Advances in Chemical Carcinogenesis: A Historical Review and Prospective

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Introduction

The American Association for Cancer Research has been the citadel for communicating research on chemical carcinogens for over a century. It therefore seems appropriate that a review of chemical carcinogenesis inaugurates a series of articles highlighting advances in understanding, treating, and preventing cancer.

At the dawn of the 20th century, we had recognized that chemicals cause cancer, but we had not yet identified individual cancer-causing molecules, nor did we know their cellular targets. We clearly understood that carcinogenesis, at the cellular level, was predominantly an irreversible process. What we lacked was knowledge of the mechanisms by which chemicals cause cancer and the molecular changes that characterize tumor progression.

We now are early in a century in which cancer is being investigated at the molecular level, and we have developed technologies that afford unprecedented power to delineate and manipulate altered pathways in cancer cells. Can we harness new insights and technologies to prevent or obliterate human cancers or delay their progression? Can we identify individuals who have a particularly high susceptibility to specific environmental carcinogens?

The history of chemical carcinogenesis is punctuated by key epidemiologic observations and animal experiments that identified cancer-causing chemicals and that led to increasingly insightful experiments to establish molecular mechanisms and to reduction of human exposure. In 1914, Boveri (1) made key observations of chromosomal changes, including aneuploidy. His analysis of mitosis in frog cells and his extrapolation to human cancer is an early example of a basic research finding generating an important hypothesis (the somatic mutation hypothesis). The first experimental induction of cancer in rabbits exposed to coal tar was performed in Japan by Yamagiwa and Ichikawa (2) and was a confirmation of Pott's epidemiologic observation of scrotal cancer in chimney sweeps in the previous century (Fig. 1; ref. 3). Because coal tar is a complex mixture of chemicals, a search for specific chemical carcinogens was undertaken. British chemists, including Kennaway (4), took on this challenge and identified polycyclic aromatic hydrocarbons, for example, benzopryene, which was shown to be carcinogenic in mouse skin by Cook, Hewett, and Hieger in 1933 (5). The fact that benzopyrene and many other carcinogens were polyaromatic hydrocarbons lead the Millers (6) to

postulate and verify that many chemical carcinogens required activation to electrophiles to form covalent adducts with cellular macromolecules. This in turn prompted Conney and the Millers (7) to identify microsomal enzymes (P450s) that activated many drugs and chemical carcinogens.

The discovery of DNA as the genetic material by Avery, MacLeod, and McCarthy (8) and the description of the structure of DNA by Watson and Crick (9) indicated that DNA was the cellular target for activated chemical carcinogens and that mutations were key to understanding mechanisms of cancer. This led to defining the structure of the principal adducts in DNA by benzo(a)pyrene (10) and aflatoxin B_1 (11). The concepts developed in investigating mechanisms of chemical carcinogenesis also led to discoveries that are relevant to other human conditions in addition to cancer, including atherosclerosis, cirrhosis, and aging.

Global epidemiologic studies have indentified environmental and occupational chemicals as potential carcinogens. The most definitive epidemiologic studies have been those in which a small group is exposed to an inordinately large amount of a specific chemical, such as aniline dyes.

Figure 1 illustrates exposure of individuals to residues from fossil fuel in chimneys, to tobacco smoke, and to fungi containing aflatoxin, and the identification of the responsible carcinogen(s). Active smoking and exposure to second-hand smoke are among the major causes of cancer mortality worldwide. Even after causative chemicals are identified, however, measurement of accumulated exposure of individuals in different environments remains an important challenge.

The fact that genetic changes in individual cancer cells are essentially irreversible and that malignant changes are transmitted from one generation of cells to another strongly points to DNA as the critical cellular target modified by tobacco smoke and environmental chemicals. DNA damage by chemicals occurs randomly; the phenotypes of associated carcinogenic changes are determined by selection.

Cancers caused by environmental agents frequently occur in tissues with the greatest surface exposure to the agents: lung, gastrointestinal tract, and skin. Recently, the study of chemical carcinogenesis has merged with studies on the molecular changes in cancer cells, thus generating biological markers to assess altered metabolic pathways and providing new targets for therapy. Although these are exciting areas, they may be peripheral to attacking the primary causes of the most common human cancers. As we catalog more and more mutations in cancer cells and more and more changes in transcription regulation, it becomes increasingly apparent that we need to understand what generates these changes. The fact that chemicals cause random changes in our genome immediately implies that our efforts need to be directed to quantifying these changes, reducing exposure, and developing approaches to chemoprevention.
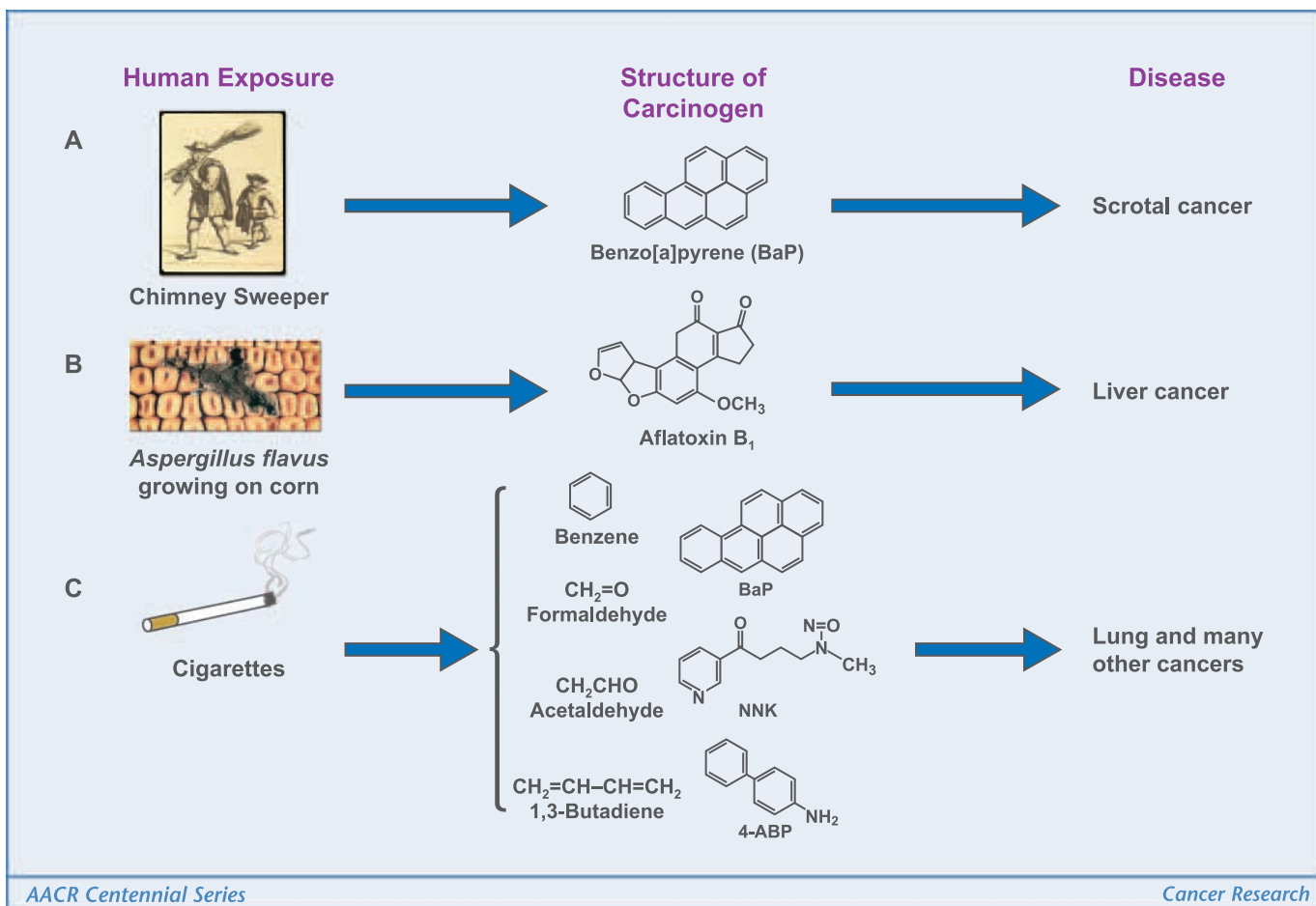
Figure 1. Exposure of humans to chemical agents and the identification of the cancer-causing molecular species. NNK, 4-N-methyl-N-nitrosamino-1-(3pyridyl)-1-butanone; 4-ABP, 4-aminobiphenyl.

