Topic Introduction

Animal Models of Chemical Carcinogenesis: Driving Breakthroughs in Cancer Research for 100 Years

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The identification of carcinogens in the workplace, diet, and environment through chemical carcinogenesis studies in animals has directly contributed to a reduction of cancer burden in the human population. Reduced exposure to these carcinogens through lifestyle changes, government regulation, or change in industry practices has reduced cancer incidence in exposed populations. In addition to providing the first experimental evidence for cancer's relationship to chemical and radiation exposure, animal models of environmentally induced cancer have and will continue to provide important insight into the causes, mechanisms, and conceptual frameworks of cancer. More recently, combining chemical carcinogens with genetically engineered mouse models has emerged as an invaluable approach to study the complex interaction between genotype and environment that contributes to cancer development. In the future, animal models of environmentally induced cancer are likely to provide insight into areas such as the epigenetic basis of cancer, genetic modifiers of cancer susceptibility, the systems biology of cancer, inflammation and cancer, and cancer prevention.

INTRODUCTION

Any model is by definition an imperfect representation of that which it seeks to emulate. Regardless of how accurate any given cancer model is, its true value should be measured by its ability to guide research and, from a practical perspective, to protect human health. Mouse, rat, and other animal models of chemically induced cancer have been remarkably faithful in revealing underlying mechanisms of carcinogenesis and pinpointing both genetic and environmental factors that influence cancer susceptibility in the human population.

There are currently two major applications of animal models of chemical carcinogenesis. One is experimental and translational cancer research, where the objectives are to learn about the causes and mechanisms of cancer, as well as chemoprevention or early detection of cancer. The second, more practical application is to test chemicals with potential human exposure for carcinogenic activity in the so-called carcinogen bioassay. We focus here on mouse models of chemical and radiation carcinogenesis, with an emphasis on combining such models with genetically engineered mouse models (GEMMs). Highlights of selected chemical carcinogenesis models, including history, applications, protocols, and future directions, will be discussed. A brief description of the history and use of the carcinogen bioassay will also be presented.

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CANCER IS A DISEASE CAUSED BY BOTH GENETIC MUTATIONS AND ENVIRONMENTAL EXPOSURES

It is widely accepted that cancer is a “genetic disease” (Vogelstein and Kinzler 2004). Much support for this concept has come from the detection of mutated genes in tumors and the use of GEMMs that are designed to carry mutations in the orthologous genes that are mutated in human cancers. These mouse models, to varying degrees of accuracy, recapitulate the pathogenesis of human cancer and provide important experimental evidence for the genetic basis of cancer (Van Dyke and Jacks 2002; Becher and Holland 2006; Frese and Tuveson 2007). Current estimates, however, indicate that inherited genetic predisposition makes a relatively minor contribution to most cancers (Lichtenstein et al. 2000), implying that most cancer-related mutations are somatically acquired through spontaneous events or as a result of environmental exposure. Human cancer has a prominent and, in some cases, overwhelming environmental etiology, indicating that cancer is also an “environmental disease” (Doll and Peto 1981; IARC 1990). Environment is defined as anything people interact with, including exposure from lifestyle choices, natural and medical radiation, sunlight, workplace exposure, drugs, and substances in the air, water, and soil (OTA 1981; IOM 2001). Thus, although GEMMs mirror some of the genetic, biologic, and pathologic features of human cancer, they do not by themselves take into account the complexity of environmental exposures that contribute to cancer. Combining chemical and other environmental exposures with genetically defined mouse models provides a useful experimental setting in which to study the interaction between host genotype and environmental exposures that ultimately dictate cancer risk.

