Review Article

Murine Preclinical Cancer Models: A Systemic Review

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ABSTRACT

In vitro cell culture and preclinical animal screening models are being used to identify and prioritize synthetic and natural agents targeting human cancer. Starting from selection and testing of potential agent, the primary step is to conduct battery of short term *in-vitro* assays. This is followed by *in vivo* evaluation of the promising & potent moieties against well-established chemical induced or spontaneous cancer models, to screen for an early indication of chemo preventive efficacy.

Keywords: Preclinical, *in-vitro* assays, cell culture.

INTRODUCTION

Cancer is one of the leading causes of death in both developed and developing countries and is therefore, of worldwide concern. According to WHO, cancer accounted for 7.9 million deaths (around 13% of all deaths) in 2007, with 38% in developed countries and 62% in developing countries. By 2030, nearly 21.4 million new cancer cases and more than 13.2 million deaths are projected to occur in the world[1].

These *in vitro* and *in vivo* preclinical data not only provide significant evidence for efficacy and potency of test agent, but also generates valuable data regarding dose-response, toxicity, and pharmacokinetic evaluation which is a prerequisite to Phase I clinical /human chemoprevention testing.

Since, animal testing plays an important preliminary role in cancer drug development process, there are certain parameters to be an ideal chemo preventive animal model. Firstly, the preclinical model should bear significant relevance to human beings in several ways including specificity for target organ and inducing cancer of similar pathology. The cancer thus produced should be genetically, histologically and molecularly in relevance to human cancer. Since, it is generally concluded that no current preclinical animal model is ideal, therefore research and development for better animal screening models is still under process of development. In the present review, we have reviewed currently available chemical induced and xenograft mouse models for screening chemoprevention efficacy and potency.

CHEMICAL INDUCED CARCINOMA MODELS

A growing number chemically induced carcinoma models are being developed and are used routinely. We will hereby explain the different methods for chemically inducing cancer in preclinical research.

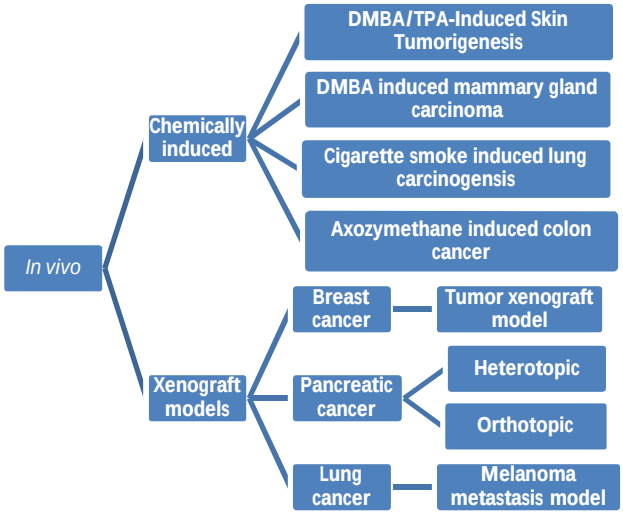

Diagram 1: Summary of Preclinical Screening Modelling For Anti Cancer Agents.

DMBA/TPA-Induced Skin Tumorigenesis

The DMBA/ TPA induced skin tumorigensis is the mouse skin model of multi-stage chemical carcinogenesis represents one of the best established *in vivo* models for the study of the sequential and stepwise development of tumors. It includes skin tumorigenesis initiation, by single topical application of DMBA (26 µg dissolved in 200 µl acetone) to the shaved dorsal skin in mice Two weeks after initiation, the mice be further treated with topical applications of TPA (6 µg in 200 µl acetone) thrice weekly for 30 weeks except a group which received acetone instead of TPA[2,3]. Skin tumors with a diameter of >1 mm be counted and recorded every week. The percentage of mice with tumors (tumor incidence) and the number of skin tumors per mouse (tumor burden) be plotted as a function of weeks on test[4].

DMBA induced mammary gland carcinoma

It is the commonest mammary gland carcinoma animal model. Twenty mice are administered 6 weekly 1.0 mg doses of DMBA in 0.2 ml of sesame oil by oral gavage, beginning at 5 weeks of age. Mice be then mated continuously to provide an oscillating hormonal environment and followed until either tumors developed or the mice become fatal. Mice bearing tumors>0.5 cm be euthanized by CO_2 inhalation and necropsied[5].

Cigarette smoke induced lung carcinogensis

This model has been used widely to evaluate the efficacy of potential chemo preventive agents in inhibiting lung cancer. As known, smoking is the primary causes of human lung cancer thereby the individual cigarette smoke carcinogens are frequently used to induce lung tumors in mice. This is commonly achieved by intraperitoneal or dietary administration of carcinogens of the polycyclic aromatic hydrocarbon (PAH) and nitrosamine class. PAHs are largely produced during the combustion of tobacco, while nitrosamines are already present in unburned tobacco and are formed as a consequence of the tobacco curing process. Benzo(a)pyrene (B(a)P), a PAH, and the nitrosamines, 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanone (NNK) and *N'*-nitrosonornicotine (NNN), are strong inducers of lung adenomas and adeno carcinomas in mice. Lung adenomas be induced by giving main-stream cigarette smoke for 120 days in Swiss albino mice (newborn). Lung adenocarcinomas be induced by administration of B (a) P, 100 mg/kg i.p. and NNK, 100 mg/kg i.p[6-9].