Chemical carcinogens cause genetic and epigenetic alterations in susceptible cells imparting a selective growth advantage; these cells can undergo clonal expansion, become genomically unstable, and become transformed into neoplastic cells. This classic view of carcinogenesis has its origin in experimental animal studies conducted in the mid 20th century. The first stage of carcinogenesis, tumor initiation, involves exposure of normal cells to chemical or physical carcinogens. These carcinogens cause genetic damage to DNA and other cellular macromolecules that provide initiated cells with both an altered responsiveness to their microenvironment and a proliferative advantage relative to the surrounding normal cells.

Early in the field of chemical carcinogenesis, investigators recognized that perturbation of the normal microenvironment by physical means, such as wounding of mouse skin or partial hepatectomy in rodents (12, 13) or chemical agents, such as exposure of the mouse skin to certain phorbol esters (14), can drive clonal expansion of the initiated cells toward cancer. In the second stage, tumor promotion results in proliferation of the initiated cells to a greater extent than normal cells and enhances the probability of additional genetic damage, including endogenous mutations that accumulate in the expanding population. This classic view of two-stage carcinogenesis (14) has been conceptually important but also an oversimplification of our increasing understanding of the multiplicity of biological processes that are deregulated in cancer. In addition, an active debate continues on the relative contribution

of procarcinogenic endogenous mechanisms—for example, free-radical–induced DNA damage (15), DNA depurination (16), DNA polymerase infidelity (17), and deamination of 5-methylcytosine (18)—compared with exposure to exogenous environmental carcinogens (19). The enhancement of carcinogens by epigenetic mechanisms such as halogenated organic chemicals and phytoestrogens (20), as well as the extrapolation of results from animal bioassays for identifying carcinogens to human cancer risk assessment, are also difficult to quantify (21). As discussed below, this debate is not merely an academic one, in that societal and regulatory decisions critical to public health are at issue. The identification of chemical carcinogens in the environment and occupational settings [benzo(*a*)pyrene and tobacco-specific nitrosamines in cigarette smoke, aflatoxin B_1 (AFB_1) residues from fossil fuel, vinyl chloride, and benzene] has led to regulations that have reduced the incidence of cancer.

Advances in Chemical Carcinogenesis

A timeline of selected experimental advances in chemical carcinogenesis that have important implications is presented in Fig. 2. First, the selected advances reflect the judgment of the authors and consultants, and remain to be modified by the readers, and, ultimately, by history. Second, the timeline shows the progression of results; an important observation generates new hypotheses that are tested by experiments with increasing

mechanistic focus. Third, the timeline is punctuated with three important molecular discoveries (DNA structure, DNA sequence, and the PCR) that refocused experiments in chemical carcinogenesis (9, 22, 23). Fourth, many technological advances have allowed conceptual ideas to be experimentally tested, including the sensitive detection of chemical carcinogens by high-pressure liquid chromatography (24) and mass spectrometry (25), detection of DNA adducts by postlabeling (26) and by specific antibodies (27), transcriptional profiling by arrays (28, 29), and quantitation of mutagenicity of carcinogens using bacterial genetics (19).

In the first half of the 20th century, the experimental focus was on identifying chemical carcinogens in complex mixtures, and on determining their metabolism and cellular targets. With the recognition that genes are encoded in DNA (9) and that DNA is transferred from one cellular generation to the next (30), research rapidly focused on the interaction of activated chemical carcinogens with DNA and on mutations that result from DNA alterations as well as the identification of key mutated (31) or deregulated genes including oncogenes and tumor suppressor genes (32). Underlying these studies was the expectation that delineation of mutated genes would identify them as specific targets for chemotherapy. The expectation that targeting individual mutated or rearranged gene products would be efficacious for cancer treatment has thus far been verified in only a limited number of situations, such as the use of imatinib for chronic myelogenous leukemia (33).

Experiments Are Generators of New Ideas and Concepts

The experimental landmarks highlighted in Fig. 2 frequently generated new experiments, and this progression has foretold some of our key concepts on the mechanisms of chemical carcinogenesis. An overriding concept has emerged that links DNA damage by reactive chemicals, the production of mutations by unrepaired DNA adducts, and the selection of cells harboring mutated genes that characterize the malignant phenotype. Studies on arylhydroxylamines provided a paradigm for tracing the metabolism of carcinogens to chemically reactive electrophiles that covalently bind to DNA. 2-Acetylaminofluorene (AAF) is metabolically activated by liver microsomal mixed–function oxygenases to N-hydroxy- and then to N-sulf oxy-AAF, a strong electrophile that forms covalent adducts with guanine moieties in DNA (34). AAF is not mutagenic in bacterial assays, whereas N-hydroxy-AAF is highly carcinogenic (34). N-hydroxy-AAF is rendered inactive by the formation of a glucuronide in the liver that is transported to the bladder and excreted (35). Unfortunately, it is subjected to acid hydrolysis in the bladder to yield active N-hydroxy-AAF, which is associated with human bladder cancer. Thus, the activation and detoxification of a chemical carcinogen in specific cells or tissues can be a major factor in determining tissue and host specificity.

Hypothesis and Experimental Verification

The testing of certain concepts in chemical carcinogenesis awaited the development of new technologies. For example, the concept of somatic mutations in cancer (1, 36) preceded by 40 years the establishment of DNA as the genetic material (8) and by 63 years the development of DNA sequencing methods (23) that directly showed clonal mutations in human cancer cells. Also, the mutator phenotype hypothesis formulated in 1974 (17) has been only recently experimentally verified (37).

Many hypotheses are still under active investigation. These include the potential importance of carcinogen-protein interactions (38), carcinogen-induced reversion to stem cell–like phenotypes (39), inherited changes in gene expression (40, 41), direct action of nongenotoxic chemicals (42), and targeted interactions of carcinogens with specific genes such as *TP53* (43–45). Other concepts focus on carcinogenesis mediated by RNA damage (46), RNA-templated DNA repair (47), specific metastasis genes (48, 49), and sequential clonal lineage pathways in cancer (50, 51).

Emerging hypothesis such as anticarcinogens (52), overlapping pathways to malignancy (53), coordinated changes in gene expression (54), epigenetic silencing by chemical carcinogens (40, 55, 56), and oncogene addiction (57) are just beginning to be explored. Finally, there are concepts for which quantitation is lacking, yet have stood the test of time based on their inherent significance; these include the importance of anaerobic metabolism by tumors (58, 59) and the initiation of tumorigenesis by the generation of oxygen-reactive species (15).

Endogenous Carcinogens

Although establishing DNA as the genetic material provided a structure that faithfully can be duplicated during each cell division, it rapidly became apparent that DNA was also subject to direct modification by X-rays (60), alkylating agents (61), and by an increasing number of environmental chemicals (62, 63). Changes in DNA by many chemical carcinogens are indirect; they first require activation by P-450 aryl hydroxylases into electrophiles to form covalent adducts with DNA and with other cellular macromolecules (64, 65). Many normally generated reactive molecules that are intermediates in metabolism modify many cellular molecules including DNA and therefore are mutagens and carcinogens. However, not all mutagens seem to be carcinogens. What was unanticipated was the magnitude of DNA modification by normal cellular processes in the absence of exposure to environmental mutagens (66, 67).

The lability of DNA in an aqueous environment was first quantified by Lindahl and Nyberg, who measured the rates of depurination (16) and deamination (18) in solution under different conditions and extrapolated these results to those predicted to be present in human cells. They calculated that each normal cell could undergo >10,000 DNA damaging events per day. Endogenously generated modifications of DNA include methylation by S-adenosylmethione, modification by lipid peroxidation products, chlorination, glycosylation, oxidation, and nitrosylation (66–71). Reactive oxygen and nitrogen species are particularly relevant because the activated species are generated by host cells, and the process of resynthesis results in the replacement of >50,000 nucleotides per cell per day (68). To maintain our genomes, we have evolved a network of DNA repair pathways to excise altered residues from DNA (Fig. 3). A major consideration is the relative contribution of environmental and endogenous DNA damage to carcinogenesis. DNA damage by environmental agents would have to be extensive and exceed that produced by normal endogenous reactive chemicals to be a major contributor to mutations and cancer. This consideration underlines the difficulty in extrapolating risk of exposure to that which would occur at very low doses of carcinogens.

