the respiratory tract and adenomas in the large intestine. Recent studies using the mouse skin carcinogenesis model suggest that conversion of a benign tumor to a malignant one requires another genetic event in that DNA-damaging and mutagenic agents enhance the frequency and hasten the conversion of benign papillomas to squamous cell carcinomas (*3*). Therefore, carcinogen–DNA adducts may be important in both the early (tumor initiation)

*Laboratory of Human Carcinogenesis, Division of Cancer Etiology, National Cancer Institute, Bethesda, MD 20205.

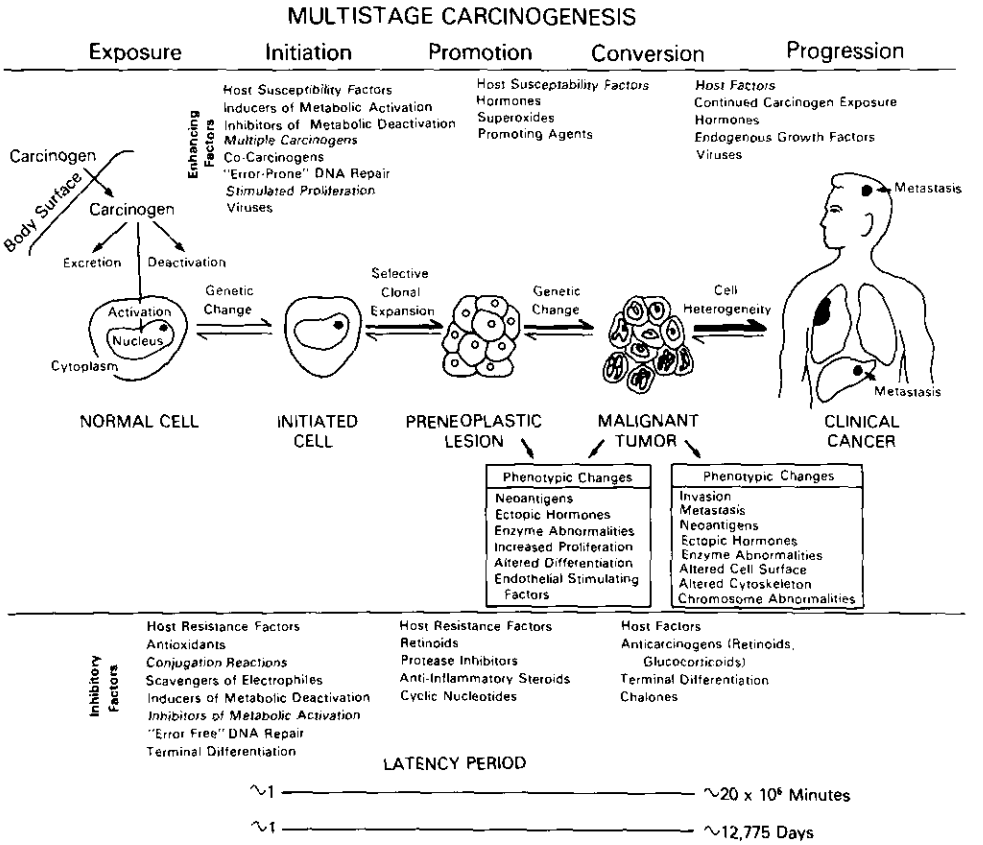

FIGURE 1. Schematic representation of the multistage process of carcinogenesis. Examples of factors that may either enhance or inhibit carcinogenesis are taken from studies of experimental carcinogenesis.

and later (tumor conversion) stages of carcinogenesis.