EARLY STUDIES AND THE USE OF ANIMALS TO IDENTIFY CARCINOGENS

More than 200 years ago, the first report linking environmental exposure and cancer was published by the English physician and surgeon Percivall Pott (Pott 1775). Dr. Pott noted that patients with scrotal skin cancer commonly reported employment as chimney sweeps in their youth. These young workers were chronically exposed to high levels of soot and tar and developed cancer after a long latency. The observation of tumor latency noted by Pott was highly prescient and predated the concept of multistage cancer. Over 100 years later, in 1895, Rehn reported observations of increased bladder cancers in workers exposed to aniline dyes (Rehn 1895). These and other seminal epidemiologic observations ultimately gave rise to the field of chemical carcinogenesis (Lawley 1994). It was not until 1915, with a paper first published in English in 1918, that a direct causal link between chemical exposure and cancer was established. Yamagiwa and Ichikawa (1918) reported that chronic application of coal tar to rabbit ears gave rise initially to benign lesions, some of which eventually developed into malignant epidermal tumors. This research was soon followed by similar studies in mice (Tsutsui 1918). In the 1930s, the first pure chemical compounds, such as polycyclic aromatic hydrocarbons (PAHs) benzo[a]pyrene, dibenz[a,h]anthracene, and 3-methylcholanthrene, were shown to induce tumors in mice, firmly establishing defined chemical entities as a cause of cancer (Cook et al. 1932; Kennaway 1955). PAHs consist of multiple fused benzene rings and are formed during incomplete combustion of organic matter. PAHs are a major component of cigarette smoke and air pollution particulates and, to this day, are widely distributed in the environment and found in certain foods. In part because of the widespread distribution of PAHs such as benzo[a]pyrene, there are few epidemiologic studies linking its exposure to human cancer, and animal models provide the main evidence of their carcinogenicity.

Following the reports of bladder cancer in workers in the dye industry, Sasaki and Yoshida (1935) succeeded in inducing liver cancer in rats after feeding them the azo dye *o*-amidoazotoluene. In 1936, Kinosita reported that 4-dimethyl-aminoazobenzene induced liver cancer (Kinosita 1936), and in 1941, bladder cancer and other cancers were found in rats exposed to 2-acetylaminofluorine (Weisberger and Weisburger 1958). Studies of azo dyes also provided the first evidence that some chemicals require metabolic activation by the host to cause cancer (Miller and Miller 1947). The

epidemiology of aromatic amines and cancer is largely the epidemiology of industry-related bladder cancer, and due in no small part to these early animal studies, the exposure of workers to these compounds has been greatly reduced. Aromatic amines are still used in industry during chemical synthesis and are found in cigarette smoke. The link between cigarette smoking and bladder cancer has been attributed to aromatic amines (Vineis and Pirastu 1997), indicating an ongoing threat from these compounds.

As additional animal studies were performed, evidence of links between cancer and environmental exposure to carcinogens continued to emerge. Between 1950 and 1975, ethyl carbamate (urethane) was used as a sedative, and large numbers of Japanese patients were exposed to this carcinogen. Urethane was shown to induce lung cancer in mice as early as 1943 (Nettleship et al. 1943), but decades passed before its deliberate use was stopped (Miller 1991). Urethane is found at low levels in many foods as a by-product of fermentation. Like many other carcinogens, urethane requires metabolic activation by the p450 system, in this case to vinyl carbamate epoxide, which can covalently bind to DNA to form mutagenic DNA adducts (Forkert 2010). The *N*-nitroso compounds represent another important class of chemical carcinogens. One of these, *N*-nitrosodimethylamine (DMN), was first shown to be carcinogenic in rats (Magee and Barnes 1956). Subsequently, many additional *N*-nitroso compounds were shown to cause cancer in animals, and nearly every species tested is susceptible to cancer induced by DMN or the related *N*-nitrosodiethylamine (DEN) (Magee and Barnes 1967). Nitrosamines are present in the environment and produced during digestion and may contribute broadly to cancer (Miller and Miller 1979). Other early animal studies establishing a causal link between environmental exposures and cancer include UV-radiation-induced skin cancer (Findlay 1928) and X-ray-induced lymphomas, ovarian cancer, and other tumors in mice (Furth and Furth 1936). Collectively, these landmark studies through the early to mid-20th century established unequivocal causal connections between chemical or radiation exposure and the subsequent development of cancer (Boyland 1969). Importantly, these studies contributed directly to a reduction in human exposure to many of these agents and a subsequent reduction in cancer risk through a variety of mechanisms, including lifestyle changes, government regulation, and alternative industry practices.

