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Preclinical Animal Models for the Development of Cancer Chemoprevention Drugs

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1. INTRODUCTION

Preclinical cell culture and animal efficacy testing models are currently used to identify, assess, and prioritize chemical agents and natural products with the aim of preventing human cancer. If little is known about a potential agent, the first step is a sequential series of short-term in vitro prescreens of mechanistic, biochemical assays. These assays provide quantitative data to help establish an early indication of chemopreventive efficacy and to assist in prioritizing agents for further evaluation in longer-term in vitro transformation bioassays and whole animal models. Promising chemical agents or combinations of agents that work through different inhibitory mechanisms are subsequently tested in well-established chemically induced or spontaneous animal tumor/cancer models, which typically include models of the colon, lung, bladder, mammary, prostate, and skin. These animal bioassays afford a strategic framework for

evaluating agents according to defined criteria, and not only provide evidence of agent efficacy, but also serve to generate valuable dose-response, toxicity, and pharmacokinetic data required prior to Phase I clinical safety testing. Based on preclinical efficacy and toxicity screening studies, only the most successful agents considered to have potential as human chemopreventives will progress into clinical chemoprevention trials.

Six key elements are necessary for the ideal animal model for chemoprevention testing: the animal model should bear relevance to human cancers, not only in terms of specific organ sites but also in producing cancerous lesions of similar pathology; the genetic abnormalities of these lesions should resemble those found in humans; the model should have relevant intermediate lesions that simulate or approximate the human cancer process both histologically and molecularly; the model should be capable of producing a consistent tumor burden of greater than 80% lesions within a reasonable period of time (less

Table 1
Animal Models in Current Use for the Screening and Development of Chemopreventive Agents

<i>Organ site</i>	<i>Species</i>	<i>Carcinogen^a</i>	<i>Endpoint measured</i>
Mammary gland	Rat	MNU	Adenocarcinomas
	Rat	DMBA	Adenocarcinomas and adenomas
Lung	Hamster	DEN	Adenocarcinomas
	Hamster	MNU	Squamous cell carcinomas
	Rat	NNK	Adenomas and squamous cell carcinomas
	Mouse	B[a]P, NNK, vinyl carbamate, DEN, uracil mustard, urethane, cigarette smoke	Adenomas and adenocarcinomas
Colon	Rat	AOM, IQ, PhIP	Aberrant crypts and adenocarcinomas
Prostate	Rat	MNU	Adenocarcinomas
Bladder	Mouse	OH-BBN	Transitional cell carcinomas
	Rat	OH-BBN	Transitional cell carcinomas
Skin	Mouse	DMBA or UV	Papillomas and squamous cell carcinomas
Ovary	Rat	DMBA	Epithelial and thecal cell carcinomas
	Rat	BOP	Thecal cell carcinomas
Esophagus	Rat	NMBA	Squamous cell carcinomas
Head and neck	Rat	4NQO	Squamous cell carcinomas
	Hamster	DMBA	Squamous cell carcinomas
Pancreas	Hamster	BOP	Ductal carcinomas

^aAbbreviations: MNU, methylnitrosourea; DMBA, dimethylbenz[*a*]anthracene; DEN, diethylnitrosamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; B[a]P, benzo[*a*]pyrene; AOM, azoxymethane; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; OH-BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; UV, ultraviolet light; BOP, *N*-nitrobis-(2-oxopropyl)amine; NMBA, *N*-nitroso-methylbenzylamine; 4-NQO, 4-nitroquinoline-1-oxide.

than 6 mo); the carcinogen or genetic defect used to produce cancer should bear relevance to that encountered by humans; and the predictive values and accuracy of the animal model for human efficacy should be >80% (i.e., agents positive in animal tests are positive in clinical trials and agents negative in animals are negative in clinical trials). While it is generally understood that no current animal model is ideal, research and development of better animal models is ongoing in many laboratories in an increasing variety of organ sites. In this chapter, a review of currently used animal models for chemoprevention efficacy testing will be presented (Table 1).