Because cancer is the result of complex interaction between multiple environmental factors and both acquired and inherited host factors (*4*), one should consider carcinogen–DNA adducts as only one piece in the puzzle. Examples of other portions of the puzzle include determinants of tumor promotion and progression. In the skin carcinogenesis studies, wide variations in susceptibility to tumor-promoting agents have been observed among animal species and among different inbred strains of a single species (Table 1) (*5*). Epidemiological studies suggest that tumor promotion may influence both tumor incidence and latency period in humans (*6*). There is also increasing amount of data which suggests that chemical carcinogens may cause both direct DNA damage, i.e., carcinogen–DNA adducts, and indirect DNA damage by causing formation of free radicals and superoxides that react with DNA and cause molecular lesions, e.g., thymine glycol (*7*). Carcinogens can damage membranes and initiate the arachidonic acid cascade and the release of lipid peroxidation aldehydes, such as 4-hydroxyalkenals (*8*) that bind to DNA. Phthalates and hypolipidemic drugs, including clofibrate, apparently act through an indirect mechanism by causing proliferation of peroxisomes and a subsequent increase in superoxides (*9*). Measures of indirect DNA damage are needed, e.g., the development of monoclonal antibodies to thymine glycol in DNA (*10*).

The carcinogenicity and mutagenicity of chemicals may be dependent on more than one metabolite. For ex-

Table 1. Sensitivity to skin carcinogenesis in different stocks and strains of mice.

	Sensitivity[a]
Complete carcinogenesis	Sencar > CD-1 > C57BL/6 ≥ BALB/c ≥ ICR/Ha Swiss > C3H
Two-stage carcinogenesis (initiation-promotion)	Sencar > > CD-1 > ICR/Ha Swiss ≥ BALB/c > C57BL/6 ≥ C3H ≥ DBA/2

[a] Data represent sensitivities of various mouse strains to benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene. Ranking represents a subjective view because dose-response data were not available for all strains (*5*).

ample, aldehydes are also produced in equimolar concentrations with alkyldiazonium ions during the metabolism of *N*-nitrosamines. The effects of such aldehydes on normal human cells are being studied (*11,12*). For example, formaldehyde inhibits DNA repair of O^6-methylguanine and potentiates the mutagenicity of an alkylating agent, *N*-methyl-*N*-nitrosourea, in normal human fibroblasts. Because formaldehyde alone also causes mutations in human cells, we propose that formaldehyde may cause genotoxicity by a dual mechanism of directly damaging DNA and also inhibiting repair of mutagenic and carcinogenic DNA lesions caused by other chemical and physical carcinogens.

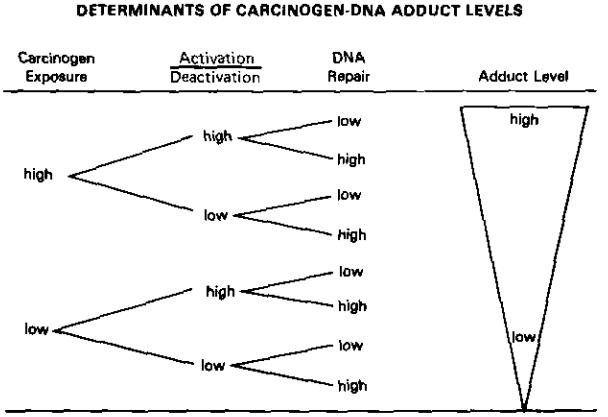

FIGURE 2. Major determinants of amounts of carcinogen–DNA adducts.

Determinants of Carcinogen–DNA Levels

The amount of carcinogen–DNA adducts detected at any time point is dependent on several dynamic factors, including carcinogen exposure, the metabolic balance between carcinogen activation and deactivation, and DNA repair rates (Fig. 2). Procarcinogens from several chemical classes are enzymatically activated to metabolites that bind to DNA in cultured human tissues (Table 2), and the predominant adducts are similar to those found in experimental animals in which the chemical is known to be carcinogenic. In humans, both wide (50- to 150-fold) interindividual variations in the amounts of adducts formed from several chemical classes of procarcinogens metabolized in cultured human tissues (Table 3) (*13*) and also severalfold variation in rates of excision DNA repair (*14*) have been observed. The activities of DNA repair enzymes may vary, too. For example, the activity of O^6-alkylguanine–DNA alkyltransferase is generally 10-fold higher in human tissues when compared to the corresponding rat tissue, and a wider interindividual variation is found in the outbred human species than among individual inbred rats (Table 4) (*15,16*). We must also be aware of the complexities inherent in measuring carcinogen–DNA adducts in the intact animal. When considering the dynamics of cell renewal and loss in the tissues, the problem of quantitative extrapolation between an individual's carcinogen exposure and the detected amount of carcinogen–DNA adducts becomes even more obvious.

