

Animal Models in Cancer Chemoprevention

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Abstract

Animal models are currently being used to check the efficacy of chemo preventive/chemotherapeutic agents. This is because the microenvironment of cultivated primary cells isolated from an animal organ/system is markedly different from *in vitro* systems. Animal models are important to study the interactions between different cell types and drug/ or xenobiotic metabolisms. To study specific cancer type, animal models can be generated either chemically or transgenetically. A number of tumor-specific animal models are available for chemo preventive studies. To examine the efficacy of chemotherapeutic drugs used for the treatment of various cancers such as prostate, skin, liver, colon etc. are being tested in the different chemically induced or transgenic animal models. However, the validity of animal models in determining the effectiveness of a chemo preventive agent in the human treatment will require the critical evaluation of the positive and negative effects of these agents on human. In this mini-review, we will throw some light on the various small mammalian animal models used in the cancer chemoprevention studies.

Keywords: Animal models; Cancer; Carcinogen; Chemoprevention; Transgenic

Abbreviations: ER: Estrogen Receptor; PI3K: Phosphatidylinositol 3-kinase; PTEN: Phosphatase and Tensin Homolog; 4NQO: 4-Nitroquinoline 1-oxide; HDAC: Histone Deacetylase; NSAID: Non-steroidal anti-inflammatory drugs; NNK: 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone Aox: Acyl-CoA Oxidase.

Introduction

Mammals including humans have very complex physiological functions which are highly integrated and tightly regulated. To study such complex integrative pathways, there is a need to have full description about the mechanisms involved in healthy and diseased conditions. Cultivated primary cells isolated from *in vivo* systems differ strongly from their corresponding cell

types i.e. cultivated cells may have hundreds of genes up-regulated or down-regulated in them when they are isolated from their micro-environment. Disadvantages of an *in vitro* system over *in vivo* include problems to speculate the chemo-preventive drug concentration/doses, problems to infer the effect of long-term exposure of a drug from *in vitro* systems, difficulty in checking the interaction between different cell types and difficulty in understanding the xenobiotic metabolism [1].

Till now no *in vitro* model has fulfilled the criterion to study these complex networks and interactions between signaling molecules in their actual conditions. Animal models display not only genetic similarities but also show similar patterns of genetic alterations. These animal models are used to address the scientific questions from basic science to therapies used for treatment. It should

also be noted that despite of huge similarities between species, these model organisms have several differences like fine regulation of gene expression, genetic differences which further translate into physiological differences [2]. Animal models are currently being used to testify and to check the efficacy of chemical agents extracted from the natural resources in the treatment of different cancer types. To study the specific form of cancer and its progression, animal models are either established by chemical induction or produced trans-genetically (transgenic animal models). Various animal models are used in the chemoprevention studies as summarized in

table 1. For the selection of an ideal Animal model in cancer chemoprevention, the following basis should be followed-

- It should show relevance to human cancer and similar pathology of the cancerous lesion.
- The genetic abnormalities and changes in gene should be similar to those observed in the case of human cancer.
- A consistent tumor burden produced in the animal model should be greater than 60% within a reasonable time period (less than 6 months) [3].

Animals	Chemically Induced/Transgenic/ Knock-Out	Cancer Type	Characteristics	Reference
TRAMP model	Transgenic	Prostate cancer	PTEN alteration fast growing nature of cancer	[3]
Sprague Dawley Female Rat	Methyl Nitrosourea (MNU)	Mammary gland	Highly sensitive to EGFR and farnesyl transferase inhibitor.	[5,6]
F344 Rats	Azoxymethane (AOM)	Head and Neck Cancer	Sensitive to EGFR Inhibitor, HDAC Inhibitor	[9]
A/J strain of female mice	Benzo[a] pyrene	Lung Cancer	Associated with KRAS mutation	[12]
Strain A mice	Vinyl Carbamate	Lung Cancer	High multiplicity and production of carcinoma	[13]
Skh:2 hairless mice	UV induced	Skin Cancer	Higher tumor multiplicity p53 mutations at dipyrindine sites sensitive to natural flavones and various NSAIDS	[15]
MMTV-Neu/p53KO mice	Transgenic	Mammary	Altered p53 like human ER negative Neu over-expressing tumor	[17]
Mdr-KO mice	Knockout	Liver cancer	β -catenin negative model CyclinD1 over-expression	[18]
MMTV-PyMT mice	Transgenic	Breast cancer	Loss of steroid hormone receptor and β 1-integrinErB2 and cyclin D1 over-expression	[19]
Aox-deficient-mouse	Knockout	Liver	Dys-regulation of gene involved in involves lipid, carbohydrates and amino acid metabolism and stress response.	[20]

