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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

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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.

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-methylycytosine (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 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 resulting 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). Alternatively, 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 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 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 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.

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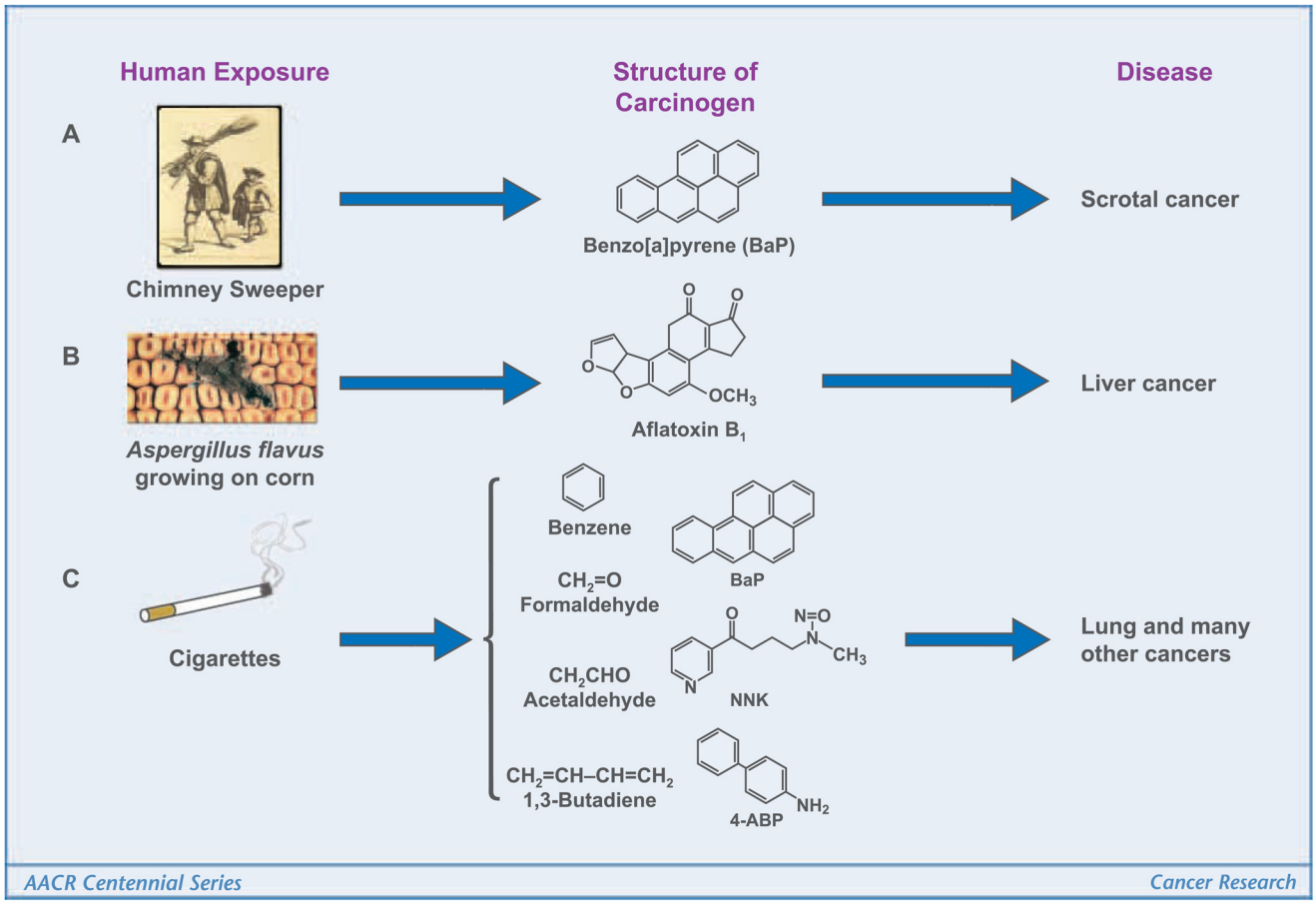

Figure 1.

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.