2. MAMMARY CANCER MODELS

A growing number of animal mammary cancer prevention models are used routinely or currently being developed. Both the 7,12-dimethylbenz[*a*]anthracene

(DMBA)- and methylnitrosourea (MNU)-induced mammary gland carcinogenesis models are routinely used for screening. The 50-d-old rat MNU-induced cancer model is popular because it typically produces 100% incidence of adenocarcinomas within 120–150 d of carcinogen treatment (1). MNU does not require metabolic activation, and therefore cannot detect agents that alter carcinogen metabolism. In this model, 50-d-old Sprague-Dawley female rats are given a single iv injection of 50 mg MNU/kg-bw (pH 5.0). The chemopreventive agent is usually started 1 wk prior to carcinogen treatment and continued until the animals are sacrificed. Tumor multiplicity is typically 4 to 6 per animal and the latency is usually 65–80 d. Agents that are positive in this model are then usually tested in older animals, where the carcinogen is administered to 100–120-d-old animals (2). Mammary glands of older

rats are more similar in terms of proliferation rates and differentiation to those of mature women. Incidence and multiplicity of cancers are lower in this case and must be compensated by having more animals per group and experimental times lengthened to 180 d. The cancers produced are hormone-dependent and are usually associated with activated *ras*. This model correctly identified the human cancer-preventive agents tamoxifen and *N*-(4-hydroxy)phenylretinamide (3–5). It should be stated that this model is highly sensitive to weight loss and decreased weight gain over the course of the experiment. For example, acute reductions of body weight gain of 6, 12, or 15% at the time of MNU treatment resulted in decreased mammary cancer multiplicities of 15, 44, and 55% respectively without chemopreventive agent administration (6,7).

The second mammary model commonly used is the DMBA model. Again, 50-d-old rats are given 12 mg of carcinogen ig and tumors arise within 120 d of carcinogen treatment (8,9). These tumors are usually encapsulated adenocarcinomas, adenomas, and fibroadenomas that arise in approx 80–100% of the animals. DMBA is a polycyclic aromatic hydrocarbon and requires activation by the cytochrome P450 enzyme system. Therefore this model can detect agents that modulate the P450 system or detoxify carcinogens via a phase 2 enzyme system (e.g., glutathione-*S*-transferases). Tumor multiplicity is usually in the range of 3–4 per animal, and latency is similar to the MNU model at 65 to 80 d.

Recently, newer transgenic models have become more prevalent. These are covered in a separate chapter by Lubet et al., Chapter 3 in this volume.

3. LUNG CANCER MODELS

Two hamster models, the diethylnitrosamine (DEN) lung and the MNU tracheal model, have been used to evaluate the efficacy of potential chemopreventive agents in inhibiting lung cancer. The DEN model induces lung adenocarcinomas following twice-weekly sc injections of 17.8 mg DEN/kg-bw starting at 7–8 wk of age and continuing for 20 wk (10). This treatment usually produces 90–100% tracheal tumors and 40–50% lung tumors in treated male Syrian golden hamsters. Serial sacrifice studies have shown that these lung tumors arise from pulmonary Clara and endocrine cells, while tracheal tumors arise from the basal cells of the trachea. Chemopreventive agents are usually administered in diet starting 1 wk prior to the first carcinogen treatment and continuing for 180 d.

The primary endpoint is percent reduction of lung tumor incidence. The pathology of these lung tumors resembles small-cell lung cancer with neuroendocrine features. In the MNU hamster tracheal model, 5% MNU in saline is administered once a week for 15 wk by a specially designed catheter that exposes a defined area of the trachea of male Syrian golden hamsters to the carcinogen (11). The chemopreventive agent is supplied in the diet, or more recently by aerosol, for 180 d beginning 1 wk prior to the first carcinogen exposure. Within this time period, 40–50% of the animals acquire tracheal squamous cell carcinomas and chemopreventive efficacy is measured as a reduction in that percentage.

Another lung cancer chemoprevention model uses the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) to induce lung tumors in rats (12). For this model, male F344 rats are given NNK (1.5 mg/kg-bw) by sc injection three times a week for 21 wk. The assay is terminated at week 98 post-carcinogen exposure; tumor incidence is determined by dividing the number of animals with cancers by the total number of animals treated. Since the tumors are very large, tumor multiplicity is not determined. The majority of animals develop lung adenomas, with fewer adenocarcinomas and occasionally a squamous cell carcinoma. In addition to lung tumors, NNK also induces nasal cavity tumors.