DNA Repair

Human cells possess an armamentarium of mechanisms for DNA repair that counter the extensiveness of DNA damage
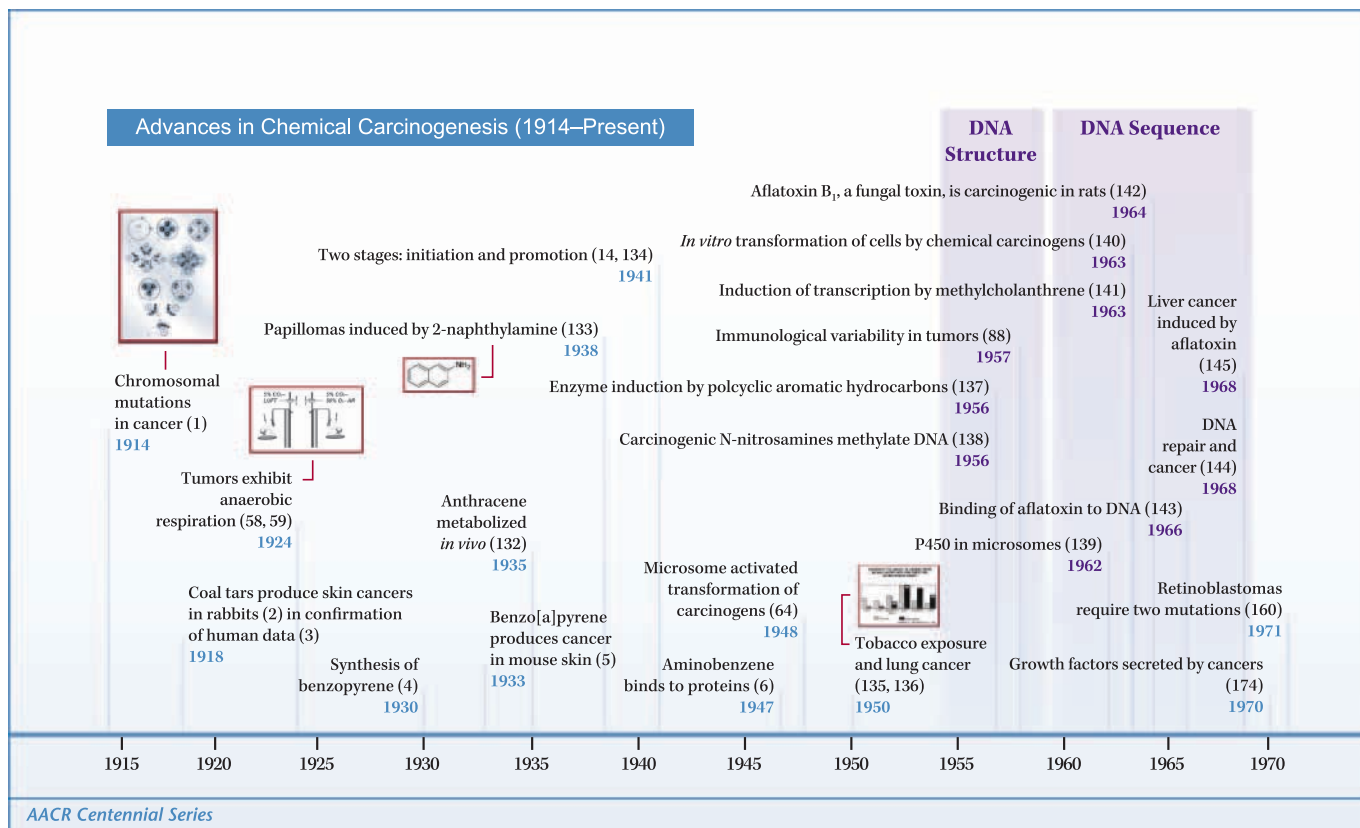
Figure 2. An overview of primary examples of events that have generated important insights into carcinogenesis.

caused both by endogenous and environmental chemicals. These mechanisms include base excision repair (BER) that removes products of alkylation and oxidation (72–74); nucleotide excision repair (NER) that excises oligonucleotide segments containing larger adducts (75); mismatch repair that scans DNA immediately after polymerization for misincorporation by DNA polymerases (76); and oxidative demethylation (77), transcription-coupled repair (TCR) that preferentially repairs lesions that block transcription (78); double-strand break repair and recombination that avoids errors by copying the opposite DNA strand (79); as well as mechanisms for the repair of cross-links between strands (80, 81) that yet need to be established.

Most DNA lesions are subject to repair by more than one pathway. As a result, only a minute fraction of DNA lesions escapes correction are present at the time of DNA replication and can direct the incorporation of noncomplementary nucleotides result-ing in mutation (Fig. 3). Unrepaired DNA lesions initiate mutagenesis by stalling DNA replication forks or are copied over by error-prone *trans*-lesion DNA polymerases (82–84). Alternative-ly, incomplete DNA repair can result in the accumulation of mutations and mutagenic lesions, such as abasic sites (85).

Integrative Cell Biology

Damage to DNA by chemical carcinogens activates checkpoint signaling pathways leading to cell cycle arrest and allows time for DNA repair processes. In the absence of repair, cells can use special DNA polymerases that copy past DNA adducts (86, 87), or undergo apoptosis by signaling the recruitment of immunologic and inflammatory host defense mechanisms. The demonstration that

each methylcholanthrene-induced tumor has a unique antigenic signature provided one of the earliest glimpses into the stochastic nature of cellular responses to carcinogens (88). The immunologic and inflammatory responses facilitate not only engulfment and clearance of damaged cells but also the resulting generation of reactive oxygen (89) and nitrogen radicals (90) that further damage cellular DNA.

Inflammation and Carcinogenesis

The concept that chronic inflammation can result in cancer is supported by Virchow's (91) histologic observation of inflammatory lymphocytes infiltrating tumors. Inflammation accompanying the "painting" of coal tar was described by Japanese pathologists in the earliest experimental study of chemical carcinogenesis (2). The classic tumor promoter, croton oil, and its most active ingredient, 12-*O*-tetradecanoylphorbol-13-acetate, are potent inflammatory agents. In addition to studies of "two-stage" skin carcinogenesis, other animal models have shown the synergistic interaction of chemical carcinogens with proinflammatory agents; for example, respiratory infection with influenza virus synergistically increases the lung cancer response in rats to a carcinogenic N-nitrosamine (92).