Measures of putative DNA repair products are being developed. Following the lead of Wogan and co-workers (*17*), who assayed aflatoxin B_1-modified guanine in the urine of rats exposed to aflatoxin B_1, Autrup et al. (*18*) detected these adducts in urine of Africans who were ingesting mycotoxin-contaminated food. Indirect DNA

Table 2. Chemical carcinogens activated to form DNA adducts by cultured human bronchus, colon, esophagus, pancreatic duct, and bladder.[a]

Carcinogen	Bronchus	Colon	Esophagus	Pancreatic duct	Bladder
Polynuclear aromatic hydrocarbons					
Benzo(a)pyrene	+[a]	+	+	+	+
7,12-Dimethylbenz(a)anthracene	+	+	+	+	0
3-Methylcholanthrene	+	+	+	0	0
Dibenz(a,h)anthracene	+	+	+	0	0
N-Nitrosamines					
Nitrosodimethylamine	+	+	+	+	0
Nitrosodiethylamine	+	+	+	0	0
Nitrosopyrrolidine	+	+	−	0	0
Nitrosopiperidine	+	−	−	0	0
Dinitrosopiperazine	+	+	0	0	0
Mycotoxins					
Aflatoxin B_1	+	+	+	0	+
T-2 Toxin	0	0	+	0	0
Hydrazines					
1,2-Dimethylhydrazine	+	+	+	0	0
Aromatic amines					
2-Acetylaminofluorene	+	0	+	0	+
Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole)	0	+	0	0	0

[a] Key: (+) detection of carcinogen binding to DNA; (−) binding not detected; (0) not tested.

Table 3. Interindividual variation in carcinogen binding to DNA in cultured human tissues.[a]

Tissue	Fold variation in carcinogen binding[b]					
	BP	AFB_1	DMNA	1,2-DMH	AAF	DMBA
Esophagus	99	70	90			
Trachea	6					
Bronchus	75	120	60	10	18	50
Peripheral lung	3					
Liver		12				
Duodenum	31					
Colon	130	150	145	80		
Bladder	68	127			114	
Endometrium	70					

[a] The highest variation among people reported for carcinogen–DNA binding in cultured human tissues (*38*).
[b] Key: BP, benzo(a)pyrene; AFB_1, aflatoxin B_1; DMNA, *N*-nitrosodimethylamine; 1,2-DMN, 1,2-dimethylhydrazine; AAF, 2-acetylaminofluorene; DMBA, 7,12-dimethylbenz(a)anthracene.

Table 4. Activities of O^6-alkylguanine–DNA alkyltransferase in extracts from human and rat tissues.

	O^6-Alkylguanine–DNA alkyltransferase activity, fmole/mg[a]				
	Human			Rat	
	Mean	Range	No. of samples	Mean[b]	No. of samples
Liver	873	411-1795	5	115	4
Colon	261	135-413	10	21	4
Esophagus	217	184-283	3	29	4
Lung	122	41-194	13	54	4
Brain	76	37-122	5	< 15	4

[a] Alkylguanine transalkylase activity was assayed by following the loss of O^6-methylguanine from ^{3}H-methylated DNA using specific antibodies for O^6-methylguanine or by quantitation of the methylated purine content by high performance liquid chromatography.
[b] The range of activities in extracts from rat tissues varied less than 20%.

damage caused by superoxides can also be assessed in urine by measuring thymine glycol formed in experimental animals, e.g., after ionizing radiation (*19*). This noninvasive technique may be useful in monitoring individual animals and eventually humans to determine if this and other assays will predict exposure and/or susceptibility.