Table 1: Various Animal Models Used In Cancer Chemoprevention Studies.

Limitations of two-dimensional in vitro cancer models are also included while selecting an animal model i.e. selection of phenotypic and genotypic cells, accumulated mutations over a time period, isolation of tumor cells from the tumor microenvironment (TME). Despite successful pre-clinical trials, the chemopreventive drugs that are subjected to trial on these animal models failed largely (only 8% succeeded to clinical trial) which is likely due to poor methodology and failure to mimic the accurate human diseased conditions [4].

Chemically Induced Rat Models for Cancer Chemoprevention

Mammary gland carcinogenesis induced by Methyl Nitrosourea (MNU) is an important model to study ER⁺ cancer in Sprague Dawley female rat. This model shows a high incidence rate of mammary gland carcinogenesis (>84%) and development of adenocarcinoma within 120-150 days of carcinogen treatment. This model is highly sensitive to EGFR and Farnesyl transferase inhibitor and is also susceptible to hormonal manipulation [5,6].

To study the chemoprevention drug efficacy, another chemoprevention model for lung cancer has been generated by tobacco-specific carcinogen, NNK in F344 strain of Rats. The majority of animals after NNK treatment displayed lung adenoma and sometimes squamous cell carcinoma after 21 weeks of subcutaneous injection [7]. Sulphur containing compounds like 6-phenyl hexyl isothiocyanate (PHITC), Phenethyl isothiocyanate (PEITC) has shown chemopreventive efficacy in this model [8] 4NQO induced head and neck cancer in rats serves as an effective model for the chemoprevention study. This model is used to study the efficacy of EGFR inhibitor and HDAC inhibitor. Tarceva, which is an EGFR inhibitor, is under human trial.

In colon cancer, cancer induced by azoxymethane (AOM) in F344 rats is an effective model to study the chemopreventive drugs like Aspirin, Cyclooxygenase-2-inhibitor, Statin, Ornithine decarboxylase inhibitor and HMG-CoA reductase inhibitor [9].

OH-BBN induced bladder cancer in rats and mice shows similar histopathological changes and invasiveness and similar gene expression changes including Cyclin, Cyclin Kinases, Survivin, Annexin II as in human bladder transitional cell carcinoma (TCC). NSAIDs including Naproxen, Indomethacin and NO-releasing NSAIDs has been reported to show striking effects in this model [3].

3,2'-dimethyl-4-aminobiphenyl (DMAB) induced prostate cancer in F344 rats after 20 weeks subcutaneous injection is an important animal model to study the promoting effects of anti-androgenic agents like 5 alpha-reductase, Casodex or C17-20 lyase inhibitor [10].

Chemically Induced Mouse Models for Cancer Chemoprevention

Carcinogen 7, 12 Dimethylbenz[a]anthracene (DMBA) induces ER/PR⁺ cancer in mouse similar to that in case of human. Long latency tumor induced by DMBA displays an 85% of incidence rate and shows a high frequency of PI3KCa and PTEN mutations [11]. This model is helpful to study agents which are known to modulate cytochrome P450 enzyme system. DMBA is a polycyclic carcinogen which is activated by cytochrome P450 system so it is helpful in studying those chemopreventive agents which target Cytochrome P450 system [3].

