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The Use of Animal Models for Cancer Chemoprevention Drug Development

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Abstract

Animal models currently are used to assess the efficacy of potential chemopreventive agents, including synthetic chemicals, chemical agents obtained from natural products and natural product mixtures. The observations made in these models as well as other data are then used to prioritize agents to determine which are qualified to progress to clinical chemoprevention trials. Organ specific animal models are employed to determine which agents or classes of agents are likely to be the most effective at nontoxic doses to prevent organ-specific forms of cancer. These results are then used to target specific organs in high risk populations in clinical trials. The animal models used are either carcinogen-induced with carcinogens specific for particular organ sites or they are transgenic/mutant animals with insertions, deletions, or mutations at targeted gene sites known to enhance cancers in a specific organ. Animal tumor models with characteristics favorable to chemoprevention studies are available for lung, colon, skin, bladder, mammary, prostate, head and neck, esophagus, ovary and pancreas. In addition to single agent dose-response testing, such models are frequently used for testing combinations of agents, testing different routes of administration, evaluating surrogate endpoint biomarkers, and generating initial pharmacokinetics and toxicology data. For some of the more standard animal models there is significant correlation with human chemopreventive trial results. There are a growing number of positive human chemoprevention trials which used agents or combinations which were positive in animal testing. The number of negative human clinical trials have been fewer, but again correlating with negative animal results. Clearly the validation of animal models to predict the efficacy of agents in human clinical trials will await further human data on positive and negative outcomes with chemopreventive agents. Whether validated or not, animal efficacy data remain central to the clinical trial decision-making process.

Keywords

Animal Models; Chemoprevention; Transgenic mice; Mutant mice

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INTRODUCTION

Animal models are currently utilized to assess the efficacy of and prioritize synthetic chemicals, chemical agents obtained from natural products and potentially natural product mixtures which may progress to clinical chemoprevention trials. The intention is to employ organ specific animal models to determine which agents are likely to be helpful in preventing specific forms of cancer. Such animal models are either well-established chemically-induced, spontaneous, or transgenic animal tumor/cancer models and typically include organ-based models for the prevention of colon, lung, bladder, mammary, prostate, pancreas, and skin. These animal bioassays afford a strategic framework for evaluating agents according to defined criteria, typically to a tumor endpoint which is the primary endpoint in most Phase III clinical prevention trials. In addition to providing evidence of agent efficacy, animal data may help 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 efficacious and least toxic agents considered to have greatest potential as human chemopreventives will progress into clinical chemoprevention trials. In the chapter to follow we have focused on models and studies which have been or are being used by the Chemoprevention Agent Development Research Group within the Division of Cancer Prevention of NCI. Even with this caveat we have not attempted to include all studies which have been performed or included all of the animal models which we have examined. In addition we have not made any attempt to summarize the great number of excellent models used by numerous researchers in the area of cancer prevention studies.

There are six key elements necessary for the ideal animal model for chemoprevention testing: (a) the animal model should bear relevance to human cancers, not only in terms of specific organ sites but also with respect to its ability to produce cancerous lesions of similar pathology; (b) the genetic abnormalities of these lesions should be similar to those found in humans (this is relatively straight-forward when a given kind of cancer is driven by a single or a few mutations, e.g., colon/intestine [APC/ β -catenin], pancreatic cancer [*KRAS* mutation] and squamous cell skin cancer [p53 mutations at sites of dipyrimidine dimers]; but this is less obvious where there are no clear driving mutations.); (c) genomic changes similar to human are preferred. Thus, in both breast and colon there have been studies clearly showing the overlap in genomic expression between animal models and specific forms of breast and colon cancer; (d) the model should have relevant intermediate lesions which simulate or approximate the human cancer process both histologically and molecularly; (e) the model should be capable of producing a consistent tumor burden in greater than 60% of animals developing the endpoint (typically cancerous lesions) within a reasonable period of time (less than 6 months); and (f) the predictive value of the animal model for human efficacy data should be high (i.e., agents positive in animal tests are positive in clinical trials and agents negative in animals should be negative in clinical trials). This is a bit problematic in prevention research where a limited number of definitive human trials have been completed. 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 article a review of currently used animal models for chemoprevention efficacy testing will be presented (Tables 1 and 2).