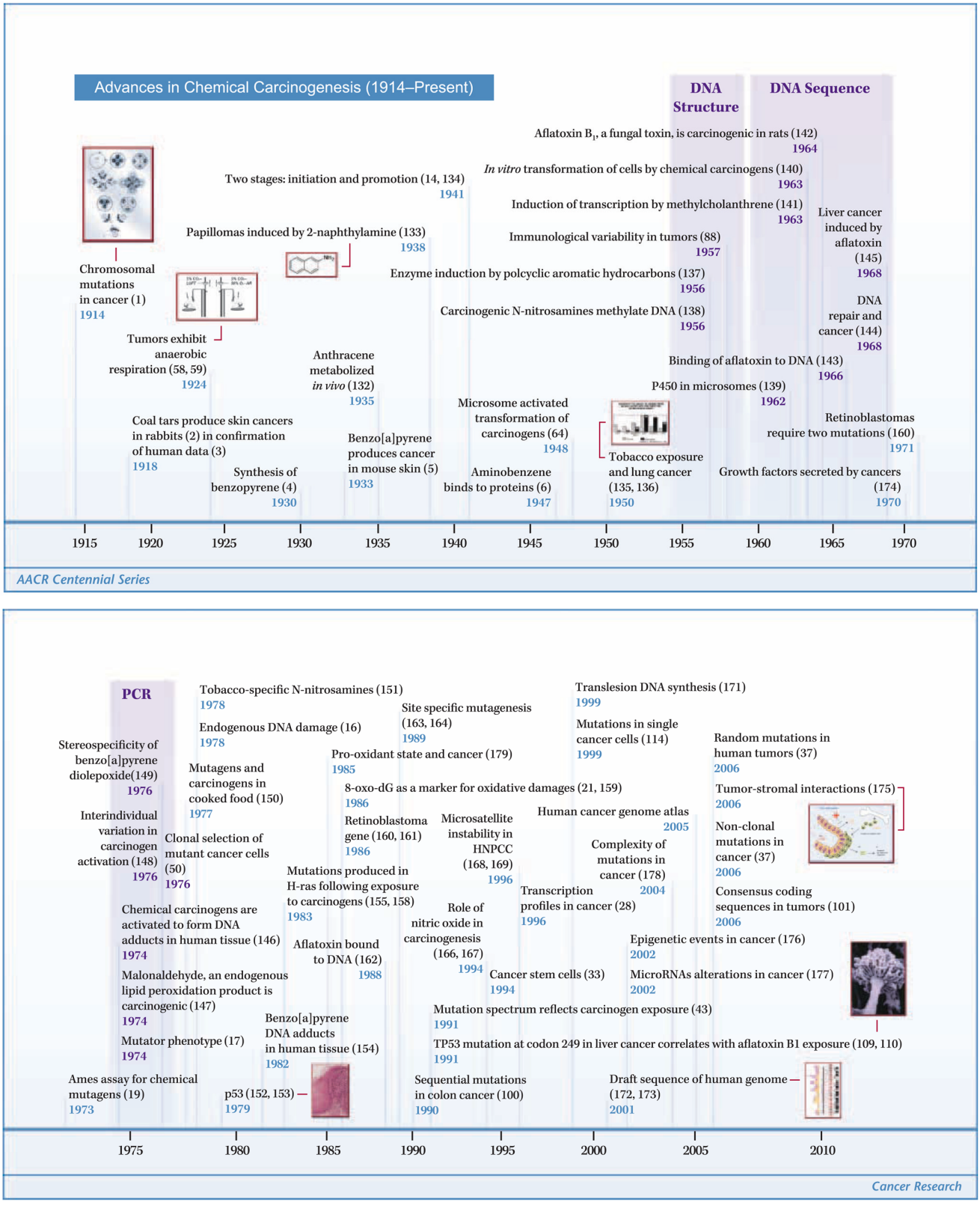

Figure 2.

An overview of primary examples of events that have generated important insights into carcinogenesis.