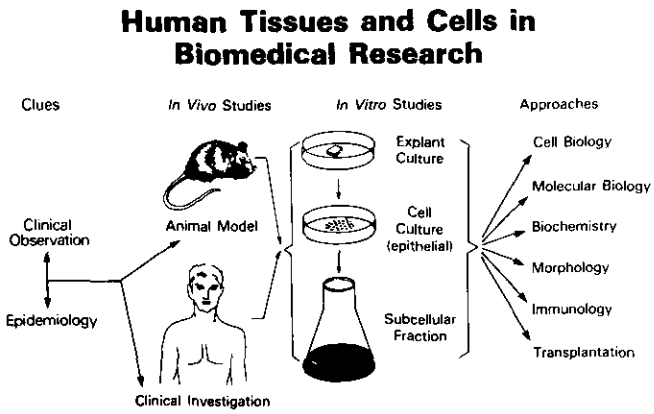

FIGURE 3. Human tissues in biomedical research. *In vitro* models provide a link between studies using animal models and clinical investigations.

Animal models provide invaluable information in studies of carcinogenesis. Extrapolation of this information from experimental animals to humans remains, however, an problematic endeavor. Most scientists consider the qualitative extrapolation to be accurate, i.e., a chemical that is carcinogenic in experimental animals is likely to be carcinogenic in humans. The current debate centers on the question of quantitative extrapolation, i.e., the carcinogenic potency of a chemical. In my opinion, this question will not be resolved by mathematical modeling but will require both a better understanding of the mechanisms of carcinogenesis obtained from comparative studies by using the strategy schematically illustrated in Figure 3. For example, responses to carcinogens, tumor promoters, anticarcinogens, etc., can be compared in tissues and cells maintained in the same controlled *in vitro* setting from humans and experimental animals. Over the last decade,

Table 5. Physical and immunological methods to identify carcinogen–DNA adducts in human biological specimens.

Assays	Estimate of sensitivity
Enzyme radioimmunoassay	1 adduct per 10^{7-8} bases
^{32}P postlabeling and nucleotide chromatography	1 adduct per 10^{7-10} bases
Synchronous fluorescence spectrophotometry	1 adduct per 10^7 bases
Radioimmunoassay	1 adduct per 10^5 bases

a large volume of experimental data has accumulated using this approach (*2*).

Laboratory observations indicating that the metabolic pathways of carcinogen activation and the predominant carcinogen-DNA adducts are similar among most animal species both strengthen our confidence in the qualitative extrapolation and suggest approaches to directly measure molecular lesions considered to be important in human carcinogenesis. For example, both immunological and physical techniques have recently been developed to measure adducts in macromolecules, including DNA isolated from carcinogen-exposed tissues and cells (Table 5). One of the advantages of these approaches is that they can be specific for both carcinogenic agent and target cell type. Antisera, both monoclonal and polyclonal, have been produced to a variety of specific carcinogen–DNA adducts (*20,21*) and also to DNA lesions caused by ionizing radiation, e.g., thymine glycol (*10*), and ultraviolet radiation, e.g., thymidine dimers (*22,23*). Highly sensitive enzyme immunoassays have been developed to measure adducts in isolated DNA from carcinogen-exposed tissues (*24*) and in histological and cytological preparations (*25–27*). These immunoassays gain their specificity from the antibody-antigen reaction which is geometrically amplified by an enzyme conjugated to one of the immunoreactants, usually the antibody (Fig. 4). P^{32}-Nucleotide postlabeling and thin-layer chromatography (*28*), high pressure liquid chromatography of DNA hydrolysates (*29–31*), and synchronous fluorescence spectrophotometry (*32*) of carcinogen–DNA adducts are physical methods that also show promise. The latter technique is obviously useful only for those carcinogens that fluorescence, e.g., polynuclear aromatic hydrocarbons. These physical techniques complement both one another and also the measurement of adducts by enzyme immunoassays.