Chronic inflammation can have a strong inherited basis, e.g. hemochromatosis, or can be acquired from infection by viruses, bacteria, or parasites or be associated with metabolic or physical conditions (93). Obesity has been considered to be a chronic inflammatory condition associated with multiple types of human cancer (94); gastric acid reflux causes chronic inflammation and can progress to Barrett's-associated esophageal adenocarcinoma (95); and colitis can progress to colon cancer (96, 97). Recent
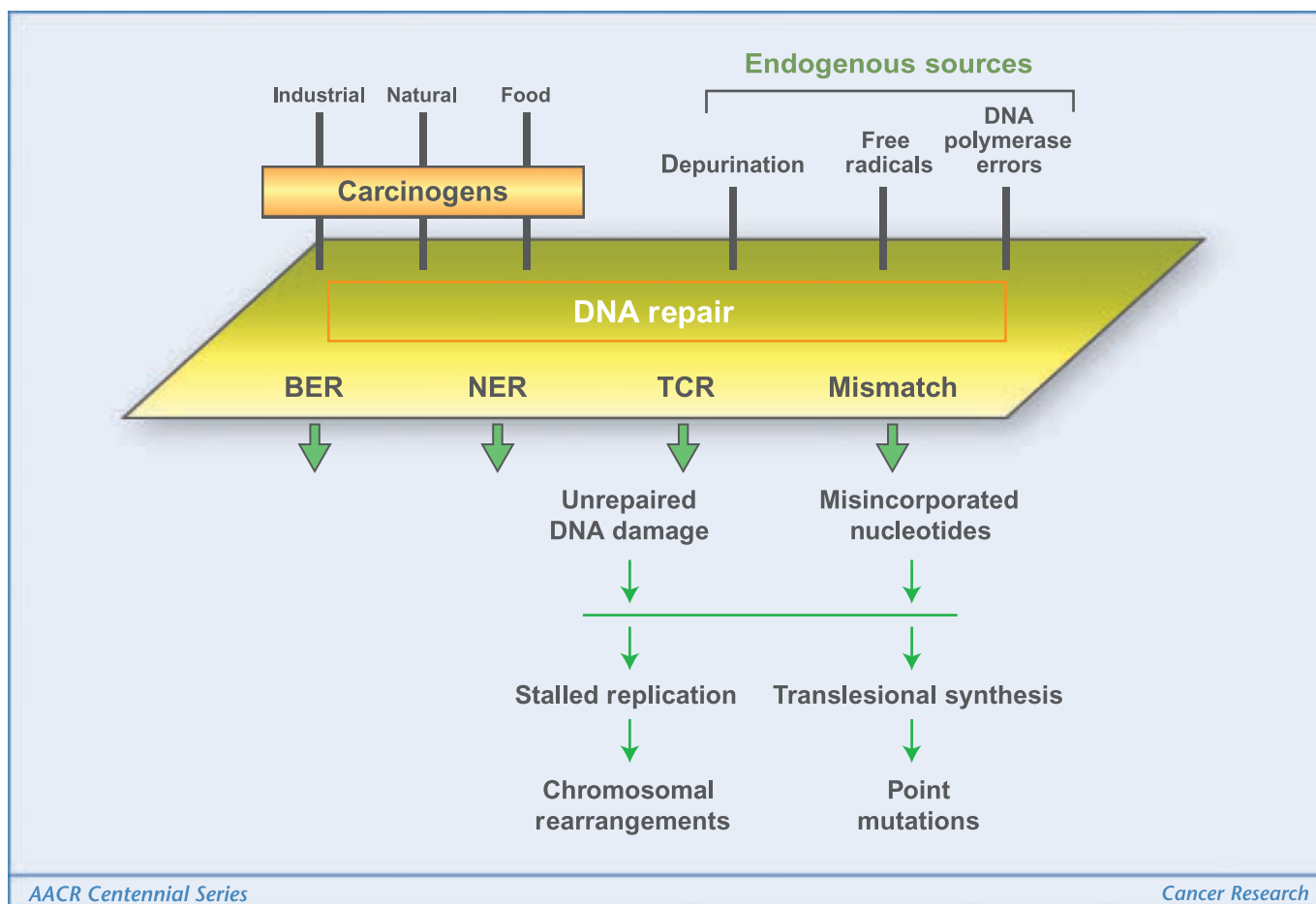
Figure 2 *Continued.*

advances have begun to uncover the underlying mechanisms of the association between chronic inflammation and cancer.

The identification of specific genes by allelic replacements and "knockouts" has facilitated the delineation of complex immune response networks that govern cellular responses to chemical carcinogens. The innate immune system is the first line of defense against pathogenic microorganisms and toxins and responds by generating free radicals, inflammatory cytokines, and the activation of the complement cascade (93, 98). In addition to reactive oxygen species, the past two decades have shown the significance of nitrogen-based free radicals, including nitric oxide and its derivatives (90, 93). The concentration and length of exposure can determine the seemingly paradoxical procarcinogenic and anti-carcinogenic activities of free radicals. As will be discussed in another article in the AACR Centennial Series, chronic activation of the innate immune system is generally procarcinogenic and adaptive immune system is anticarcinogenic (98).

Multistage Carcinogenesis

In humans, there is a 20- to 50-year lag from when an individual is exposed to a carcinogen to the clinical detection of a tumor. For most adult epithelial tumors, there is an exponential increase in cancer incidence as a function of age (99), suggesting that tumor progression proceeds in a series of sequential steps. This multistep process has been studied most extensively in colon cancer, with the progression from hyperplastic epithelium, to adenomas, to carcinomas, and to metastasis (100). Analysis of cancers at different stages, from adenomas to anaplastic tumors, suggests a sequential order of mutations and genome rearrangements:

mutations in APC, DNA hypomethylation, activation of k-*ras*, loss of heterozygosity on chromosome 18q, and loss of p53. This concept of sequential mutations has been challenged by new findings including the complexity of somatic mutations occurring in breast and colon cancers (101) and the demonstration that only a small fraction (6.6%) of colon cancers contain the three most frequently identified mutations (102). Nevertheless, this formalism may identify potential therapeutic targets.

Another not necessarily exclusive concept—that cancers exhibit a mutator phenotype—presents a more stochastic picture: Each cancer cell in a tumor harbors thousands of different mutations, and yet only a small subset of cells preferentially proliferates during tumorigenesis, owing to random mutations that confer a selective advantage (102). Evidence for this concept is the recent demonstration that the frequency of nonclonal mutations in human cancers is >200-fold greater than that in adjacent normal tissues in renal cell carcinoma, sarcoma, ovarian carcinoma, and adenocarcinoma of the colon (36, 103). The genetic variability of cancer cells produced by mutator mutations increases the likelihood that a clinical tumor will contain many cells resistant to chemotherapy and is consistent with the utility of therapeutic combinations (104).

Chemical Carcinogens and Induced Somatic Mutations as Biomarkers in Molecular Epidemiology

Decades of laboratory research in chemical carcinogenesis have provided a solid foundation for the analysis of chemical-specific macromolecular adducts and related somatic mutations in humans as biomarkers of carcinogen exposure. A paradigm for validating

Figure 3. Mutations Result from Incomplete DNA Repair. DNA damage in cells results from environmental agents (carcinogens) and endogenous sources. Most damage is removed by DNA repair processes: BER, NER, or TCR. Misincorporated nucleotides are removed by mismatch repair. Lesions that are not repaired can stall DNA replication resulting in double-strand breaks and chromosomal rearrangements. Alternatively small adducts can be bypassed by family-Y DNA polymerases.

causal relationships between biomarkers of carcinogens exposure and a cancer risk biomarker is shown in Fig. 4 (105). AFB_1, a fungal toxin, is a prototypical example of an environmental chemical carcinogen that has been validated using this strategy. Benzo(a)-pyrene, a polycyclic aromatic hydrocarbon (53), 4-aminobiphenyl (106), an aromatic amine dye, and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific N-nitrosamine (107), are other key examples.

Initially, epidemiologic studies revealed a positive association between dietary AFB_1 exposure and the incidence of hepatocellular carcinoma. Parallel laboratory studies of carcinogenicity in multiple animal species, elegant chemical and biochemical analysis of AFB_1 biometabolism, the identification of AFB_1 DNA adducts, and determination of AFB_1 mutagenic activity buttressed the evidence for national and international organizations to classify AFB_1 as a human carcinogen (117). Results from these experimental animal and laboratory studies were then systematically and successfully translated to assess AFB_1 exposure and biological effects in humans. Independent studies conducted in geographic regions of high AFB_1 exposure and high incidence of hepatocellular carcinoma, such as China and The Gambia (108), were confirmatory and added to the weight of the evidence that AFB_1 is a human carcinogen. The causal linkage between AFB_1 and hepatocellular carcinoma was further strengthened by the association between

AFB_1 exposure and a specific transversion mutation (Arg -> Ser) in the third nucleotide of codon 249 of the *p53* tumor suppressor gene in hepatocellular carcinoma (109, 110). In separate cohorts from Qidong, China, and The Gambia, a synergistic interaction between AFB_1 exposure (urinary AFB_1 nucleotide-biomarker or serum Ser 249 p53 mutations) and biomarkers of hepatitis B virus (HBV) infection (111) in the risk of hepatocellular carcinoma was reported (108, 112).

Many questions remain to be answered. For example, the molecular mechanism(s) of the synergistic interaction between AFB_1 and HBV is still uncertain (113). Do the immunosuppressive and oxidative stress effects of AFB_1 contribute to the increased carcinoma risk? Does HBV gene incorporation in the genome of hepatocytes increase their likelihood of oncogenic transformation by AFB_1?

Impact of New Technologies

Recent advances in molecular methodologies are phenomenal, and they increasingly are being applied to understanding the interaction of chemical carcinogens with cellular constituents and metabolism. Cloning of DNA has facilitated the identification of specific genes mutated in human cancers. Chemical methods, including mass spectrometry, allow us to measure carcinogen

alteration with unprecedented sensitivity and specificity. Mass spectrometry is being coupled with site-specific mutagenesis to define how specific alterations in DNA produce cognate mutations. Sequencing of the human genome and the identification of DNA restriction enzymes opens up the field of molecular epidemiology, focusing in part on individual susceptibility to carcinogens. Array technology facilitates analysis of carcinogen-induced alterations in the expression of both protein coding and noncoding genes.