MAMMARY CANCER MODELS

The primary animal model used routinely for screening potential mammary cancer prevention agents is the methylNitrosourea (MNU)-induced mammary gland carcinogenesis model which develop ER+ cancers. This rat model develops a high incidence (>85% of rats induced) and multiplicity (typically 3–5 tumors/animal) of adenocarcinomas within 120–150 days of carcinogen treatment.¹ MNU is a direct-acting carcinogen and does not require metabolic activation, and therefore it is unaffected by preventive agents that work by altering carcinogen

metabolism. In this model 50 day-old Sprague-Dawley female rats are given a single intravenous (i.v.) injection of 50 mg MNU/kg body weight (pH 5.0). The chemopreventive agent is usually started five days after the carcinogen treatment and continued until the animals are sacrificed. The resulting tumors are similar to well-differentiated ER+ human breast adenocarcinomas with respect to both histology and gene expression. They are susceptible to many of the hormonal manipulations that can modulate human ER+ cancers, including selective estrogen receptor modulators (SERMs), aromatase inhibitors, ovariectomy, and pregnancy.² More than 15 years ago our group in the Division of Cancer Prevention reported a series of studies showing the striking preventive efficacy of aromatase inhibitors in this animal model.^{3–5} These results agree with the recent clinical data showing efficacy of aromatase inhibitors in blocking development of contralateral breast cancers in the adjuvant setting, suggesting that these agents are also likely to be effective in preventing first primary ER+ breast cancers in postmenopausal women.⁶ In addition to sensitivity to hormonal agents this model has proved to be applicable to testing agents that do not directly affect the hormonal axis. The model has been highly sensitive to various RXR agonists, EGFR inhibitors (gefitinib, erlotinib [Tarceva®], and lapatinib), and farnesyl transferase inhibitors. Interestingly, EGFR inhibitors have been highly effective in treatment of human ER+ mammary tumors. Erlotinib doses are presently being optimized in order to overcome the rash associated with this class of agents; this should encourage its use in prevention studies. This model correctly identified the human cancer preventive agents tamoxifen, raloxifene, aromatase inhibitors, and N-(4-hydroxy)phenylretinamide.^{7–9}

Another ER+ mammary model used less frequently is the dimethylbenz[a]anthracene (DMBA) model. Again, 50 day old rats are given 12 mg of carcinogen intragastrically and tumors arise within 120 days of carcinogen treatment.^{10,11} These tumors are adenocarcinomas, adenomas and fibroadenomas in approximately 80–100% of the animals. DMBA is a polycyclic hydrocarbon and requires activation by the cytochrome P450 enzyme system. Therefore, this model can detect agents which modulate the P450 system or detoxify carcinogens via phase 1/2 enzymes (e.g., glutathione-S-transferases).

Recently, in light of the clinical success in preventing ER+ breast cancers, the focus of *in vivo* screening in breast cancer models has been shifted to identify agents useful against hormonally nonresponsive breast cancer, *i.e.*, ER– cancers. Two major subtypes of human ER– breast cancer are: (1) basal-like, which is frequently found in *BRCA1* mutation-associated cancers; and (2) Her2-amplified, which corresponds to Neu-amplified (Neu+/p53altered) breast cancer in the mouse.¹² These two types have significantly different cells of origin, etiologic origins, and gene expression patterns, and different responses to therapies. For the basal-like and *BRCA1* type tumors, there are two effective animal models. One is a relatively complex p53 knockout mouse model which also has *BRCA1* knocked out in the breast tissue.¹³ In agreement with studies of human *BRCA1* mutation-carrying patients, early ovariectomy reduced the formation of ER– cancers in this mouse model. RXR agonists are now being tested in this model. Most importantly and directly paralleling the human experience, these tumors are susceptible to the effects of the poly ADP ribose polymerase (PARP) inhibitors.¹⁴ A second model for the basal-like ER– subtype involves the induction of tumors by SV40 T-antigen in C3(1) mice.¹⁵ Although the T-antigen is not directly relevant to human breast cancer, gene array analysis by Perou and coworkers has shown significant similarities between these mouse tumors and human ER– tumors with a basal pattern.¹⁵ Studies have also been recently initiated with a third mouse model of the second human ER– subtype, the ER–, Her2+ subtype, in which the mouse tissue overexpresses Neu (mouse Her2) in the presence of an altered p53). The tumors produced in this model are quite similar to human ER– Her2-amplified tumors. Confirming the results of others, our investigators have found that both EGFR 1/2 inhibitors (lapatinib) and EGFR 1 inhibitors (erlotinib and gefitinib) are effective in preventing mammary cancers. The result with lapatinib is expected since EGFR 2, one of its targets, is highly