In the benzo[a]pyrene model, A/J strain of female mice and 16 weeks of treatment displayed 100% rate of incidence of pulmonary adenomas. In this model, aerosol administration of chemo-preventive drug has an

advantage over diet or oral gavage. This carcinogen is associated with KRAS mutation. Budesonide (glucocorticoid) has shown striking effects in this model which led it to clinical trials [12].

Vinyl carbamate carcinogen in 8-9 weeks old treated strain A mice shows high multiplicity and production of carcinoma in a larger proportion, showing more than 30% of carcinoma lesions. NSAIDs like Piroxicam were used in this type of model to check the efficacy [13].

Compared to other types of cancer, the generation of prostate cancer model is very difficult. MNU/Testosterone treated mice shows a higher rate of cancer incidence and is useful for the chemopreventive study of anti-androgen, Prasterone etc. [14].

UV induced skin cancer in Skh:2 hairless mice is also an effective model to study the chemopreventive role of natural flavones and various NSAIDs like Celecoxib, Piroxicam etc. This model shows a 100% incidence rate of cancer after UV-exposure and has a higher tumor multiplicity [15].

Liver cancer induced in mice by diethylnitrosamine and Phenobarbital which shows 100% hepatoma is recently being used as a model for the therapeutic drug treatment studies of liver cancer. Bicyclol which is an anti-hepatitis drug has been shown to inhibit the malignant transformation of liver cancer in this model [16].

Transgenic/ Knock-Out Models For Cancer Chemoprevention

MMTV-Neu/p53KO transgenic mice have EGFR (Neu) expression under the control of MMTV promoter and also have a heterozygous knockout of p53. This model resembles around 20-25% of human cancers having p53 mutation. EGFR inhibitor, RXR agonist like Bexarotene and UAB-30 are showing high susceptibility to this model [17].

The transgenic mouse prostate (TRAMP) model, where PTEN alteration is present shows chemopreventive susceptibility to the drug such as Toremifen. This model has an advantage to study the human prostate cancer because of its fast-growing nature and thus making it an important model for the effective drug study [3].

Mdr2 Knockout mice (Mdr-KO) is a transgenic model used to study hepatocellular carcinoma (HCC). This model shows several dysregulated pathways and gene

expression as in human HCC. It serves as a β -catenin negative model and shows CyclinD1 over-expression. Selenium and anti-oxidant agents have shown efficacy to this model [18].

MMTV-PyMT transgenic mouse model is an important model used to study breast cancer metastasis. This model shows a gradual loss of steroid hormone receptor and β 1-integrin. In late metastatic stage, it shows overexpression of ErB2 and Cyclin D1 which has close similarities with human breast cancer. Dipyridamole (DPM) shows potential chemopreventive effects in this model [19].

Aox-deficient-mouse is another model used to study the pathogenesis of liver cancer and the contribution of peroxisome receptors. The dysregulated genes in this model are those which involves lipid, carbohydrates, amino acid metabolism and stress response. This model is important to understand the correlation between hepatic metabolic functions and formation of tumor [20].

Conclusion

A number of animal models are used to determine the efficacy of a chemo preventive agent. Based on the preclinical studies using animal models; the effective dose, xenobiotic study and toxicity of the chemo preventive agents are tested in real-time. However there is a lot of scope and need of improvement in the existing animal models in order to give a precise reflection of the process of the progression of the various cancers. Further, there is a need for the development of animal models for the determination of the efficacy of chemo preventive agents in the treatment of cancers of other organs such as brain, cervix, hematopoietic system etc. The use of animal models in determining the efficacy of a chemotherapeutic drug further requires the data from human trial which will represent the pros and cons of using the chemotherapeutic agent in human. All this remains centralized to the clinical trial process. Our lab is currently working on testing the efficacy of various chemotherapeutic drugs in the liver, oral, skin and prostate cancer using established animal models.

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