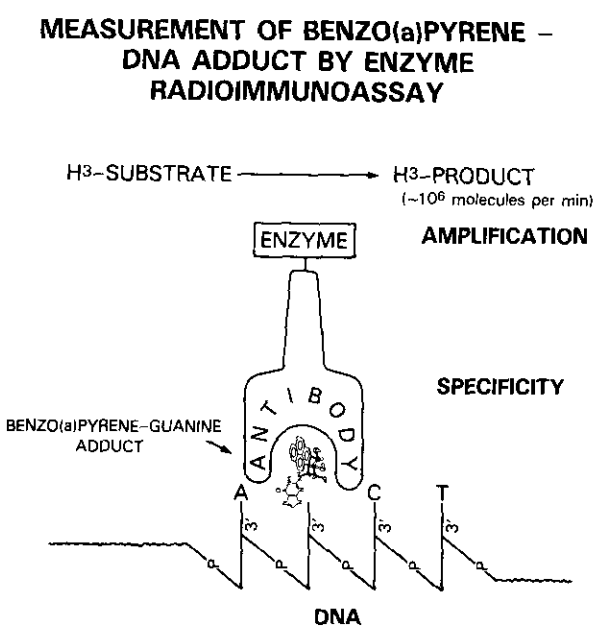

FIGURE 4. Two principles of enzyme radioimmunoassays are specificity and amplification.

In preliminary studies (*33,34*), benzo(a)pyrene diol epoxide–DNA adducts have been detected in tissue and peripheral blood samples from people exposed to benzo(a)pyrene. There is wide interindividual variation in the amounts of adducts measured, which may be a reflection of differences in environmental exposure to benzo(a)pyrene, ratio of metabolic activation and deactivation, and DNA repair rates. Ongoing investigations are assessing the contribution of each of these factors in determining the amounts of adducts. Although there is a positive association between adduct levels and tumor-initiating potency in many, but not all, studies using animal models (*35*), it is not known whether such an association exists in human carcinogenesis.

Future Research Needs

First, we need to validate current methodology to detect carcinogen–DNA adducts as to specificity, sensitivity, interlaboratory reproducibility, etc. This will require a coordinated effort similar to that developed for validating other "short-term" assays. Because humans are usually exposed to a variety of chemical carcinogens at poorly defined doses and timeframes, the amount of adduct measured at any one time will be a composite of past exposure and the other determinants of carcinogen–DNA adducts discussed above. In addition, methods are needed to detect specific adducts in complex mixtures of carcinogen–DNA adducts. Both the immunological and physical assays have this potential. Mixtures of antibodies to an array of adducts can be used as an initial screen. The physical assays, synchronous fluorescence spectrophotometry and ^{32}P-postlabeling and nucleotide chromatography, may be especially suited for analysis of a mixture of adducts in a biological sample. "Fingerprints" of computer-generated contour maps of spectra obtained by three-dimensional synchronous fluorescence spectrophotometry and two-dimensional autoradiograms of chromatographed ^{32}P-labeled nucleotides may be stored in computer libraries similar to those established for data obtained by mass spectroscopy and by nucleotide and amino acid sequencing techniques. The Laboratory of Human Carcinogenesis, DCE, National Cancer Institute, has initiated such a library of contour maps generated by synchronous fluorescence spectrophotometry. We plan to obtain spectral data in future of carcinogen–DNA adducts, carcinogen–nucleotide adducts, carcinogen–base adducts, and carcinogens and their metabolites that will be available in the future to investigators worldwide through currently available telecommunication links.

Current dogma has directed our efforts to measuring adducts formed by the direct interaction between the activated carcinogen metabolite(s). However, as discussed above, carcinogens may also exert their oncogenic effects via indirect damage to macromolecules, such as carcinogen-induced formation of superoxides

which cause DNA damage, including thymine glycol and other altered nucleic acid structures. This induction of the prooxidant state, i.e., increased concentrations of active oxygen, organic peroxides and radicals, may also be of importance in tumor promotion (*7*). Additional research is needed in this important area.

Second, new methodologies to measure carcinogen–DNA adducts are still needed. Ultratrace mass spectral technique is one that is currently being developed (*36*). We should also consider the potential value of measuring adducts at different levels of biological organization (Table 6). Immunocytochemistry and immunofluorescent microscopy should be valuable techniques for measuring adducts in individual cells. Detection of adducts and DNA damage in specific genes, e.g., oncogenes, and DNA sequences, e.g., transcriptional control regions (*37*), is a research goal that could strengthen the association among gene rearrangements, point mutations, and activation of oncogenes. Because the putative mechanistic link between carcinogen–DNA adducts and tumor initiation is not well understood, research in this area remains of fundamental importance.