amplified. The result with EGFR 1 inhibitors is more surprising, given the absence of EGFR 1 overexpression in this model. Nevertheless, the efficacy of the EGFR 1 inhibitors may reflect the fact that EGFR 1 and 2 form heterodimers; thus, the inhibition of EGFR 1 prevents formation of the heterodimer, which is the active entity. These findings are particularly important, since combining an EGFR inhibitor and a hormonal agent such as a SERM or aromatase inhibitor has potential to inhibit 75–80% of breast cancers. In addition, the model has proven to be susceptible to both RXR agonists and metformin while not responding to agents such as resveratrol or atorvastatin. Finally, treatment of FVB mice with medroxyprogesterone acetate (MPA) followed by dimethyl-benzanthrene (DMBA) results in the formation of ER– tumors. Using these various models a variety of potentially useful nonhormonal agent classes have been identified or confirmed to have anti-cancer properties. These include: EGFR inhibitors; farnesyltransferase inhibitors (FTIs); various RXR agonists, such as the RXR agonist UAB 30, which, unlike others, does not increase triglycerides; histone deacetylase inhibitors; and peroxisome proliferator-activated receptor (PPAR) γ agonists.¹⁶ The limited efficacy of statins, resveratrol, tea polyphenols, and various NSAIDs in this model has also been demonstrated.¹⁷ In fact, the results with statins were given a press release by the *Cancer Prevention Journal* and were reported by more than 1000 media outlets (newspapers, magazines, television, and radio).

Although tumors defined by histopathology are the primary endpoints for these assays, we have found that altered proliferation and apoptosis in lesions can be used as endpoints to identify highly effective agents following short-term exposure.^{18–20} This approach parallels presurgical studies in humans showing that altered proliferation can be used as an indicator of efficacy.²¹ This presurgical method in humans demonstrated the preventive efficacy of SERMS and aromatase inhibitors in humans. Additionally, anatomic (magnetic resonance imaging [MRI]) and functional imaging (positron emission imaging [PET]) are being explored to define effective agents, although functional or MRI imaging is likely to require the existence of clearly defined lesions. It is expected that these alternate endpoints will prove applicable for screening agents and will be translatable to human chemoprevention phase 2 trials.

LUNG CANCER MODELS

The Mouse Lung Adenoma Model in A/J mice has been used frequently because it is very efficient, consistent and reliable. Various carcinogens can cause lung adenomas (most with *KRAS* mutations) in this model, including benzo[a]pyrene (B[a]P), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), vinyl carbamate, diethylnitrosamine, uracil mustard and urethane. In the B[a]P model female mice of the A/J strain at 5–6 weeks of age are given either a single intraperitoneal (i.p.) dose of 100 mg B[a]P/kg body weight or three intragastric gavages of 2 mg B[a]P in 0.2 ml vegetable oil with 3–4 days between dosings. The animals are then held for about 16 weeks to allow the development of pulmonary adenomas. Typically 8–10 adenomas arise per animal with 100% incidence, i.e. 100% of treated animals develop tumors. In this model, the chemopreventive agents can be given in the diet, by gavage 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.^{22,23} In fact, pulmonary tumors were nearly 90% inhibited with glucocorticoids such as budesonide delivered by aerosol for only 1 minute per day for six days per week for 16 weeks. These promising results led to a clinical trial with aerosolized budesonide. With most carcinogens (B[a]P, NNK, etc.) a small percentage (<10%) of adenomas eventually become carcinomas after a period of a year or more; however, with vinyl carbamate a large percentage of tumors become adenocarcinomas. To increase the relevance of this model to human lung cancer, alterations of the major tumor suppressor genes p53 and/or p16 have been incorporated. The resulting models develop

adenocarcinomas with much greater frequency, exhibit more chromosome amplifications and deletions, and more closely resemble human adenocarcinomas by gene array analysis. RXR agonists, tea catechins, mTOR inhibitors, glucocorticoids, and Antitumor B (a mixture of Chinese herbs) all show efficacy in these models in terms of suppressing adenocarcinoma development. These agents are still somewhat effective even when initiated when small adenomas already exist.^{24–28} This delayed intervention design appears to be more relevant to proposed phase 2 and 3 clinical trials in current and former smokers. Work has also been initiated with a model of lung carcinogenesis involving a *GPCR5A* gene knockout mouse. This gene encodes a retinoic acid inducible protein that appears to be a G-protein coupled receptor. Unlike other lung models, the resulting adenocarcinomas do not have *KRAS* mutations.

There are two models for squamous cell carcinoma of the lung. The first is an MNU hamster tracheal model which is used to detect agents to prevent squamous cell cancers. In this model 5% MNU in saline is administered once a week for 15 weeks by a specially designed catheter that exposes a defined area of the trachea of male Syrian Golden hamsters to the carcinogen.²⁹ The chemopreventive agent is supplied in the diet, or more recently by aerosol, for 180 days, beginning one week prior to the first carcinogen exposure. Forty to 50% of the animals acquire tracheal squamous cell carcinomas within this time period, and chemopreventive efficacy is measured as a reduction in that percentage. Aerosolized agents have been used, and difluoromethylornithine (DFMO) and 5-fluorouracil showed some efficacy in this model.³⁰ A second model was recently developed in an existing mouse model of squamous cell lung cancer by induction with the carcinogen *N*-nitroso-tris-chloroethylurea (NTCU); this new model carries an altered p53 gene.³¹ Tests in this model have demonstrated the efficacy of Antitumor B, tea polyphenols, and PPAR γ agonists, but showed minimal activity of glucocorticoids in inhibiting lung carcinogenesis. Current clinical trials with both Antitumor B and tea polyphenols are using a bronchial dysplasia endpoint. The tumors in most of these studies have been scored by histopathologic analysis. Development of squamous cell lung cancer models is particularly important since these are the cancers presently being evaluated in most phase 2 clinical chemoprevention trials.