THE CARCINOGEN BIOASSAY: MICE AND RATS AS THE UNSUNG HEROES IN CHEMICAL SAFETY ASSESSMENT

Evidence of chemical carcinogenesis in animal models and epidemiologic studies, combined with the burgeoning development of the chemical industry and the resultant widespread environmental contamination and exposures, led directly to the initiation of animal testing programs by the National Cancer Institute (NCI) in the 1970s to identify potential carcinogens and to establish safe levels of exposure. The NCI Carcinogenesis Bioassay Program operated until formation of the National Toxicology Program (NTP), whose mission is to coordinate federal toxicology programs and to refine toxicology and carcinogenicity tests (National Toxicology Program 2011). Chemicals that are selected for study are subjected to a 2-yr bioassay involving both sexes of two species, usually inbred and outbred strains of mice or rats, and exposure at several predetermined doses (Fung et al. 1995). In addition, the NTP employs GEMMs on a case-by-case basis in short-term, more hypothesis-based cancer bioassays (Pritchard et al. 2003). Laboratory tests in animals are a major source of the evidence that is used to set regulatory standards for potential human exposure. This research is summarized in the *Handbook of Carcinogenic Potency and Genotoxicity Databases* (Gold and Zeiger 1997), which includes data from over 5000 experiments and 1298 chemical agents in over 1000 papers and 400 technical reports from the NCI/NTP.

Attesting to the value of carcinogen testing in animals, some 30 substances first shown to cause cancer in animals were subsequently linked to human cancer through epidemiologic studies; these substances include estrogen, formaldehyde, diethylstilbestrol, dichlorodiphenyltrichloroethane (DDT), vinyl chloride, 2,3,7,8-TCDD, radon gas, beryllium, asbestos, 4-aminobiphenyl, bis(chloro-

methylether, and 1,3 butadiene (Huff 1993; Rall 2000). Viewed another way, ~25% of the substances that are causally or strongly associated with human cancer were first identified as carcinogens in animals.

Accumulated evidence from the rodent bioassay permits some generalizations when comparing human with rodent cancer susceptibility. Cancer rates are low in humans and high in rodents in the liver (excepting hepatitis C-associated liver cancer), kidney, forestomach, and thyroid gland. Cancer rates are high in both species in the lung, mammary gland, the hematopoietic system, bladder, oral cavity, and skin. Cancer rates are high in humans and low in rodents in prostate gland, pancreas, colon/rectum, and cervix/uterus.

Extrapolating data from the rodent carcinogen bioassay to acceptable levels of human exposure involves consideration of the weight of evidence, mechanisms of action, threshold levels, and dose-response patterns of the test compounds. As part of this process, it is useful to classify chemical carcinogens into two broad categories: genotoxic and nongenotoxic. These categories may be further divided into eight subclasses: direct-acting carcinogens, procarcinogens, solid-state carcinogens, hormones, immunosuppressors, co-carcinogens, and promoters (Weisburger and Williams 1981). Much work remains to determine the mechanism of action for many of these compounds and to establish safe levels of exposure.

Collectively, the findings from the NCI/NTP bioassays have been used by federal and state regulatory agencies to reduce exposure to mutagenic carcinogens. Another outcome of these studies was the development and use of the Ames test and other short-term genotoxic assays widely used by regulatory agencies to screen chemicals before human exposure.