Third, additional studies are needed to evaluate carcinogen–DNA adducts as qualitative and quantitative indicators of disease (Table 7). Animal models will continue to be essential for these studies. Monitoring of individual animals and relating the results of the assays with the tumor incidence, type, location, and latency period in each animal is an area of high priority. Measurements of carcinogen-DNA adducts should be incorporated into a battery of other assays (Table 8) (*1,2*). *In vitro* models will also be needed for investigations comparing response in tissues and cells from humans to those of experimental animals (Fig. 3).

Fourth, biochemical epidemiological studies are yielding important information, including preliminary results indicating that carcinogen–DNA adducts can be detected in people exposed to carcinogens. More attention can now be directed at more complex experimental designs, e.g., studying the adduct removal rates in people who have ceased smoking tobacco. Cancer patients receiving chemotherapy are an example of a study population in which the dose and regimen of exposure to DNA-damaging agents is well defined. It should be emphasized that (a) laboratory-epidemiological studies are more complicated than those using animal models because of ethical, medical, and legal concerns and (b) carcinogen–DNA adducts are probably related to tumor initiation and to perhaps tumor conversion, which are only two stages in multistage carcinogenesis.

Fifth, the investigations mentioned above will require sustained and substantial financial support from private, industrial, and governmental sources. Both animal and clinical studies are intrinsically long-term in nature and thus costly.

Table 6. Location of carcinogen–DNA adduct localization at different levels of biological organization.

Level	Location
Tissue and cell	Target tissue and cell
	"Indicator" cells
	Nucleus and mitochondria
Nucleus	Matrix
	Nucleosome—linker and core
	Replicon
DNA	Genomic
	Repetitive sequences
	Gene
	Intragene

Table 7. Research goal: evaluate carcinogen–DNA adducts as indicators of disease risk.

Model type	Research goal
Animal models	Adduct levels in indicator vs. target cells
	DNA repair rates of carcinogen–DNA adducts
	Carcinogen exposure vs. adduct levels
	Adduct levels in individual animals
	Relationship between adduct levels and tumor type, location, incidence, and latency period
In vitro models	Relationship between adduct levels and *endpoints of mutagenicity and* carcinogenicity
	Interspecies comparisons between experimental animal and human cells

Table 8. Potential markers for early biological or biochemical responses to carcinogens in humans.

Types of markers	Examples
Chromosomal abnormalities	Sister chromatid exchanges, chromosomal breaks, translocations, and aberrations (peripheral lymphocytes and target tissues)
Markers for point mutations	HGPRT and thymidine kinase in peripheral lymphocytes
Markers for altered gene expression	Ectopic hormones, cytokeratins, embryonic proteins
Reproductive toxicity	Sperm abnormalities: morphology or density
Membrane changes	Monoclonal antibodies to antigens on tumor cells

The comments of Drs. Kirsi Vahakangas and Dean Mann and the secretarial aid of Norma Paige are appreciated.

REFERENCES

1. Perera, F. P., and Weinstein, I. B. Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation. J. Chron. Dis. 35: 581–600 (1982).
2. Harris, C. C., Vahakangas, K., Autrup, H., Trivers, G. E., Shamsuddin, A. K. M., Trump, B. F., Boman, B. M., and Mann, D. L. Biochemical and molecular epidemiology of human cancer risk. In: The Pathologist and the Environment (D. Scarpelli and J. Craighead, Eds.), Academic Press, New York, in press.
3. Hennings, H., Shores, R., Wenk, M. L., Spangler, E. F., Tarone, R., and Yuspa, S. H. Malignant conversion of mouse skin tumours is increased by tumour promoters. Nature 304: 67–69 (1983).
4. Harris, C. C. Role of carcinogens, cocarcinogens, and host factors in human cancer risk. In: Human Carcinogenesis (C. C. Harris