Another lung cancer chemoprevention model uses the tobacco-specific carcinogen NNK to induce lung tumors in rats.³² For this model male F344 rats are given NNK (1.5 mg/kg body weight) by subcutaneous (s.c.) injection three times a week for 21 weeks. The assay is terminated at week 98 post-carcinogen exposure and the tumor incidence is determined by dividing the number of animals with cancers by the total number of animals treated. Since the tumors are so large, the tumor multiplicity cannot be 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. The protocol for the NNK strain A/J mouse model uses female mice 6 weeks of age.³³ The mice are given a single dose of 10 μ M NNK in saline by i.p. injection. Typically 6–8 adenomas per animal develop within the 16 week bioassay period, with 100% incidence; most of the published data derive from this 16–20 week period. By 52 weeks the adenocarcinoma incidence is about 70–80%. Although the multiplicity is about 15–17 tumors per animal, only one of these lesions is typically an adenocarcinoma, with the rest being solid alveolar adenomas. In this model *N*-acetyl-l-cysteine and beta carotene had no effect on cancer incidence or multiplicity resulting from exposure of respiratory epithelium to a tobacco smoke carcinogen, a result which positively correlates with that found in human studies.³⁴

Vinyl carbamate in 0.2 ml saline was given to 8–9 week old strain A mice by a single i.p. injection of 60 mg/kg body weight. At 24 weeks there are typically 20–30 lung tumors per animal and about 12% are carcinomas and at one year about 30% of lesions are carcinomas.³⁵ This model, with its high multiplicity and capability of producing carcinomas in a large proportion of injected mice, is attractive for lung cancer prevention studies.

Over the past 5–8 years major efforts have been undertaken to induce lung tumors with cigarette smoke in wild type A/J mice, A/J mice with a dominant-negative p53 mutation, and Swiss albino mice. The appeal of such an approach, although it is expensive, is that it employs the “relevant” carcinogen. This is obviously necessary in order to study any agent that has potential to inhibit smoke-induced initiation. Recently tobacco smoke has been used by DeFlora and coworkers³⁶ to induce lung adenomas in Swiss albino mice. This animal model is important since it mimics the cancer induction process in humans by a complex mixture of chemical carcinogens and promoting agents. Newborn Swiss albino mice are exposed to cigarette smoke by inhalation for 120 days starting 12 hr after birth. Both benign and malignant lung tumors are produced by this model. The tumor incidence of control animals is 0%, while in smoke-exposed animals lung tumors begin developing at about 75 days of age, and the incidence increases to about 80% after 181–230 days. The mean lung multiplicities are between 6.1 and 13.6 tumors per mouse. Recently it was shown that N-acetyl-cysteine, budesonide and phenethyl isothiocyanate have cancer-preventing effects in this model.³⁷

A transgenic model developed by Berns and co-workers³⁸ appears to produce lung cancers similar to human small cell lung cancer. The model involves conditional knockout of p53 and Rb. Recent studies have shown that the RXR agonist bexarotene (Targretin[®]) and Polyphenon E are effective agents in this model (unpublished results).

COLON/INTESTINAL CANCER MODELS

Most preclinical chemopreventive screening and efficacy studies of gastrointestinal cancers in the NCI’s Chemopreventive Agent Development Research Group (CADRG)’s program have focused on colon carcinogenesis, have used rats induced with the carcinogen azoxymethane (AOM), and have measured either adenomas and adenocarcinomas^{39,40} or early preinvasive lesions^{41,42} as primary efficacy endpoints. The aberrant crypt foci (ACF) assay, because it requires relatively few animals and takes 6–8 weeks, is used as an initial screen. Previous data using the AOM model strongly supported the development of the ACF assay and exploration of its utility as an early biomarker of carcinogenesis in phase 2 trials of subjects at risk for colorectal cancer. A wide variety of agents (*e.g.*, NSAIDs, NO-NSAIDs, COX-2 inhibitors, statins, ornithine decarboxylase inhibitors, and p53 modulating agents) have proven effective in preventing AOM-induced cancers, even when administered in the presence of small colonic lesions. Although our initial studies in the adenocarcinoma model employed early initiation of treatment (around the time of AOM administration), we subsequently found that initiating treatment after ACF formation still exhibits high efficacy in reducing development of invasive cancers. This “delayed efficacy” has also been observed in clinical studies of colon adenoma prevention and in other animal models (*e.g.*, the OH-BBN [N-butyl-N-(4-hydroxybutyl) nitrosamine]-induced mouse bladder). NSAIDs, including COX-2 selective agents, and most recently NO-NSAIDs, have been the most consistently effective agents in this model. Results in this model and in Min/+ mice^{43,44} were cited in approval of celecoxib for use in patients with familial adenomatous polyposis (FAP) and in the rationale for initiating the two phase 3 studies in which celecoxib was shown to reduce sporadic colon adenomas with high efficacy^{45,46}. High-dose, but not low-dose, aspirin was also highly active in preventing colon tumors,⁴⁷ a result that has been confirmed in humans.^{48,49} The striking efficacy of COX inhibitors *in vivo* and the general relevance of COX inhibition in multiple organs (esophagus, leukoplakia, bladder, and skin) encourage further efforts in this area despite the cardiac toxicity associated with higher doses of celecoxib. Given the striking efficacy of the COX1/2 inhibitors we have continued to look for agents which are effective but may incur less cardiotoxicity. The primary candidates are naproxen, low dose aspirin, low dose celecoxib, and the NO-releasing NSAIDs, such as NO-aspirin. Recently published data show that both naproxen and NO-naproxen are highly effective in preventing colon and bladder cancer.⁵⁰ Because of the extensive preclinical prevention studies in the colon and the finding that both NSAIDs and