CHEMICAL CARCINOGENESIS IN EXPERIMENTAL CANCER RESEARCH

In addition to their role in identifying potential human carcinogens, chemical and radiation carcinogenesis studies have made seminal contributions to many cornerstone principles of experimental oncology, many predating the molecular biology era (Pitot 1983). For example, it was noted that many chemicals did not cause cancer at the site of exposure but were often active at distal sites, often in the liver. In 1947, the Millers noticed aromatic amine liver carcinogens bound to liver proteins (Miller and Miller 1947) and speculated that binding to such macromolecules was important for the carcinogenicity of these compounds. Following up on early skin tumor painting studies, Heidelberger showed that PAHs bound to the DNA of mouse skin, and Lawley established a correlation between carcinogenicity and DNA binding, implicating DNA as the relevant target (Brookes and Lawley 1964; Goshman and Heidelberger 1967). Because chemical carcinogens were covalently bound to DNA, these and other findings led to two important concepts: that many carcinogens are inactive in their native form and require metabolic activation to become active and carcinogenic, and that mutation of DNA may be a key event in their mechanism of action as carcinogens.

Berenblum and Shubik applied 7,12-dimethylbenz[*a*]anthracene (DMBA) and croton oil in sequence to induce skin tumors in mice and from their analysis concluded that carcinogenesis occurs through at least two discrete stages: initiation, an irreversible event mediated in this case by DMBA, and promotion, a reversible process involving chronic exposure to an irritant such as croton oil (Berenblum and Shubik 1947). The phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was later shown to be the active ingredient in croton oil (Verma and Boutwell 1980). The stages of carcinogenesis were subsequently expanded to include malignant progression, a process in which benign neoplasms convert to malignant, invasive lesions, sometimes with metastatic dissemination. It is now generally accepted that cancer arises as a multistep process, an idea that can be traced back to the writings of Dr. Pott (Pott 1775).

After the finding of carcinogens bound to DNA and the correlation between DNA binding and carcinogenicity (Brookes and Lawley 1964), it was not until the discovery of mutated oncogenes and tumor suppressor genes in both human tumors and chemically induced rodent tumors (Balmain and Pragnell 1983; Zarbl et al. 1985; Brown et al. 1990; Burns et al. 1991) that the link initially

discovered in 1915 between environmental exposures to carcinogens, mutations, and cancer was completed. Collectively, these findings helped to solidify the concepts of mutagens as carcinogens and the genetic basis of cancer. When viewed through the lens of Nowell's clonal evolution model of cancer development (Nowell 1976), it is easy to see the major impact of these concepts, even if unstated, on the design and interpretation of many oncology experiments even to this day. The study of chemical carcinogenesis models has contributed to any other fundamental concepts in cancer biology and genetics, and many of the first carcinogens to be identified are still widely used as experimental agents.

COMBINING CHEMICAL CARCINOGENESIS AND GENETICALLY ENGINEERED MODELS OF CANCER

Over the past two decades, GEMMs have been increasingly used to model cancer. These models are the subject of numerous articles and recent reviews (Van Dyke and Jacks 2002; Becher and Holland 2006; Frese and Tuveson 2007). Given that cancer is the product of complex interplay between environmental exposure and genetic susceptibility, the combined use of chemical carcinogenesis and genetically engineered models is a logical approach to unraveling these interactions. The combined effect of genetics with chemical exposure can easily be seen in the increased spectrum of tumors observed in some GEMMs after exposure to carcinogens or radiation. The tumor suppressor p53 provides one early illustrative example. p53 knockout mice were originally reported to be susceptible to spontaneous lymphomas and sarcomas but not epithelial tumors (Donehower et al. 1992; Jacks et al. 1994). This observation was curious, because p53 is frequently mutated in both human and mouse epithelial tumors. To address this conundrum, p53-deficient mice were subjected to the DMBA/TPA multistage skin tumor protocol. Interestingly, p53-deficient mice did not develop more skin tumors, but those tumors that did develop progressed much more rapidly to malignant, invasive, and metastatic carcinomas (Kemp et al. 1993). This pointed to a role for p53 in suppressing malignant progression, a conclusion that has been borne out in a number of subsequent studies (Jackson et al. 2005; Lewis et al. 2005). Thus, p53 plays a stage-specific role in epithelial cancer, and chemical exposure was required to reveal this phenotype. Because Ras mutations were found in skin tumors from both wild-type and p53-deficient mice, these experiments also illustrated cooperation between mutations in Ras and p53 during malignant progression.