On the horizon are techniques that can measure single molecules of carcinogens in cells, random mutations in individual cells, analysis of the dynamics of how molecules breathe and work, and bioinformatics and genetic maps to delineate complex interacting functional pathways in cells. Underlying this progress in understanding chemical carcinogenesis is a cascade of advances in molecular biology that makes it feasible to quantify DNA damage by chemical agents, mutations, and changes in gene expression.

Determining the structure of DNA, DNA sequencing, and the PCR revolutionized cell biology, including carcinogenesis. Advances in detection of DNA damage, including postlabeling of DNA (26), immunoassays (27), and mass spectrometry (25), have allowed the detection of a single altered base in 10^9 nucleotides using human nuclear DNA. This technology can be extended to analyze DNA or RNA in a single cell (114). Advances in cell biology, including array technology (28) and proteomics (115, 116), make it feasible to assess global changes in RNA and protein expression during carcinogenesis. Together, these technologies underlie systems biology, making it increasingly feasible to map biochemical pathways in cancer cells from DNA, to RNA, to proteins, to function.

The Next 100 Years

We have made enormous strides in identifying chemical carcinogens and deciphering their mechanisms of action. We have increasingly focused on DNA as a target, considering the fact that, at the cellular level, cancer is an inherited disease: Once a cancer, perhaps always a cancer. The international efforts to classify chemicals as either potential or actual human carcinogens (117) are not without controversy, but in most cases are firmly grounded in epidemiology. The need to identify chemical carcinogens in advance of human exposure and epidemiologic evidence is obvious.

Increasing emphasis on mechanistic data and knowledge of similarities and differences among animal species is a timely development. For example, the renal carcinogenicity of gasoline in the male rat proceeds by a mechanism not likely to be relevant to humans (118). Like carcinogenesis, chemoprevention is initiated by epidemiologic observations, verified by animal experiments, and amplified by mechanistic and structural studies (119–121). The dose-response relationship between carcinogen exposure and the induction of cancer continues to be a topic of intense scientific and public debate (61, 105, 122–126). The default assumption of a linear dose-response relationship is a conservative position in the interest
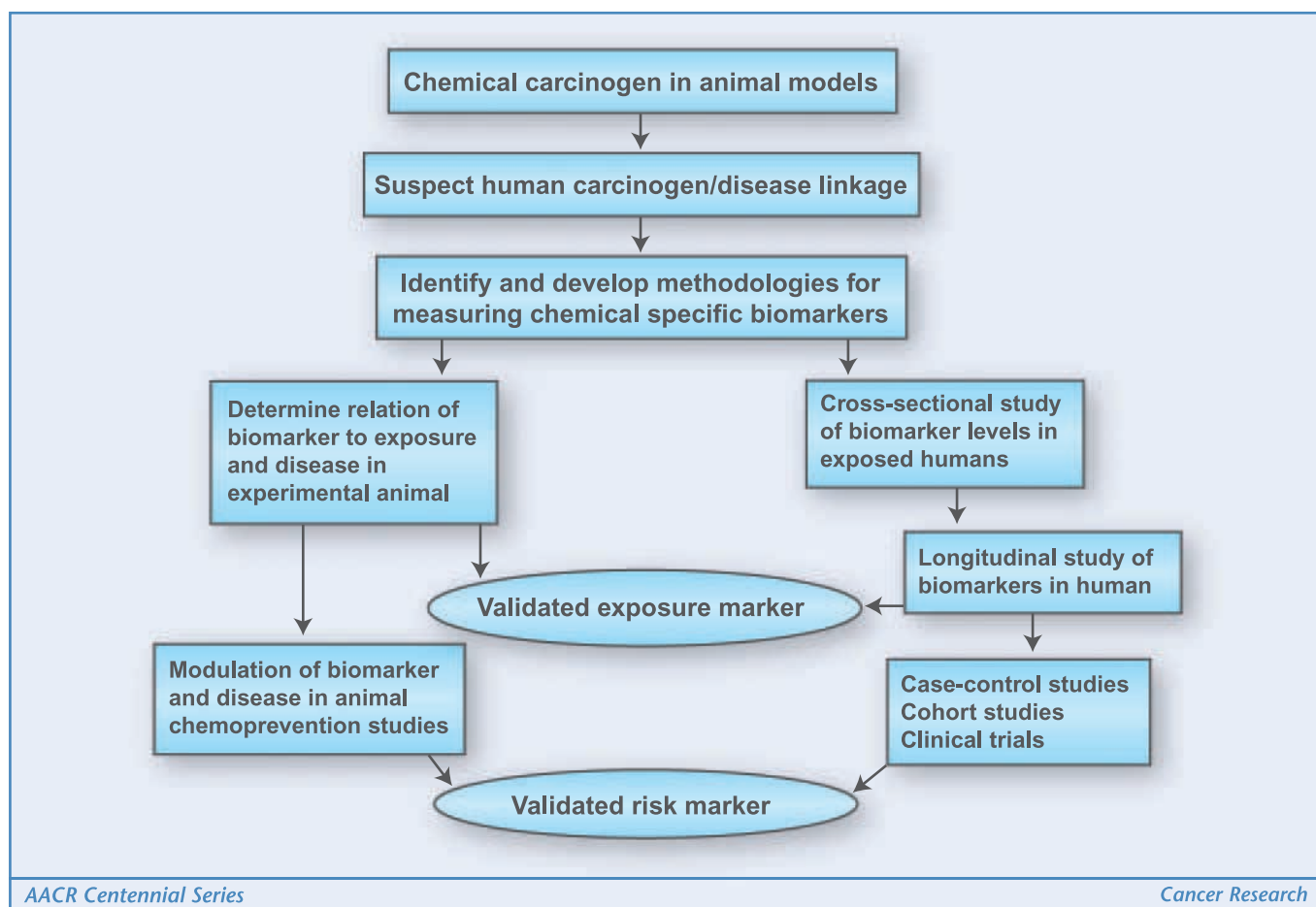

Figure 4. A modified molecular epidemiologic approach for validating causal relationships between carcinogen exposure and cancer risk.

of public health that needs to be continually evaluated as mechanistic data accumulate in the future.

The field of chemical carcinogenesis has a rich history of scientific accomplishment that underpins much of cancer biology, cancer risk assessment, public health policy, and life-style and occupational causes of cancer. The concepts of gene-environment interactions and interindividual variation in the molecular epidemiology of human cancer risk were generated by the synthesis of chemical carcinogenesis, cellular and molecular biology, and cancer epidemiology (127). Functional genetic polymorphisms in DNA repair and xenobiotic metabolizing enzymes are examples of an inherited basis of interindividual differences in cancer susceptibility (127, 128).

Many of the biomarkers of cancer risk and detection are based on the knowledge of chemical carcinogenesis, including carcinogen-DNA adducts, somatic mutations, and mutation spectrum linking carcinogen exposure and DNA adduction with mutation. Chemical-viral interactions can have synergistic effects, for example, dietary AFB_1 and HBV in hepatocellular carcinogenesis. Animal models of chemical carcinogenesis continue to play a critical role in the field of cancer chemoprevention and in our understanding the mechanisms of inflammation-associated cancer and the contributions of microRNA in cancer.