DFMO are relatively effective in existing animal models, lower doses of these agents in combination were also examined. The objective was to determine whether low dose combinations might avoid toxicities associated with these agents: NSAIDs (gastric) and DFMO (ototoxicity). We found that numerous NSAIDs/COX 2 inhibitors [piroxicam, aspirin, celecoxib] plus DFMO were more effective in combination than lower doses of either agent given alone. These data supported implementation of the study of the strikingly effective combination of sulindac and DFMO in a clinical adenoma prevention trial demonstrating almost a 70% decrease in overall adenomas and >80% decrease in advanced adenomas.

Three genetically engineered models of intestinal cancer that mimic germline mutations predisposing subjects to colorectal cancer have been developed for prevention screening. The first two mouse models (Min/+ and APC 1638 mice), both containing germline mutations in the APC gene, similar to patients with FAP, develop multiple intestinal lesions. Agents that prevent adenomas and adenocarcinomas in the AOM-induced rat model, also reduce polyp formation in these mice.^{43,51–53} As noted above, positive results in Min/+ mice contributed to the scientific rationale for evaluating celecoxib in FAP patients. The third model is the MSH2 mismatch repair-deficient mouse that carries a conditional homozygous deletion of the *MSH2* gene; the deletion is confined to the colon. These mice are generated using the *loxP*/CRE system, in which an *MSH2* gene flanked by two recombinase-sensitive lox sites is combined with a CRE recombinase gene under control of the colon-specific villin promoter. This model corresponds to human hereditary non-polyposis coli (HNPCC) and is presently being employed to evaluate a series of NO-releasing NSAIDs and their parental counterparts.

BLADDER CANCER MODELS

The primary models used to evaluate prevention of bladder cancer are OH-BBN-induced rats and mice.⁵⁴ The resulting tumors are invasive and have histology similar to bladder transitional cell carcinoma (TCC) in humans. The model has been characterized both by mutation analysis and gene array procedures. Gene expression changes similar to those in human bladder tumors, including alterations in expression of FHIT, survivin, Ki67, annexin II, cyclins and cyclin kinases, and the various S100 calcium binding proteins were found.⁵⁵ Furthermore comparison of gene array expression between human bladder cancer and the OH-BBN tumors showed that tumors from the model were similar to invasive human bladder cancer. Striking efficacy has been observed in this model for NSAIDs (*e.g.*, indomethacin, naproxen, NO-naproxen, celecoxib), various EGFR inhibitors, and tea polyphenols,^{50,54–56} and NO-releasing NSAIDs are currently being tested. Both celecoxib and Polyphenon E have progressed to clinical trials. As noted above for colon tumors, high efficacy is observed when intervention with agents is initiated after preinvasive or even microscopically invasive lesions already exist, suggesting that these agents affect later stages of carcinogenesis. In fact, naproxen, NO-naproxen,⁵⁰ and gefitinib (Iressa®) have recently been shown to inhibit the development of large palpable tumors more effectively than the development of microscopic adenocarcinomas, implying that these agents preferentially inhibit tumor progression compared to their effect on the growth of early lesions. Currently, two newer p53-driven models are being evaluated for the efficacy of p53-rescue compounds. These models include the Ha-*ras*-activated p53^{+/-} and the uroplakin II-SV40 large T transgenic mice.