and H. Autrup), Academic Press, New York, 1983, pp. 941–970.
5. Slaga, T. J., Fischer, S. M., Weeks, C. E., Klein-Szanto, A. J. P., and Reiners, J. Studies of mechanisms involved in multistage carcinogenesis in mouse skin. In: Mechanisms of Chemical Carcinogenesis (C. C. Harris and P. A. Cerutti, Eds.), A. R. Liss, New York, 1982, pp. 207–227.
6. Doll, R., and Peto, R. (Eds.). The Causes of Cancer. Oxford Press, New York, 1981.
7. Cerutti, P. A. Prooxidant states and promotion. Science 227: 375–381 (1985).
8. Esterbauer, H. Aldehydic products of lipid peroxidation. In: Free Radicals, Lipid Peroxidation and Cancer (D. C. H. McBrien and T. F. Slater, Eds.), Academic Press, New York, 1982, pp. 101–158.
9. Reddy, J. K., and Lalwani, N. D. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipodemic drugs and industrial plasticizers to humans. CRC Crit. Revs. Toxicol. 12: 1–58 (1984).
10. Leadon, S. A., and Hanawalt, P. C. Monoclonal antibody to DNA containing thymine glycol. Mutat. Res. 112: 191–200 (1983).
11. Grafstrom, R. C., Curren, R. D., Yang, L. L., and Harris, C. C. Genotoxicity of formaldehyde in cultured human bronchial fibroblasts. Science 228: 89–91 (1985).
12. Saladino, A. J., Willey, J. C., Lechner, J. F., Grafstrom, R. C., and Harris, C. C. Effects of formaldehyde, acetaldehyde, benzoyl peroxide and hydrogen peroxide on cultured normal human bronchial epithelial cells. Cancer Res., 45: 2522–2526 (1985).
13. Harris, C. C., Trump, B. F., Grafstrom, R., and Autrup, H. Differences in metabolism of chemical carcinogens in cultured human epithelial tissues and cells. J. Cell. Biochem. 18: 285–294 (1982).
14. Setlow, R. B., Variations in DNA repair among humans. In: Human Carcinogenesis (C. C. Harris and H. Autrup, Eds.), Academic Press, New York, 1983, pp. 231–254.
15. Grafstrom, R. C., Pegg, A. E., Trump, B. F., and Harris, C. C. O^6-Alkylguanine-DNA alkyltransferase activity in normal human tissues and cells. Cancer Res. 44: 2855–2857 (1984).
16. Myrnes, B., Giercksky, K.-E., and Krokan, H. Interindividual variation in the activity of O^6-methylguanine-DNA methyltransferase and uracil-DNA glycosylase in human organs. Carcinogenesis 4: 1565–1568 (1983).
17. Bennett, R. A., Essigman, J. M., and Wogan, G. N. Excretion of aflatoxin-guanine adduct in the urine of aflatoxin B_1-treated rats. Cancer Res. 41: 650–654 (1981).
18. Autrup, H., Bradley, K., Shamsuddin, A. K. M., Wakhisi, J., and Wasunna, A. Detection of putative adduct with fluorescence characteristics identical to 2,3-dihydro-2-(7'-guanyl)-3-hydroxyaflatoxin B_1 in human urine collected in Murang'a District, Kenya. Carcinogenesis 4: 1193–1195 (1983).
19. Cathcart, R., Schwiers, E., Saul, R. L., and Ames, B. N. Thymine glycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. Proc. Natl. Acad. Sci. (U.S.) 81: 5633–5637 (1984).
20. Muller, R., and Rajewsky, M. F. Antibodies specific for DNA components structurally modified by chemical carcinogens. J. Cancer Res. Clin. Oncol. 102: 99–113, 1981.
21. Poirier, M. C. Antibodies to carcinogen-DNA adducts. J. Natl. Cancer Inst. 67: 515–519 (1981).
22. Strickland, P. T., and Boyle, J. M. Characterization of two monoclonal antibodies specific for dimerised and nondimerised adjacent thymidines in single-stranded DNA. Photochem. Photobiol. 34: 595–601 (1981).