For the past several years, the mouse lung adenoma model has been more frequently used because it is very efficient, consistent, and reliable. Several carcinogens can cause lung adenoma, including benzo[*a*]pyrene (B[*a*]P), NNK, vinyl carbamate, DEN, uracil mustard, and urethane. In the B[*a*]P model, female Strain A/J mice at 15 wk of age are given either a single i.p. dose of 100 mg B[*a*]P/kg-bw or three ig gavages of 2 mg B[*a*]P in 0.2 mL vegetable oil with 3–4 d between dosings. The animals are then held for about 16 wk for development of pulmonary adenomas. Typically, 8–10 adenomas arise per animal with 100% incidence. In this model, the chemopreventive agent can be given either in the diet or by aerosol administration. Aerosol administration has major advantages over diet for agents with known toxicity to gastrointestinal organs and poor metabolic profiles (i.e., they are rapidly metabolized and excreted). For example, striking results have been observed by administering budesonide, a glucocorticoid, by aerosol for very short periods of time (13,14). With most carcinogens (B[*a*]P, NNK, etc.) a small percentage (<10%) of adenomas eventually become carcinomas after a period of 1 yr or more.

The protocol for the NNK Strain A/J mouse model calls for female mice 6 wk of age (15) to be given a single dose of 10µm NNK in saline by i.p. injection. Typically 6–8 adenomas per animal develop within the 16-wk bioassay with 100% incidence. At 52 wk, adenocarcinoma incidence is about 70–80% with a multiplicity of 15–17 tumors (mostly solid alveolar adenomas plus a few adenocarcinomas) per animal. In this model, *N*-acetyl-*L*-cysteine and β-carotene had no effect on cancer incidence or multiplicity resulting from exposing respiratory epithelium to a tobacco smoke carcinogen, a result that positively correlates with that found in human studies (16).

Vinyl carbamate was given to 8–9-wk-old Strain A mice by a single i.p. injection of 60mg/kg-bw in 0.2 mL saline. At 24 wk, there are typically 20–30 lung tumors per animal and about 12% are carcinomas; at 1 yr there are about 30% carcinomas (17). This model, with its high tumor multiplicity and capability to produce significant frequencies of carcinomas, is attractive for lung cancer prevention studies.

Recently, tobacco smoke has been used to induce lung adenomas in the Strain A mouse model (18). This animal model is important because it mimics the cancer induction process in humans by a complex mixture of chemical carcinogens and promoting agents. Strain A mice are exposed to cigarette smoke by inhalation of tobacco smoke for 5 mo followed by a 4-mo smoke-free recovery period. Both benign and malignant lung tumors are produced by this model. The tumor incidence of control animals is about 30%, while the tumor incidence in smoke-exposed animals is about 80% (19).

4. COLON CANCER MODELS

The azoxymethane (AOM)-induced aberrant rat colon crypt model has become a primary whole-animal screening assay for potential chemopreventive agents due to its short time course, low cost, and requirement for only a small amount of test agent. Aberrant colon crypts are single and multiple colonic crypts containing cells exhibiting dysplasia (20–23). The aberrant crypts are induced in 8-wk-old F344 rats by two injections of 15 mg AOM/kg-bw 1 wk apart. Protocols A and B are used. Under Protocol A, animals are fed the test agent diet from 1 wk before the first AOM injection to 3 wk after the first injection, for a total of 4 wk. Protocol B was designed to test the effects of the chemopreventive agent on the post-initiation phase of colon carcinogenesis. Rats receive the chemopreventive agent from 4 to 8

wk after the first AOM exposure. The AOM rat colon tumor model treats the animals similarly initially, but holds these animals on the chemopreventive agent diet until about 40 wk post-carcinogen, when cancer incidence is approx 70% and multiplicity is 1–2 tumors per animal. Both benign and malignant tumors are found at sacrifice. In the mouse model, methoxyacetyl-methane acetate, the ultimate carcinogenic metabolite of AOM, is given by ip injection (20 mg/kg-bw) once a week for 4 wk. Colon tumors appear within 38 wk after dosing (24). Celecoxib, a COX-2-specific inhibitor recently approved to prevent polyps in humans, was positive in the AOM rat colon tumor model using both early and late interventions (25). Other carcinogens used in primarily rat models are 2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (26). Other animal models for colon cancer are discussed in Chapter 4 in this book by Lipkin et al.

5. PROSTATE CANCER MODELS

The MNU Bosland model, named after its developer, is a rat model that develops a high incidence of dorsolateral prostate cancer (27). Male Wistar-Unilever rats are treated with 50 mg of cyproterone acetate at 8–9 wk of age, then receive daily injections of 100 mg testosterone propionate/kg-bw for 3 d. Sixty hours after the first testosterone dose, rats receive a single iv injection of MNU. Two weeks later, each rat is implanted sc with two silastic tubes containing 40 mg of crystalline testosterone. These tubes are replaced after 6 mo. The rats are sacrificed at 13 mo after the MNU injection, and the prostates are examined histologically for microscopic and macroscopic tumors in each area of the prostate and associated tissues. Typically 60–80% of the rats develop carcinomas in the dorsolateral prostate within the 13-mo time frame. It is possible to define the site of origin for small lesions (hyperplasia, carcinoma *in situ*, and small carcinomas). However, for large lesions it is often impossible to determine where the tumor originated. In this model, adenocarcinomas are malignant but rarely metastasize to distant sites.