PROSTATE CANCER MODELS

Unlike breast, colon, skin and lung cancer, for which clear models exist, the development of prostate cancer models has been more difficult. Also, in contrast to colon, pancreas and skin, where the driving mutations are clearly defined, or breast, where tumors in animal models have been compared to subtypes of human breast cancers in terms of genomics, models of prostate cancer pose greater difficulties. Most human prostate cancer will not progress to kill the

individual, and the existence of one or two driving mutations is not obvious. One prostate cancer prevention model, the Bosland model, named after its developer,⁵⁷ uses MNU/ testosterone-treated rats. These treated animals develop a high incidence of primarily microscopic cancers in the dorsolateral prostate. This model of hormonally driven prostate carcinogenesis has proven useful in detecting the potential chemopreventive activity of agents such as anti-androgens, retinoids and prasterone (dehydroepiandrosterone, or DHEA) and its analog fluasterone.^{58,59} However, the cancers have a long latency period, the model is expensive and requires substantial amounts of test agent, and the resulting tumors are microscopic. Two mouse prostate models driven by SV-40 T antigen have been explored for identifying prostate chemopreventive agents. The transgenic adenocarcinoma of the mouse prostate (TRAMP) model employs a probasin promoter; a second model uses C3(1)/T-antigen to target the prostate.⁶⁰ One characteristic of these models is that the tumors grow fairly rapidly, unlike most human prostate tumors. Nevertheless, other investigators have found that a number of agents that show cancer preventive activity in human prostate are effective in the TRAMP model (e.g., tea polyphenols and the SERM, toremifene). Loss of the phosphatase and tensin homologue (PTEN) tumor suppressor gene is seen early in human prostate cancer, so mouse models with PTEN alterations are also being evaluated to determine their applicability to screening.⁶¹ Most recently it appears that a model employing a knockout of PTEN, combined with a translocation of the transcriptional activator *ETS*-related gene, *ERG*, onto an androgen responsive promoter, may be a particularly appealing prostate model to pursue.

SKIN CANCER MODELS

Compounds effective in preventing skin carcinogenesis have typically been identified in the classical two-stage DMBA-TPA (12-*O*-tetradecanoylphorbol 13-acetate) mouse skin cancer model.^{62,63} Both CD-1 and the SENCAR mice are highly susceptible to skin tumor induction by a single DMBA dose and multiple doses of TPA applied topically over a 20 week period. Skin papillomas appear as early as 6 weeks after the DMBA treatment, eventually progressing to squamous cell carcinomas by 18 weeks.⁶⁴ Other carcinogens, most notably benzo[a]pyrene, have also been used in this model to induce skin cancers.⁶⁵ Approximately 10 years ago the CADRG started to use a UV-induced mouse skin cancer model to test chemopreventive agents; this model is extremely relevant to the etiology of human skin cancer. Thus, the resulting tumors are induced by UV light, as are human squamous cell skin cancers, and the driving mutations are p53 mutations that arise at the site of thymidine dimers. SKH-1 hairless mice are given multiple exposures to UV irradiation over a 24 week period and develop skin lesions approximately 30 weeks later. Test chemopreventive agents are administered either in the diet or applied topically to the skin. Using the protocol above, 100% of the mice developed skin tumors by 34–36 weeks and had a tumor multiplicity of about 4 tumors per animal.⁶⁶ A number of NSAIDs and green tea polyphenols have proven effective in this model.^{67,68} Thus, NSAIDs (e.g., nimesulide, piroxicam, indomethacin, and celecoxib), DFMO, and tea polyphenols were effective in preventing skin tumors, primarily squamous cell carcinomas, in UV-exposed SKH-1 hairless mice.^{69,70} Most importantly, a clinical trial in humans has shown that celecoxib (which was identified as active in this model) inhibits the development of both squamous and basal cancers by more than 50%. In another skin cancer model, mice with a *PTCH* gene knocked out are highly susceptible to UV-induced basal cell carcinomas. These mice have been shown to respond to a number of agents, including NSAIDs, RAR receptor agonists, CP31398 (a p53 stabilizer) and cyclopamine.^{71,72} The retinoid receptor agonists and COX-2 inhibitors are presently in clinical trials.

OVARY CANCER MODELS

There is no established animal model for ovarian cancer chemoprevention studies. A potential model employs a thread soaked in DMBA that is surgically implanted into the ovary of a rat.

⁷³ This model employs Wistar-Furth rats at 7–8 weeks of age. Sterile silk thread is immersed in melted DMBA which allows about 200 ug/thread to be adsorbed. The thread is then passed twice through the left ovary of the rats. In this model about one half of the cancers are epithelial in nature while the other half are granulosa-theca tumors. Nearly 80% of DMBA-exposed mice develop ovarian tumors at 300 days post carcinogen exposure. The NSAID piroxicam is partially effective in this model. In contrast, neither celecoxib nor bexarotene is effective. A recently reported *BRCA1* model⁷⁴ will also be explored since *BRCA1* mutation carriers provide one of the few clearly defined populations with a high ovarian cancer risk. Ovarian cancer is rarely observed in most species, with the notable exception of the domestic hen, which like humans, spontaneously develops ovarian cancer. This is a promising new addition to ovarian cancer models where cancer presumably arises due to incessant ovulation and where progestins have been effective inhibitors of ovarian cancer.⁷⁵ Furthermore, ovarian cancer in the hen appears to be p53 driven; hence, the inhibitory effect of specific p53 rescue compounds is currently being evaluated.