p53-deficient mice are also susceptible to DMN-induced hemangiosarcomas (Harvey et al. 1993), ionizing radiation-induced lymphomas and sarcomas (Kemp et al. 1994), and UV-induced skin cancer (Ziegler et al. 1994; Jiang et al. 1999), and are sensitive to other chemicals and agents (Tennant et al. 1999). The susceptibility of mice deficient in Arf, a tumor suppressor that regulates p53, to DMBA/TPA-induced skin cancer progression (Kelly-Spratt et al. 2004), chemically induced hemangiosarcomas (Busch et al. 2012), and lung cancer (Busch et al. 2013) (see Protocol: **Induction of Lung Tumors in Mice with Urethane** [Gurley et al. 2014a]) collectively highlight the Arf/p53 signaling axis as an important barrier to chemical- and radiation-induced cancer.

Another notable example illustrating the value of combining chemical and radiation carcinogenesis with GEMMs involved the cyclin-dependent kinase inhibitor p27 (CDKN1B). While its role as a CDK inhibitor made p27 a candidate tumor suppressor, the initial paucity of mutations in *CDKN1B* in tumors cast doubts on its importance in human cancer (Philipp-Staheli et al. 2001). Further, p27 knockout mice displayed only modest susceptibility to spontaneous tumor formation, which was restricted to the pituitary gland (Fero et al. 1996; Kiyokawa et al. 1996). These findings were difficult to reconcile with the clear association of low levels of p27 protein expression with poor prognosis in breast, lung, prostate, colon, and other cancers (Chu et al. 2008). This puzzle was at least partially solved when p27-deficient mice were challenged with chemical carcinogens or ionizing radiation. Following exposure, both p27 null and heterozygous mice showed a marked susceptibility to cancer relative to wild-type littermates in a wide range of tissues, including the lung, small intestine, colon, prostate, and hematopoietic tissue (Fero et al. 1998; Di Cristofano et al. 2001; Philipp-Staheli et al.

2002; Kelly-Spratt et al. 2009). In addition to establishing a causal and pan-tissue role for p27 in tumor suppression, analysis of tumors from $p27^{+/-}$ mice showed no mutation or loss of heterozygosity in the remaining wild-type *Cdkn1b* allele, providing clear evidence of tumor suppressor gene haploinsufficiency. Simultaneously, Donehower reported that p53 also showed evidence of haploinsufficient tumor suppression (Venkatachalam et al. 1998). Before these landmark experiments, tumor suppressor genes were widely, if not universally, thought to function in a genetically recessive manner, requiring mutational hits on both alleles before cancers could develop. In contrast, these studies showed that loss of just a single allele was sufficient. Haploinsufficiency has now been described for many tumor-suppressor genes (Payne and Kemp 2005; Scuoppo et al. 2012) and may be the rule rather than the exception. Thus, combining chemical carcinogenesis and GEMMs was instrumental in modifying one of the central dogmas of cancer genetics. As a postscript, recent data from cancer genome sequencing projects have found that *CDKN1B* is one of the most significantly mutated genes in human breast cancer (Ellis et al. 2012), underscoring the predictive value of mouse models to identify tumor suppressor genes.

An important lesson from these and numerous other examples is that the spontaneous tumor spectra of GEMMs only tell part of the story, and appropriate environmental or dietary exposures should be routinely considered in the phenotypic analysis of GEMMs. The different spectra of tumors observed between mice and humans has been attributed to inherent differences between the species and has been used to argue against the value of mice as a model of human cancer (Anisimov et al. 2005). At least some of these differences, however, are almost certainly due to very different environmental exposures between laboratory mice and humans. Another potentially confounding difference when comparing mouse to human cancer susceptibility is that mouse model experiments are frequently performed on one or two inbred strain backgrounds, whereas the human population is genetically diverse. It is well established that genetic background affects cancer risk in both humans and mice (Balmain 2002; Dragani 2003).