Many questions in the field of chemical carcinogenesis remain to be answered. Are stem cells mutated by chemical carcinogens and become precursors of human cancer? Do chemical carcinogens generate epigenetic changes during carcinogenesis? These and other questions, many to be formulated by future studies, will continue to excite investigators in chemical carcinogenesis research, enhance our understanding of carcinogenesis, and, as a result, improve prevention, cancer detection, and treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

References

1. Boveri T. Zur Frage der Entstehung maligner Tumoren. Jena: Gustave Fischer Verlag; 1914.
2. Yamagiwa K, Ichikawa K. Experimental study of the pathogenesis of carcinoma. J Cancer Res 1918;3:1–21.
3. Pott P, Cancer Scroti, In: Chirurgical observations relative to the cataract, the polypus of the nose, the cancer of the scrotum, the different kinds of ruptures, and the modification of the toes and feet. London: Hawes, Clarke, Collins; 1775. p. 63–8.
4. Kennaway EL. Further experiments on cancer-producing substances. Biochem J 1930;24:497–504.
5. Cook JW, Hewett CL, Hieger I. The isolation of a cancer-producing hydrocarbon from coal tar. Parts I, II, and III. J Chem Soc 1933;24:395–405.
6. Miller EC, Miller JA. The presence and significance of bound amino azodyes in the livers of rats fed p-dimethyl-aminoazobenzene. Cancer Res 1947;7:468–80.
7. Conney AH, Miller EC, Miller JA. The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. Cancer Res 1956;16:450–9.
8. Avery OT, MacLeod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumoccal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumoccus type III. J Exp Med 1944;79:137–58.
9. Watson JD, Crick FH. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. Nature 1953;171:737–8.
10. Carrell JC, Carrell TG, Carrell HL, Prout K, Glusker P. Benzo[a]pyrene and its analogues: structural studies of molecular strain. Carcinogenesis 1997;18:415–22.
11. Croy RG, Essigman JM, Reinhold VN, Wogan GN. Identification of the Principal Aflatoxin B1-DNA Adduct Formed *in vivo* in Rat Liver. PNAS 1978;75:1745–9.
12. Hennings H, Boutwell RK. Studies on the mechanism of skin tumor promotion. Cancer Res 1970;30:312–20.
13. Fausto N, Camobekk JS, Riehle KJ. Liver Regeneration. Hepatology 2006;43:S45–53.
14. Berenblum I. The mechanism of carcinogenesis. A study of the significance of cocarcinogenic action and related phenomena. Cancer Res 1941;1:807.
15. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett 1991;291:9–19.
16. Lindahl T, Nyberg B. Rate of depurination of native deoxyribonucleic acid. Biochemistry 1972;11:3610–8.
17. Loeb LA, Springgate CF, Battula N. Errors in DNA replication as a basis of malignant change. Cancer Res 1974;34:2311–21.
18. Lindahl T, Nyberg B. Heat-induced deamination of cytosine residues in deoxyribonucleic acid. Biochemistry 1974;13:3405–10.
19. Ames BN, Durston WE, Yamasaki E, Lee FD. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci U S A 1973;70:2281–5.
20. Martin JH, Crotty S, Nelson PN. Phytoestrogens: perpetrators or protectors? Future Oncology 2007;4:3007–1.
21. Swenberg JA, Richardson FC, Boucheron JA, et al. High- to low-dose extrapolation: critical determinants involved in the dose response of carcinogenic substances. Environ Health Perspect 1987;76:57–63.
22. Mullis KB, Faloona FA. Specfic synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol 1987;155:335–50.
23. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977;74:5463–7.
24. Esaka Y, Inagaki S, Goto M. Separation procedures capable of revealing DNA adducts. J Chromatography B 2003;797:321–9.
25. Singh R, Farmer BP. Liquid chromatography-electrospray-ionization-mass spectrometry: the future of DNA adduct detection. Carcinogenesis 2006;27:178–96.
26. Randerath K, Reddy MV, Gupta RC. 32P-labeling test for DNA damage. Proc Natl Acad Sci U S A 1981;78:6126–9.
27. Poirier MC, Yuspa SH, Weinstein IB, Blobstein S. Detection of carcinogen-DNA adducts by radioimmunoassay. Nature 1977;270:186–8.
28. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995;270:467–70.
29. Kallionieni A, Kallioniemi O-P, Sudar D, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258:818–21.
30. Meselson M, Stahl FW. The replication of DNA in Escherichia coli. Proc Natl Acad Sci U S A 1958;44:671–82.
31. Sukumar S, Notario V, Martin-Zanca D, Barbacid M. Induction of mammary carcinomas in rats by nitrosomethylurea involves malignant activation of H-ras-1 locus by single point mutations. Nature 1983;306:658–61.
32. Weinberg RA. The Biology of Cancer, Garland Science, London, 2006.
33. Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces hematologic and cytogenetic reponses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 2002;99:3530–9.
34. Singer B, Grunberger D. The Molecular Biology of Mutagens and Carcinogens. New York: Plenum Press; 1983.
35. Cramer JW, Miller JA, Miller EC. N-hydroxylation: A new metabolic reaction observed in the rat with the carcinogen 2-acetylaminofluorene. J Biol Chem 1960;235:885–8.
36. Boveri T. Uber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Veh Dtsch Zool Ges 1902;Wurzburg.
37. Bielas JH, Loeb KR, Rubin BP, True LD, Loeb LA. Human cancers express a mutator phenotype. Proc Natl Acad Sci U S A 2006;103:18238–42.
38. Mott DM, Sani BP, Sorof S. The content of the principal protein target of a hepatic carcinogen in liver tumors. Cancer Res 1973;33:2721–5.
39. Sell S, Pierce GB. Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Lab Invest 1994;70:6–22.
40. Weinstein IB, Begemann M, Zhou P, et al. Disorders in cell circuitry associated with multistage carcinogenesis: exploitable targets for cancer prevention and therapy. Clin Cancer Res 1997;3:2696–702.
41. Pogribny IP, Rusyn I, Beland FA. Epigenetic aspects of genotoxic and non-genotoxic hepatocarcinogenesis: Studies in rodents. Environ Mol Mutagen 2008;49:9–15.
42. van Delft JH, van Agen E, van Breda SG, Herwijnen MH, Staal YC, Kleinjans JC. Discrimination of genotoxic

from non-genotoxic carcinogens by gene expression profiling. Carcinogenesis 2004;25:1265–76.

43. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991;253:49–53.

44. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on the TP53 mutation patterns and tumor phenotype: lessons from recent developments in th IARC TP53 database. Hum Mutat 2007;28:622–9.

45. Levine AJ, Momand J, Finlay CA. The P53 tumour suppressor gene. Nature 1991;351:453–6.

46. Weinstein IB, Grunberger D, Fujimura S, Fink LM. Chemical carcinogens and RNA. Cancer Res 1971;31:651–5.

47. Storici F, Bebenek K, Kunkel TA, Gordenin DA, Resnick MA. RNA-templated DNA repair. Nature 2007;447:338–41.

48. Steeg PS, Bevilacqua G, Kopper L, et al. Evidence for a novel gene associated with low tumor metastatic potential. J Natl Cancer Inst 1988;80:200–4.

49. Bernards R, Weinberg RA. Metastasis genes: a progression puzzle. Nature 2002;418:823.

50. Nowell PC. The clonal evolution of tumor cell populations. Science 1976;194:23–8.

51. Shibata D, Navidi W, Salovaara R, Li Z-H, Aaltonen LA. Somatic microsatellite mutations as molecular tumor clocks. Nat Med 1996;2:676–81.

52. von Borstel RC, Higgins JA. Janus carcinogens and mutagens. Mutat Res 1998;402:321–9.

53. Rustgi AK. The genetics of hereditary colon cancer. Genes Dev 2007;21:2525–38.

54. English JM, Cobb MH. Pharmacological inhibitors of MAPK pathways. Trends Pharmacol Sci 2002;23:40–5.

55. Klaunig JE, Kamendulis LM, Xu Y. Epigenetic mechanisms of chemical carcinogenesis. Hum Exp Toxicol 2000;19:543–55.

56. Wilson VL, Jones PA. Inhibition of DNA methylation by chemical carcinogens *in vitro*. Cell 1983;32:239–46.

57. Weinstein IB, Joe A. Oncogene addiction. Cancer Res 2008;68:3077–80.

58. Warburg O, Posener K, E. Negelein. The metabolism of the cancer cell. Biochemiche Ztschr 1924;152:319–44.

59. Weinhouse S, Warburg O, Burk D, Schade AL. On respiratory impairment in cancer cells. Science 1956;124:269–70.

60. Little JB. Radiation carcinogenesis. Carcinogenesis 2000;21:397–404.

61. Wheeler GP, Skipper HE. Studies with mustards. III. *In vivo* fixation of C14 from nitrogen mustard-C14H3 in nucleic acid fractions of animal tissues. Arch Biochem Biophys 1957;72:465–75.