ESOPHAGUS CANCER MODELS

Esophageal cancers can be induced in F344 rats by the repeated administration of *N*-nitroso-*N*-methylbenzylamine (NMBA). This model results in the formation of squamous cell cancer, while the most common human esophageal cancer is adenocarcinoma. Also this model is one of the first to use computerized spectral analysis to detect very early changes in the cancer process.⁷⁶ Studies using 4-NQO (4-nitroquinoline-1-oxide) in the drinking water are currently under way to evaluate the effect of p53 rescue compounds in this p53-driven model of oral-esophageal carcinogenesis.

More recently, an esophageal anastomosis (rat surgical) model has been developed that mimics acid reflux disease in human Barrett's esophagus, and leads to esophageal adenocarcinomas.^{77,78} As in other gastrointestinal organs, COX-2 appears to be a significant target in these esophagus models; NSAIDs, COX-2 selective agents, and lipoxygenase inhibitors are effective. This potential role of COX-2 inhibition agrees with the finding that persons at high risk of developing esophageal adenocarcinomas who take NSAIDs exhibit a strong reduction in the progression of their premalignant esophageal tissue to adenocarcinoma.⁷⁹ Currently, a proton-pump inhibitor and a combined COX/LOX inhibitor are being tested in this model. The NSAID sulindac was effective in a transgenic model of esophageal cancer.⁸⁰

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. The induction of cancer in rat tongue by 4-NQO has become a model for chemoprevention studies of head and neck cancer.^{81,82} Oral lesions produced in rats by 4-NQO resemble human lesions in that many are ulcerated and appear as endophytic abnormalities of the tongue.⁸³ The rats at 5 weeks of age begin exposure to 4NQO in their drinking water (20 ppm) and continue for a period of ten weeks. Chemopreventive agent administration is usually in the diet and begins two weeks after the end of 4NQO treatment and continues for an additional 22 weeks. The oral tissues are examined for histological evidence of hyperplasia, dysplasia, and cancer. Recent reports indicate that these cancers can be prevented by celecoxib and piroxicam, but not zileuton.⁸⁴

Additional data suggest strong inhibition by EGFR inhibitors and moderate efficacy with the PPAR γ agonist, rosiglitazone, and the histone deacetylase (HDAC) inhibitor, suberoylanilide hydroxamic acid (SAHA). Both a PPAR γ agonist, pioglitazone, and the EGFR inhibitor, Tarceva, have progressed to human trials.

PANCREATIC CANCER MODEL

As mentioned above, pancreatic cancer, like human colon cancer, is driven by a signature mutation, in this case in the *KRAS* gene. The *N*-nitrosobis(2-oxopropyl)amine (BOP)-induced hamster model of ductal carcinomas has been used for a number of years to evaluate agents with potential cancer preventive activity in the pancreas. In fact, the resulting tumors routinely have mutations in the *KRAS* gene. In this model, after treatment with BOP and chemopreventive agents, the pancreas is sectioned and scored for histological lesions: hyperplasias, dysplasias, and cancers. Recently, striking preventive activity of NO-releasing aspirin was shown in this model.⁸⁵ A new transgenic model for pancreatic cancer using mice bearing the LSL-*KRAS* transgene is being developed. Ductal pancreatic cancers arise in 80–100% of the transgenic mice by five months of age. Atorvastatin and NO-releasing aspirin appear to have significant chemopreventive activity in this model (Rao, unpublished results).

CONCLUSIONS

Preclinical animal models have been used extensively in the efficacy testing of potential chemopreventive agents. Standardized statistical methodology has been established to evaluate and compare the data from most of these animal model experiments based on the various endpoints.⁸⁶ For some of the more standard animal models there is significant correlation with human efficacy. For example, the animal ER+ breast cancer model responds to SERMs, aromatase inhibitors, pregnancy, 4HPR [4-hydroxy(phenyl)retinamide] and EGFR inhibitors in a manner similar to human ER+ breast cancers. The AOM colon and Min/+ mouse models respond to both NSAIDs/Coxibs and DFMO, results that have been borne out in human trials of these agents. The animal UV-induced skin cancer model predicted the efficacy of celecoxib in a human phase 2 trial. Finasteride results in animal studies correlate with outcomes for prostate cancer reduction seen in human trials. For colon cancer, aspirin and calcium results in animals correlate with human data. Anethole trithione, a drug used to treat dry mouth, and 13-cis-retinoic acid have exhibited efficacy in human lung and head and neck cancers. To date there have been two important negative correlations between animal and clinical studies: β -carotene for lung cancer and selenium and vitamin E for prostate cancer. Additional correlates are mentioned in the heart of the paper.