The Lobund rat model has been used by many investigators to study prostate cancer and its prevention (28). Similarly to the experimental protocol above, MNU is injected once i.v.; one wk later, testosterone pellets are implanted onto the animals' backs. The animals are sacrificed 427 d later when 25% have tumors of the accessory sex organs. Recently, however, histopathological

analysis of the tumors found that cancers of the dorso-lateral prostate are the least frequent and at later stages are overgrown by cancers from other glands, such as the seminal vesicle (29).

6. BLADDER CANCER MODELS

The *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (OH-BBN)-induced mouse and rat bladder cancer models have been in use for many years with inherent advantages and disadvantages. In mice, bladder tumors induced by OH-BBN typically are transitional cell carcinomas, morphologically similar to those found in human bladder cancer (30). These cancers are highly invasive and aggressive in nature. Intragastric administration of 7.5 mg OH-BBN over an 8-wk period to 50-d-old male DBF mice (C57BL/6 X DBS/2F₁) typically results in a 40–60% tumor incidence at 6–8 mo post-carcinogen with an average multiplicity of 0.5–0.7 tumors/mouse. A twice-weekly carcinogen treatment for 8 wk to female F344 rats results in similar transitional cell carcinomas at 8 mo, but the tumors are more papillary and slowly growing. In the rat, incidence of premalignant lesions (hyperplasias and papillomas) is near 100%, and cancer incidence is roughly 60%. Nonsteroidal antiinflammatory drugs (NSAIDs) are profoundly effective in inhibiting bladder cancer in these animal models (31); recently the COX-2 inhibitor celecoxib caused a greater than 90% reduction in bladder cancers in both mice and rats (32). The drug development process for the chemopreventive agents 2-difluoromethylornithine (DFMO) and oltipraz, using animal models for bladder cancer inhibition, has been reviewed (33,34).

7. SKIN CANCER MODELS

Compounds effective in preventing skin carcinogenesis have typically been identified in the classical two-stage DMBA-12-*O*-tetradecanoylphorbol-13-acetate (TPA) mouse skin cancer model (35,36). Both CD-1 and SENCAR mice are highly susceptible to skin tumor induction by a single DMBA dose and multiple doses of TPA applied topically over a 20-wk period. Skin papillomas appear as early as 6 wk post-carcinogen treatment, eventually progressing to squamous cell carcinomas by 18 wk (37). Other carcinogens, most notably B[a]P, have also been used in this model to induce skin cancers (38). More recently, a UV mouse skin model is gaining in use for testing chemopreventive agents due to its high relevance to the etiology of human skin cancer. Skh hairless mice are given multiple exposures to UV

irradiation over a 24-wk period and develop skin lesions in approx 30 wk. Chemopreventive test agents are either administered in the diet or applied topically to the skin. Using this protocol, 100% of the mice develop skin tumors by 34–36 wk and have tumor multiplicities of about four tumors per animal (39). A number of NSAIDs and green tea polyphenols have proven effective in this model (40,41).

8. OVARIAN CANCER MODELS

There is no established animal model for ovarian cancer chemoprevention studies. A potential model employs surgical implantation of thread soaked in DMBA into ovaries of Wistar-Furth rats at 7–8 wk of age (42). Sterile silk thread immersed in melted DMBA allows about 200 µg DMBA to be adsorbed to the thread, which is then passed twice through the left ovary of the rats. This model appears promising due to finding that about half of the cancers are epithelial in nature, while the other half are granulosa/thecal tumors. The frequency of tumors is near 80% at 300 d post-carcinogen exposure. More than half of the cancers are poorly differentiated adenocarcinomas and the balance are mainly thecal/granulosa cell tumors (Dr. Keith Crist, Medical College of Ohio, unpublished results).

A second model currently under development also involves a carcinogen, *N*-nitrobis-(2-oxopropyl)amine (BOP), administered to Lewis rats (modified from 43,44). At 8 and 14 d of age, the female rats are injected sc with 0.8 mg of BOP, and at 45 d of age the animals are put onto diets containing chemopreventive agents. The study typically is terminated when the animals are about 8 mo old. The cancers produced are predominately granulosa/thecal tumors (Dr. Clinton Grubbs, University of Alabama, Birmingham, unpublished results). However, only about 10% of all human ovarian cancers are granulosa/thecal in nature.