23. Eggset, G., Volden, G., and Krokan, H. UV-induced DNA damage and its repair in human skin $_{in\ vivo}$ studied by sensitive immunohistochemical methods. Carcinogenesis 4: 745–750 (1983).
24. Harris, C. C., Yolken, R. H., and Hsu, I. C. Enzyme immunoassays: applications in cancer research. In: Methods in Cancer Research (H. Busch and L. C. Yeoman, Eds.), Academic Press, New York, 1982, pp. 213–242.
25. Adamkiewicz, J., Nehs, P., and Rajewsky, M. F. Immunological methods for detection of carcinogen-deoxyribonucleic acid adducts. In: Methods of Monitoring Human Exposure to Carcinogenic and Mutagenic Agents (K. Hemminki, M. Sorsa and H. Vainio, Eds.), Plenum Press, New York, in press.
26. Heyting, C., Van der Laken, C. J., Van Raamsdonk, W., and Pool, C. W. Immunohistochemical detection of O^6-ethyldeoxyguanosine in the rat brain after *in vivo* applications of N-ethyl-N-nitrosourea. Cancer Res. 43: 2935–2941 (1983).
27. Poirier, M. C., Stanley, J. R., Beckwith, J. B., Weinstein, I. B., and Yuspa, S. H. Indirect immunofluorescent localization of benzo(a)pyrene adducted to nucleic acids in cultured mouse keratinocyte nuclei. Carcinogenesis 3: 345–348 (1982).
28. Randerath, K., Reddy, M. V., and Gupta, R. C. ^{32}-P-labeling test for DNA damage. Proc. Natl. Acad. Sci. (U.S.) 78: 6126–6129 (1981).
29. Lo, K. M., Franklin, W. A., Lippke, J. A., Henner, W. E. and Haseltine, W. A. New methods for the detection of DNA damage to human cellular DNA by environmental carcinogens and antitumor drugs. In: Indicators of Genotoxic Exposure, Banbury Report 13 (B. A. Bridges, B. E. Butterworth and I. B. Weinstein, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, 1982, pp. 253–276.
30. Herron, D. C., and Shank, R. C. Adducts in human DNA following dimethinitrosamine poisoning. In: Indicators of Genotoxic Exposure, Banbury Report 13 (B. A. Bridges, B. E. Butterworth, and I. B. Weinstein, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, 1982, pp. 245–252.
31. Rahn, R. O., Chang, S. S., Holland, J. M., and Shugart, L. R. A fluorometric-HPLC assay for quantitating the binding of benzo(a)pyrene metabolites to DNA. Biochem. Biophys. Res. Commun. 109: 262–268 (1982).
32. Vahakangas, K., Trivers, G., Rowe, M., and Harris, C. C. Benzo(a)pyrene-diol epoxide-DNA adducts detected by synchronous fluorescence spectrophotometry. Environ. Health Perspect., 62: 101–104 (1985).
33. Perera, F. P., Poirier, M. C., Yuspa, S. H., Nakayama, J., Jaretzki, A., Curnen, M. M., Knowles, D. M. and Weinstein, I. B. A pilot project in molecular cancer epidemiology: determination of benzo(a)pyrene-DNA adducts in animal and human tissues by immunoassays. Carcinogenesis 3: 1405–1410 (1982).
34. Shamsuddin, A. K. M., Sinopoli, N. T., Hemminki, K., Boesch, R. R., and Harris, C. C. Detection of benzo(a)pyrene-DNA adducts in human white blood cells. Cancer Res., 45: 66–68 (1985).
35. Wogan, G. N., and Gorlick, N. J. Chemical and biochemical dosimetry of exposure to genotoxic chemicals. Environ. Health Perspect. 62: 5–18 (1985).
36. Griese, R., Private communication.
37. Boles, T. C., and Hogan, M. E. Site-specific carcinogen binding to DNA. Proc. Natl. Acad. Sci. (U.S.) 81: 5623–5627 (1984).
38. Vahakangas, K., Autrup, H., and Harris, C. C. Interindividual variation in carcinogen metabolism, DNA damage and DNA repair. In: Methods of Monitoring Human Exposure to Carcinogenic and Mutagenic Agents. IARC Monograph, International Agency for Research on Cancer, Lyon, in press.