There are at least four other general points that can be deduced from our animal cancer prevention studies: 1) For many of the agents one can initiate treatment long after the initiation of cancer either by a carcinogen or by a transgene and still observe a preventive effect; this is important because the intervention in most phase 2 and phase 3 prevention trials is relatively late in the cancer process. 2) Virtually no agent is strongly effective in all organ sites; thus, NSAIDs/celecoxib are highly effective in colon, skin, bladder, esophagus and head and neck cancers but routinely less effective in cancers of the breast, lung and prostate. 3) Combinations of agents or altered routes of administration of certain drugs may lower toxicities while retaining efficacy. 4) The animal models may be quite useful in determining potential pharmacodynamic markers for clinical trials. However, such studies have only scratched the surface and have not been fully exploited for this purpose. Clearly, there is much room for improving the current animal models to reflect the etiology and progression of the human cancer process. A need also exists to develop animal models for testing cancer preventive agents in other organs including brain, kidney, cervix, lymphatics, the hematopoietic system, and skin (melanoma). The validation of animal models to predict the efficacy of agents in human clinical trials will await further human data on positive and negative outcomes with chemopreventive agents. Animal efficacy data remain central to the clinical trial decision-making process.

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TABLE 1

Carcinogen-induced Animal Models in Current Use for the Screening and Development of Chemopreventive Agents

Organ Site	Species	Carcinogen/Genetic Modification *	Endpoint Measured
Mammary Gland	Rat	MNU	Adenocarcinomas
	Rat	DMBA	Adenocarcinomas and Adenomas
Lung	Hamster	MNU	Squamous cell carcinomas
	Mouse	B[a]P, NNK, vinyl carbamate, uracil, urethane, cigarette smoke	Adenomas and Adenocarcinomas
	Mouse	NTCU	Squamous cell carcinomas
Colon	Rat	AOM	Aberrant crypts and Adenocarcinomas
Prostate	Rat	MNU	Adenocarcinomas
Bladder	Mouse, Rat	OH-BBN	Transitional cell carcinomas
Ovary	Rat	DMBA	Epithelial and thecal cell carcinomas
	Chicken	Spontaneous	Epithelial carcinomas
Skin	Mouse	DMBA or UV	Papillomas and squamous cell carcinomas
Esophagus	Rat	NMBA	Squamous cell carcinomas
	Rat	Surgery, DS, Fe++	Adenocarcinomas
Head and Neck	Rat	4NQO	Squamous cell carcinomas
	Hamster	DMBA	Squamous cell carcinomas
Pancreas	Hamster	BOP	Ductal carcinomas

* Abbreviations: MNU, methylnitrosourea; DMBA, dimethylbenz[a]anthracene; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; B[a]P, benzo[a]pyrene; AOM, azoxymethane; 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; DS, dextran sulfate; NTCU, *N*-nitroso-tris-chloroethylurea.

TABLE 2**Genetically Engineered Animal Models Currently Used for Chemopreventive Agent Development**

Organ Site	Species	Genetic Modification	Endpoint Measured
Mammary	Rat	Neu retrovirus	Adenocarcinoma
	Mouse	MMTV, Neu+, and p53 knockout	Adenocarcinoma (model for human Her2)
	Mouse	<i>BRCA1</i> and p53 knockout	Adenocarcinoma (model for Human <i>BRCA1</i> -mutation-associated cancer)
	Mouse	SV40 T antigen (TRAMP)	Adenocarcinoma (model for human basal cell cancer)
Lung	Mouse	<i>TP53</i> mutants plus B[a]P or NNK	Adenocarcinoma
	Mouse	Altered <i>TP53</i> and <i>GPCR5a</i> knockout (NNK)	Small cell lung cancer
Pancreas	Rat	<i>LSL-K-ras</i>	Adenocarcinoma
Colon	Mouse	Min (<i>APC</i> mutant)	Intestinal polyps
	Mouse	<i>APC</i> 1638, <i>MSH2</i> knockout	Intestinal polyps, Colon polyps
Prostate	Mouse	SV40 T antigen (TRAMP)	Carcinoma
	Mouse	<i>PTEN</i> knockout plus TRAMP	Carcinoma
Skin, cervix	Mouse	E6/7 (Keratin 14 promoter)	Carcinoma
Skin	Mouse	<i>PTCH</i> knockout (UV)	Basal cell carcinoma
	Mouse	<i>HGF</i> , <i>H-ras</i> , and <i>TP53</i> knockouts	Melanoma

B[a]P, benzo[a]pyrene; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; MMTV, mouse mammary tumor virus.