CHEMICAL CARCINOGENESIS MODELS TO IDENTIFY GENETIC MODIFIERS OF CANCER RISK

Inbred strains differ widely in their susceptibility to spontaneous or chemically induced neoplasia in most if not all tissues, including the lung, liver (Drinkwater and Ginsler 1986; Dragani et al. 1995a), skin, and colon (Demant 2003). In most cases, treatment with tissue-specific chemical carcinogens to induce tumors is an essential strategy for the discovery, mapping, and eventual identification of genetic modifiers of cancer risk. For example, topical application of the carcinogen DMBA was used to develop the highly susceptible SENCAR mouse strain (Slaga 1986) and to map and characterize multiple skin cancer susceptibility loci (*Skts*) using both inbred strains and interspecies *Mus musculus* × *Mus spretus* crosses (Nagase et al. 1995; Angel and DiGiovanni 1999; Quigley et al. 2009). Urethane or *N*-ethyl-*N*-nitrosourea (ENU) treatment of A/J and other strains of mice was used to identify lung cancer susceptibility loci, including the *Pas*, *Par*, and *Sluc* alleles (Lynch 1926; Dragani et al. 1995b; Tripodis et al. 2001; Liu et al. 2006). Treatment of inbred strains with DEN, ENU, or urethane to induce liver tumors revealed dramatic differences in susceptibility to hepatocarcinogenesis, leading to the identification of *Hcs* and *Hcr* modifier alleles (Drinkwater and Ginsler 1986; Dragani et al. 1995a; Poole et al. 1996). The carcinogens 1,2-dimethylhydrazine (DMH) and azoxymethane (AOM) as well as the germline-susceptible strain *Apc^{Min}* were used to identify small intestine and colon cancer susceptibility alleles *Mom* and *Scs* (Dietrich et al. 1993; van Wezel et al. 1999; Bissahoyo et al. 2005). Going forward, in-depth analysis of these genetic modifiers will provide insight into *gene* × *gene* or epistatic interactions that predict cancer risk, novel mechanisms in cancer progression, and, by extrapolation, human cancer susceptibility.

In addition to revealing mechanisms of *genotype* × *environment* interaction and identifying genes that modulate environmentally induced cancer, the enhanced susceptibility of some GEMMs to

chemical agents is valuable to regulatory agencies. For example, transgenic Ras and p53 heterozygous knockout mice are routinely used by the NTP in the carcinogen bioassay, thus reducing time, saving money, and allowing increased throughput to test additional potential human carcinogens (Tennant et al. 1996, 1999; Ashby 2001). Because of the broad susceptibility of p27-deficient mice to epithelial cancer, it has been argued that p27-deficient mice would also be useful for the carcinogen bioassay (Payne and Kemp 2003).

COMMON MODELS OF CHEMICAL CARCINOGENESIS

Probably the most extensively researched animal model of cancer is the multistage chemical carcinogenesis model of mouse skin (see Abel et al. [2009] for detailed protocols). In addition to having an unbroken experimental history that goes back for the better part of a century, this model has a number of experimental advantages, including simplicity, the ability to observe and quantify the number and growth rate of tumors, the ability to study initiation, promotion, progression, and metastasis in sequence, and the ability to study the genes associated with these stages. One of the more useful attributes of the DMBA/TPA skin cancer protocol is that the induced tumors harbor mutations in the oncogene Ras and the tumor suppressor p53 with remarkable consistency. Since Quintanilla and Balmain discovered mutations in *Hras* in chemically induced skin tumors 30 years ago (Balmain and Pragnell 1983; Quintanilla et al. 1986), studies in many laboratories have confirmed that DMBA/TPA-induced tumors almost uniformly contain the identical A>T mutation in codon 61 of *Hras*. This remarkable consistency lends itself to the progressive accumulation of knowledge about genetic, biologic, and environmental modifiers of oncogenic Ras (Kemp 2005). Dozens, if not hundreds, of different inbred mouse strains and GEMMs have been subjected to the DMBA/TPA protocol, providing a rich repository of knowledge on the genetics and biology of multistage cancer. Virtually any general feature of cancer biology can be or has been explored with this model, including inflammation, angiogenesis, epigenetics, and chemoprevention, to name just a few. Recent examples include dissection of the role of Arf, p53, and EMT in metastasis (Ruddell et al. 2008; Tsai et al. 2012) and the tracing of cell lineage to determine the clonal origin of cancer (Driessens et al. 2012).