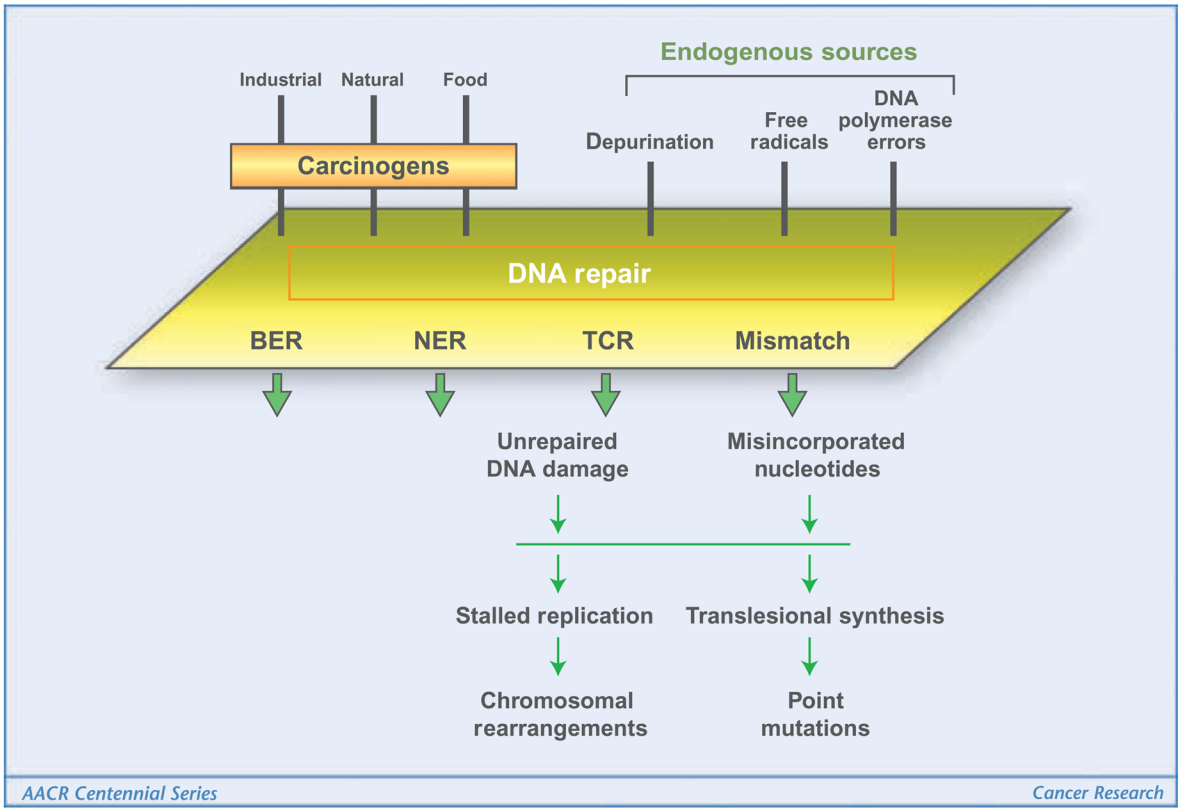

Figure 3.

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.

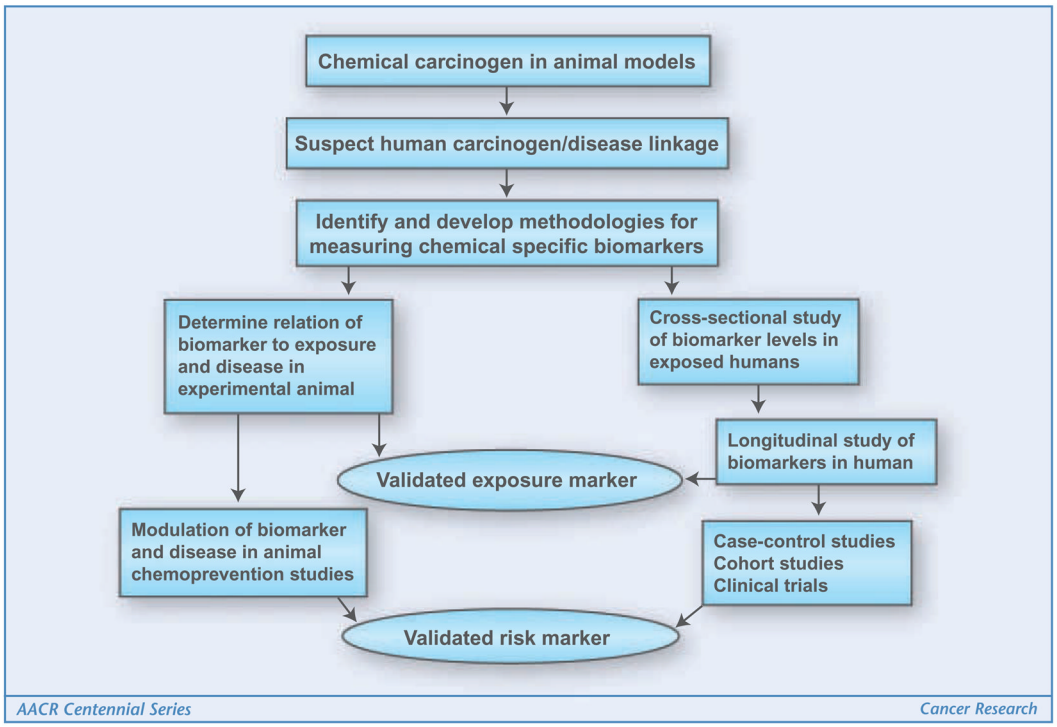

Figure 4.

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