62. Poirier MC. Chemical-induced DNA damage and human cancer risk. Nat Rev Cancer 2004;4:630–7.

63. Luch A. Nature and nurture - lessons from chemical carcinogenesis. Nat Rev Cancer 2005;5:113–25.

64. Mueller GC, Miller JA. The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates. J Biol Chem 1948;176:535–44.

65. Lu AY, Levin W, West SB, et al. Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds, and endogenous substrates. VI. Different substrate specificities of the cytochrome P450 fractions from control and phenobarbital-treated rats. J Biol Chem 1973;248:456–60.

66. Lindahl T. Instability and decay of the primary structure of DNA [see comments]. Nature 1993;362:709–15.

67. Loeb LA. Endogenous carcinogenesis: molecular oncology into the twenty-first century-presidential address. Cancer Res 1989;49:5489–96.

68. Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. Science 2001;29:1284–9.

69. Bohr VA, Smith CA, Okumoto DS, Hanawalt PC. DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell 1985;40:359–69.

70. Cerutti PA. Preoxidant states and tumor promotion. Science 1985;227:375–81.

71. Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucl Acids Res 1984;12:2137–45.

72. Roth RB, Samson LD. 3-methyladenine DNA glycosylase-deficient Aag null mice display unexpected bone marrow alkylation resistance. Cancer Res 2002;62:656–60.

73. Gerson SL. Clinical relevance of MGMT in the treatment of cancer. J Clin Oncol 2002;20:2388–99.

74. Duncan J, Hamilton L, Friedberg EC. Enzymatic degradation of uracil-containing DNA. J Virol 1976;19:338–45.

75. Setlow RB, Carrier WL. The disappearance of thymine dimers fromDNA: an error-corecting mchanism. Proc Natl Acad Sci U S A 1963;51:226–31.

76. Modrich P. Mechanisms and biological effects of mismatch repair. Annu Rev Genet 1991;25:229–53.

77. Sedgwick B. Repairing DNA-methylation damage. Nat Rev Mol Cell Biol 2004;5:148–57.

78. Hanawalt PC. Transcription-coupled repair and human disease. Science 1994;266:1957–8.

79. Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, Ellenberger T. DNA Repair and Mutagenesis. 2nd ed. Washington (DC): ASM Press; 2005.

80. Zheng H, Wang X, Legerski RJ, Glazer PM, Li L. Repair of DNA interstrand cross-links: interactions between homology-dependent and homology-independent pathways. DNA Repair 2006;5:566–74.

81. Kuraoka I, Kobertz WR, Ariza RR, Biggerstaff M, Essigmann JM, Wood RD. Repair of an interstrand DNA cross-link initiated by ERCC1-XPF repair/recombination nuclease. J Biol Chem 2000;275:26632–6.

82. Masutani C, Kusumoto R, Yamada A, et al. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. Nature 1999;399:700–4.

83. Friedberg EC, Wagner R, Radman M. Specialized DNA polymerases, cellular survival, and the genesis of mutations. Science 2002;296:1627–30.

84. McCulloch SD, Kunkel TA. The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. Cell Res 2008;18:148–61.

85. Loeb LA. Apurinic sites as mutagenic intermediates. Cell 1985;40:483–4.

86. Masutani C, Kusumoto R, Iwai S, Hanaoka F. Mechanisms of accurate translesion synthesis by human DNA polymerase eta. EMBO J 2000;19:3100–9.

87. Sweasy JB, Lauper JM, Eckert KA. DNA polymerases and human diseases. Radiat Res 2006;166:693–714.

88. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. J Natl Cancer Inst 1957;18:769–78.

89. Klebanoff SJ. Phagocytic cells: Products of oxygen metabolism. In: Gallin JI, Goldstein IM, Snyderman R, editors. Inflammation: Basic Principles and Clinical Correlates. New York: Raven Press, Ltd.; 1988. p. 391–444.

90. Ohshima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat Res 1994;305:253–64.

91. Virchow R. Die krankhaften Geschwulste: 30 Vorlesungen gehalten wahrend des Wintersemesters 1862–1863. Berlin: Hirschwald; 1863.

92. Schreiber H, Nettesheim P, Lijinsky W, Richter CB, Walburg HE, Jr. Induction of lung cancer in germfree, specific-pathogen-free, and infected rats by N-nitrosoheptamethyleneimine: enhancement by respiratory infection. J Natl Cancer Inst 1972;49:1107–14.

93. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276–85.

94. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–808.

95. Barrett MT, Sanchez CA, Prevo LJ, et al. Evolution of neoplastic cell lineages in Barrett oesophagus. Nat Genet 1999;22:106–9.

96. Rubin CE, Haggitt RC, Burmer GC, et al. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. Gastroenterology 1992;103:1611–20.

97. Hussain SP, Amstad P, Raja K, et al. Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. Cancer Res 2000;60:3333–7.

98. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 2006;6:24–37.

99. Cairns J. The origin of human cancers. Nature 1981;289:353–7.

100. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.

101. Sjoblom T, Jones S, Wood LD, et al. The concensus coding sequences of human breast and colorectal cancers. Science 2006;314:268–74.

102. Smith G, Carey FA, Beattie J, et al. Mutations in APC, Kirsten-ras, and p53 - alternative genetic pathways to colorectal cancer. Proc Natl Acad Sci U S A 2002;99:9433–8.

103. Zheng L, Dai H, Zhou M, et al. Fen1 mutations result in autoimmunity, chronic inflammation and cancers. Nat Med 2007;13:812–9.

104. Beckman RA, Loeb LA. Efficiency of carcinogenesis with and without a mutator mutation. Proc Natl Acad Sci U S A 2006;103:14140–5.

105. Wogan GN, Hecht SS, Felton JS, Conney AH, Loeb LA. Environmental and chemical carcinogenesis. Semin Cancer Biol 2004;14:473–86.

106. Vineis P, Pirastu R. Aromatic amines and cancer. Cancer Causes Control 1997;8:346–55.

107. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999;91:1194–210.

108. Kirk GD, Bah E, Montesano R. Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis and prevention from The Gambia, West Africa. Carcinogenesis 2006;27:2070–82.

109. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature 1991;350:429–31.

110. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris C. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 1991;350:427–8.

111. Blumberg BS, Larouze B, London WT, et al. The relation of infection with the hepatitis B agent to primary hepatic carcinoma. Am J Pathol 1975;81:669.

112. Ross RK, Yuan J-M, Yu MC, et al. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet 1992;339:943–6.

113. Groopman JD, Kensler TW, Wild CP. Protective interventions to prevent aflatoxin-induced carcinogenesis in developing countries. Annu Rev Public Health 2008;29:187–203.

114. Klein CA. Single cell amplification methods for the study of cancer and cllular ageing. Mech Ageing Dev 2005;126:147–51.

115. Anderson NL, Hofmann JP, Gemmell A, Taylor J. Global approaches to quantitative analysis of gene-expression patterns observed by use of two-dimensional gel electrophoresis. Clin Chem 1984;30:2031–6.

116. Aebersold RH, Leavitt J, Saavedra RA, Hood LE, Kent SB. Internal amino acid sequence analysis of proteins separated by one- or two-dimensional gel electrophoresis after *in situ* protease digestion on nitrocellulose. Proc Natl Acad Sci U S A 1987;84:6970–4.

117. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluation of Carcinogenicity. Monographs Volumes 1 to 99, Lyon: IARC: 1971–2006: http://monographs.iarc.fr.

118. Melnick RL, Kohn MC, Huff J. Weight of evidence versus weight of speculation to evaluate the α2u-globulin hypothesis. Environ Health Perspect 1997;105:904–6.

119. Sporn MB, Dunlop NM, Newton DL, Smith JM. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 1976;35:1332–8.

120. Weisburger JH. Practical approaches to chemoprevention of cancer. Drug Metab Rev 1994;26:253–60.

121. Lu YP, Lou YR, Liao J, et al. Administration of green tea or caffeine enhances the disappearance of UVB-induced patches of mutant p53 positive epidermal cells in SKH-1 mice. Carcinogenesis 2005;26:1465–72.

122. Preston RJ. Mechanistic data and cancer risk assessment: the need for quantitative molecular endpoints. Environ Mol Mutagen 2005;45:214–21.

123. Calabrese EJ. Toxicological awakenings: the rebirth of hormesis as a central piller of toxicology. Toxicol Appl Pharmacol 2005;204:1–8.