Table 1 highlights selected examples of tissue-specific (liver, lung, breast, colon) or pan-tissue models of chemical carcinogenesis that have been widely used in regulatory settings and experimental cancer research. Further details are provided in Protocol: **Induction of Lung Tumors in Mice with Urethane** (Gurley et al. 2014a), Protocol: **Induction of Liver Tumors in Mice with *N*-Ethyl-*N*-Nitrosourea or *N*-Nitrosodiethylamine** (Gurley et al. 2014b), and Protocol: **Induction of Colon Cancer in Mice with 1,2-Dimethylhydrazine** (Gurley et al. 2014c).

In summary, the ease of applying chemical carcinogens to any strain background or GEMM facilitates the study of *gene* $\times$ *gene* and *gene* $\times$ *environment* interactions and is valuable for revealing

TABLE 1. Common models of chemical carcinogenesis

Tissue	Agent	Route
Pan tissue	Ionizing radiation	Whole-body exposure
Skin	ENU	i.p. injection
	DMBA	Topical application
	B [a]P	Topical application
	UV	Topical exposure
Liver	DEN	i.p. injection
	ENU	i.p. injection
	Phenobarbital	Drinking water
Lung	Urethane	i.p. injection
	NNK	i.p. injection
Breast	DMBA	Oral gavage
Colon	AOM, DMH	i.p. injection

ENU, *N*-ethyl-*N*-nitrosourea; DMBA, 7,12-dimethylbenz[*a*]anthracene; DEN, *N*-nitrosodiethylamine; NNK, nicotine-derived nitrosamine ketone; AOM, azoxymethane; DMH, 1-2-dimethylhydrazine; i.p., intraperitoneal.

latent tumor predisposition of GEMMs. The versatility and widespread use of animal models of chemical carcinogenesis, which began in the middle part of the last century, engenders progressive accumulation of insight into the genetics and biology of cancer.

THE FUTURE OF CHEMICAL CARCINOGENESIS

Animal models of chemical carcinogenesis have made a major contribution toward establishing the precept that the most effective way to prevent cancer is to reduce exposure to mutagenic carcinogens. However, many important chemicals and agents that are carcinogenic are not obviously mutagenic, including hormones, phenobarbital, asbestos, some metals, and chlorinated hydrocarbons such as DDT and polychlorinated biphenyls (PCBs). Application of these agents to selected GEMMs that carry mutations in genes that influence the response to these agents may help unravel additional mechanisms of carcinogenesis. It can reasonably be anticipated that some of these mechanisms will involve epigenetic events. Abundant epidemiologic and molecular data point to epigenetic mechanisms as an important component in the link between early environmental exposure and later risk of diseases such as cancer (Jirtle and Skinner 2007); examples of carcinogens that induce epigenetic changes include nickel, arsenic, and diethylstilbestrol (Anderson 2004; Herceg 2007). Novel approaches that combine environmental exposures with GEMMs are needed to determine both the environmental causes of epigenetic alterations and the mechanistic role of these alterations in cancer development. Clonal analysis of cancer has confirmed that tumors are genetically and phenotypically heterogeneous (Driessens et al. 2012; Greaves and Maley 2012). This heterogeneity likely contributes to treatment failure, and modeling this heterogeneity in mice is a significant challenge. Because models of chemical carcinogenesis recapitulate important aspects of the etiology of human cancer, and chemically induced tumors are often highly heterogeneous, these models may serve as a useful venue to study tumor heterogeneity and the implications for tumor response to therapy. Animal models of cancer that combine both genetic and environmental elements will continue to play a critical role in the quest to reduce the burden of cancer on the human population.

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