9. ESOPHAGUS CANCER MODELS

Esophageal cancers can be induced in rats by the administration of *N*-nitroso-*N*-methylbenzylamine (NMBA). Studies conducted in China indicate that *N*-nitro compounds and their precursors are possible etiological factors in human esophageal cancers (45). In this model, male F344 rats are given NMBA (0.5 mg/kg-bw) by sc injection three times a week for 5 wk (46). The use of this model for chemoprevention studies has recently been reviewed by leaders in its development (47). The chemopreventive agents are given either in diet or in drinking water for the full 25 wk of the experiment.

Then the animals are sacrificed and the esophagi removed and cut longitudinally, fixed in neutral buffered formalin, and examined under a dissecting microscope. This model is one of the first to use computerized image morphometry to validate the modulation of very early changes only recognized by the computer against the inhibition of the cancer endpoint (48).

More recently, an esophageal anastomoses model has been developed that parallels acid reflux disease in human Barrett's esophagus (49,50).

10. HEAD AND NECK CANCER MODELS

Tumors of the head and neck are relatively common epithelial tumors in humans. Typically, tumors of this origin are associated with exposure to tobacco smoke. Cancer induction in rat tongue by 4-nitroquinoline-1-oxide (4NQO) has become a model for chemoprevention studies of head and neck cancer (51,52). Oral lesions produced in rats by 4NQO are similar to human lesions since many are ulcerated and endophytic tongue lesions (53). At 5 wk of age, rats begin exposure to 4NQO in their drinking water (20 ppm) and continue for a period of 10 wk. Chemopreventive agent administration, usually in the diet, begins 2 wk after the end of 4NQO treatment and continues for an additional 22 wk. Oral tissues are histologically examined for evidence of hyperplasia, dysplasia, and cancer. There are reports that these cancers can be prevented by garlic (54).

The DMBA-induced Syrian hamster cheek pouch cancer model is another widely accepted model of oral cancer (55,56). Carcinogenesis protocols with DMBA induce premalignant changes and squamous cell carcinomas that closely resemble human lesions (57,58). Cheek pouches of 6-wk-old noninbred Syrian hamsters are exposed topically to 0.5% DMBA in mineral oil three times a week for 14 wk. The hamster cheek pouch is an anatomically easily accessible pocket that can be readily everted for local carcinogen/preventive agent treatment and macroscopic follow-up. An advantageous and unique feature of this cheek pouch model is the possibility of timed-sequence follow-up of the evolution of intraepithelial neoplasia after DMBA treatment by simply everting the cheek pouch and visually monitoring or biopsying tissue for measurement of the endpoints. Macroscopic/molecular biochemical endpoints can eventually be correlated with histopathological studies of biopsy samples. Thus the cheek pouch tissue is amenable to using a variety of sampling techniques.

11. PANCREAS CANCER MODEL

Human pancreatic cancer has an extremely low 1-yr survival rate and current therapies are usually ineffective. One animal model being used to screen agents with potential cancer preventive activity is the hamster BOP model (59,60). Pancreatic tumors induced in Syrian hamsters resemble human pancreatic cancer in many aspects. In response to BOP, these rodents develop pancreatic ductal (ductular) carcinomas. In this model, male Syrian golden hamsters are injected sc with BOP in normal saline three times at weekly intervals. The test chemopreventive agents are administered in the diet beginning 2 wk after the last BOP exposure. Typically, a 48-wk period following the last carcinogen exposure is needed to develop sufficient tumors in the pancreas. The pancreases are histologically sectioned and scored for hyperplasias, dysplasias, and cancers.

12. CONCLUSIONS

Preclinical animal models have been used extensively in efficacy testing of potential chemopreventive agents (61–64). Standardized statistical methodology has been proposed to evaluate data from most of these animal model experiments based on the various endpoints (65). Clearly, there is much room for improving current animal models to reflect the etiology and progression of the human cancer process. There is also a need to develop animal models for testing cancer preventive agents in other organs, including brain, kidney, cervix, and lymphatic cancers. Validation of animal models for predicting efficacy of agents in human clinical trials will await further human data on positive and negative chemopreventive agents. To date, accuracy has been remarkably high, with positive correlations for tamoxifen and 4-HPR for breast cancer and aspirin and celecoxib for colon cancer, and negative correlation for β -carotene for lung cancer.

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