124. Farber E. Chemical carcinogenesis. N Engl J Med 1981;305:1379–89.

125. Sugimura T. Studies on environmental chemical carcinogenesis in Japan. Science 1986;233:312–8.

126. Heidelberger C. Chemical carcinogenesis. Annu Rev Biochem 1975;44:79–121.

127. Perera FP, Weinstein IB. Molecular epidemiology: recent advances and future directions. Carcinogenesis 2000;21:517–24.

128. Nebert DW, Dalton TP. The role of cytochrome P450 enzymes inendogenous signaling pathways and environmental carcinogenesis. Nat Rev Cancer 2006;12: 947–60.

129. Yuspa SH, Poirier MC. Chemical carcinogenesis: from animal models to molecular models in one decade. Adv Cancer Res 1988;50:25–70.

130. Balmain A, Harris CC. Carcinogenesis in mouse and human cells: parallels and paradoxes. Carcinogenesis 2000;21:371–7.

131. Harris CC. Chemical and physical carcinognesis: Advances and perspectives for the 1990s. Cancer Res 1991;51:5023–44s.

132. Boyland E, Levi AA. Metabolism of polycyclic compounds. I. Production of dihydroxydihydroanthracene from anthracene. Biochem J 1935;29:2679–83.

133. Hueper WC, Wiley FH, Wolfe HD. Experimental production of bladder tumors in dogs by administration of β-napthylamine. J Ind Hyg Toxicol 1938;20:46–84.

134. Sugimura T and Fujimora S. Tumor production in glandular stomach of rats by N-methyl-N′-nitro-N-nitrosoguanidine. Nature 1967;216:943–4.

135. Doll R, Hill AB. Smoking and carcinoma of the lung: preliminary report. Br Med J 1950;2:739–48.

136. Wynder EL, Graham EA. Tobacco smoking as a possible etiologic factor in bronchogenic carcinoma. JAMA 1950;143:329–36.

137. Conney AH, Miller EC, Miller JA. The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. Cancer Res 1956;16:450–9.

138. Magee PN, Barnes JM. The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. Br J Cancer 1956;10:114–22.

139. Omura T, Sato R. A new cytochrome in liver microsomes. J Biol Chem 1962;237:1375–6.

140. Berwald Y, Sachs L. *In vitro* cell transformation with chemical carcinogens. Nature 1963;200:1182–4.

141. Loeb LA, Gelboin HV. Stimulation of amino acid incorporation by nuclear RNA from normal and methylcholanthrene treated rats. Nature 1963;199:809–10.

142. Barnes JM, Butler WH. Carcinogenic activity of aflatoxin to rats. Nature 202; 1964;202:1016.

143. Sporn MB, Dingman CW, Phelps HL, Wogan GN. Aflatoxin B1: binding to DNA *in vitro* and alteration of RNA metabolism *in vivo*. Science 1966;151:1539–41.

144. Cleaver JE. Defective repair replication of DNA in xeroderma pigmentosum. Nature 1968;218:652–6.

145. Alpert ME, Hutt MS, Davidson CS. Hepatoma in Uganda. A study in georaphic pathology. Lancet 1968;1: 1265–7.

146. Harris CC, Genta VM, Frank AL, et al. Carcinogenic polynuclear hydrocarbons bind to macromolecules in cultured human bronchi. Nature 1974;252:68–9.

147. Shamberger RJ, Andreone TL, Willis CE. Antioxidants and cancer. IV. Initiating activity of malonaldehyde as a carcinogen. J Natl Cancer Inst 1974;53:1771–3.

148. Harris CC, Autrup H, Connor R, Barrett LA, McDowell EM, Trump BF. Interindividual variation in binding of benzo[a]pyrene to DNA in cultured human bronchi. Science 1976;194:1067–9.

149. Yang SK, McCourt DW, Roller PP, Gelboin HV. Enzymatic conversion of benzo[a]pyrene leading predominantly to the diol-epoxide r-7,t-8-dihydroxy-t-9, 10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene through a single enantiomer of r-7,t-8-dihydroxy-7,8-dihydrobenzo[a]pyrene. Proc Natl Acad Sci U S A 1976;73:2594–8.

150. Sugimura T, Nagao M, Kawachi T. Mutagen-carcinogens in food with special reference to highly mutagenic pyrolytic products in broiled foods. In: Hiatt HH, Watson JD, Winsten JA, editors. Origins of human cancer. New York: Cold Spring Harbor; 1977. p. 1561–77.

151. Hecht SS, Chen CB, Hirota N, Ornaf RM, Tso TC, Hoffmann D. Tobacco-specific nitrosamines: formation from nicotine *in vitro* and during tobacco curing and carcinogenicity in strain A mice. J Natl Cancer Inst 1978; 60:819–24.

152. Lane DP, Crawford LV. T antigen is bound to a host protein in SY40-transformed cells. Nature 1979; 278:261–3.

153. Linzer DI, Levine A. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcnoma cells. Cell 1979;17:43–52.

154. Perera FP, Poirier MC, Yuspa SH, et al. A pilot project in molecular cancer epidemiology: determination of benzo[a]pyrene-DNA adducts in animal and human tissues by immunoassays. Carcinogenesis 1982;3: 1405–10.

155. Shih C, Weinberg RA. Isolation of a transforming sequence from a human bladder carcinoma cell line. Cell 1982;29:161–9.

156. Pulciani S, Santos E, Lauver AV, Long LK, Robbins KC, Barbacid M. Oncogenes in human tumor cell lines: molecular cloning of a ransforming gene from hman bladder carcinoma cells. Proc Natl Acad Sci U S A 1982; 79:2845–9.

157. Shukumar S, Notario V, Martin-Zanca D, Barbacid M. Induction of mammary carcinomas in rats by nitroso-methylurea involves malignant activation of H-ras-1 locus by single point mutations. Nature (Lond) 1983;306.

158. Balmain A, Pragnell IB. Mouse skin carcinomas induced *in vivo* by chemical carcinogens have a transforming Harvey-ras oncogene. Nature 1983;303: 72–4.

159. Grollman A, Moriya M. Mutagenesis by 8-oxoguanine: an enemy within. Trends Genet 1993;9:246–9.

160. Knudson AG, Jr. Hereditary cancer, oncogenes and antioncogenes. Cancer Res 1985;45:1437–43.

161. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986; 323:643–6.

162. Wogan GN. Detection of DNA damage in studies on cancer etiology and prevention. IARC Sci Publ 1988;:32–51.

163. Loechler EL, Green CL, Essigmann JM. *In vivo* mutagenesis by O6-methylguanine built into a unique site in a viral genome. Proc Natl Acad Sci U S A 1984;81: 6271–5.

164. Basu AK, Loechler EL, Leadon SA, Essigmann JM. Genetic effects of thymine glycol: site-specific mutagenesis and molecular modeling studies. Proc Natl Acad Sci U S A 1989;86:7677–81.

165. Denissenko MF, Pao A, Tang MS, Pfeifer GP. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in p53. Science 1996; 274:430–2.

166. Esumi H, Tannenbaum SR. U.S.-Japan Cooperative Cancer Research Program: seminar on nitric oxide synthase and carcinogenesis. Cancer Res 1994; 54:297–301.

167. Carter EA, Derojas-Walker T, Tamir S, Tannenbaum SR, Yu YM, Tompkins RG. Nitric oxide production is intensely and persistently increased in tissue by thermal injury. Biochem J 1994;304:201–4.

168. Perucho M. Cancer of the microsatellite mutator phenotype. Biol Chem 1996;377:675–84.

169. Fishel R, Lescoe MK, Rao MRS, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993;75: 1027–38.

170. DeRisi J, Penland L, Brown PO, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 1996;14:457–60.

171. Masutani C, Araki M, Yamada A, et al. Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity. EMBO J 1999;18:3491–501.

172. Lander ES, Linton LM, Birren B, et al. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature 2001;409:860–921.

173. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. Science 2001;291: 1304–51.

174. Aaronson SA, Todaro GJ. Transformation and virus growth by murine sarcoma viruses in human cells. Nature 1970;225:458–9.

175. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol 2006;1:119–50.

176. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002;3: 415–28.

177. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002; 99:15524–9.

178. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. Nat Rev Cancer 2004;4:177–83.

179. Cerutti P. Prooxidant states and tumor promotion. Science 1985;227:375–81.