Axozymethane induced colon cancer

The azoxymethane (AOM)-induced aberrant rat colon crypt model has become a primary whole-animal screening assay for potential chemo preventive agents due to its short time course, low cost, and requirement for only a small amount of test agent.

Seven-week-old male Sprague-Dawley rats weighing approximately 225-300g should be placed in disposable plastic cages. At eight weeks old, AOM (15 mg/kg) be injected subcutaneously into rats once weekly for two consecutive weeks. At the end of each study, the colons be extracted from the sacrificed rats, cleaned with phosphate buffer solution (PBS) and cut into the proximal, middle and distal regions of the large intestine. To quantitate the aberrant crypt foci (ACF), methylene blue staining be conducted by dipping the colonic segments in 10% formalin buffer fixative solution for 24 h followed by methylene blue dye (0.1% w/v) staining for 20–30 min. A light microscope with a 40X magnification should be used to quantitate ACFs on the colon[10].

XENOGRAFT MOUSE MODELS

Various animals models have been developed to mimic and study human cancer. These models are used to investigate the factors involved in malignant transformation, invasion and metastasis, as well as to examine response to therapy. One of the most widely used models is the human tumor xenograft. In this model, human tumor cells are transplanted, either under the skin or into the organ type in which the tumor originated, into immune compromised mice that do not reject human cells. For example, the xenograft will be readily accepted by athymic nude mice, severely compromised immune deficient (SCID) mice, or other immune compromised mice[12]. Depending upon the number of cells injected, or the size of the tumor transplanted, the tumor will develop over 1–8 weeks (or in some instances 1–4 months, or longer), and the response to appropriate therapeutic regimes can be studied *in vivo.*

Xenograft modal of pancreatic cancer

The current available therapies for pancreatic cancer in humans are mostly ineffective and therefore it has an extremely low survival rate. One important animal model which is used to screen agents with potential cancer preventive activity is tumor xenograft model. This model is recently the most common choice for the preclinical cancer screening due to its advantages in mimicking genetic and epigenetic abnormalities in comparison to human beings. It is depicted by the injection of human tumor cells grown from culture into a mouse or by the transplantation of a human tumor mass into a target mouse. This xenograft has to be made readily acceptable to the host animals by compromising the immune system. There are two main types of human xenograft mouse models used for pancreatic cancer research, heterotopic and orthotopic, defined by the location of the implanted xenograft.

Heterotopic xenograft model

For many years, the subcutaneous xenograft model has been the most widely used preclinical murine model for cancer research because it is rapid, inexpensive, reproducible, and has been considered sufficiently in preclinical studies to test anti-cancer drugs. In heterotopic subcutaneous mouse model, the xenograft is implanted between the dermis and underlying muscle and is typically located on the flank, on the back or the footpad of the mice. The subcutaneous model also has the advantages of providing visual confirmation that mice used in an experiment have tumors prior to therapy; and provides a means of assessing tumor response or growth over time, compared to intracavitary models where animal survival is the sole measure of response[14].

Numerous researchers have reported to use tumor engraftment in nude mice for studying the possible response to standard chemotherapy treatment and new pharmacological blocking agents with significant results and thereby suggesting the new and potential treatment options for pancreatic cancer[15, 16].

The main drawback of the heterotopic model is that during drug regimens these models often do not mimic significant effect of human disease as subcutaneous microenvironment is not relevant to that of the organ site

of primary. Additionally, subcutaneous tumor models rarely form metastases. The gross observations from these tumor models suggests that they do not represent proper sites for human tumours and are not predictive when used to test responses against anti-cancer entities[12,17,18].

Orthotopic xenograft model

In this model, orthotropic tumours are transplanted to the target organ. Orthotropic tumor model has emerged as the most preferred model for cancer research due to its increased clinical relevance.

Anaesthetized mice 6-8 week old are used in the standard procedure. The pancreatic lobes are visualized by the incision of the abdominal skin and muscle. The tumour cells are injected in the gently retracted pancreas. After revival surgery the mice should be monitored and weighed daily to evaluate the tumor progression and its response towards the treatment[13].

In basis research, this model has been used frequently to study gene expression profiling of liver metastases and tumour invasion in pancreatic cancer[19].

Xenograft model of lung cancer: Melanoma metastasis model

In standard melanoma metastasis model procedure, mice are injected with 1 X10^6 B16 melanoma cell (IV) on day 0 and with either phosphate-buffered saline (PBS) or test/std drug (i.p) on days 0, 2, 4, 7, 9 and 11. The experimental animals should be killed on day 14 and surface lung metastasis be counted using dissecting microscope [20].

Xenograft model of breast cancer: Tumor Xenograft model

In standard procedure for breast carcinoma Xenograft experiments, subcutaneous injection of 5 X 10^6 BT474MI cells on day 1 in 0.1 ml PBS mixed with 0.1 ml Matrigel (as a substrate for cell culture). BALB/c / *FcRγ*–/– BALB/c or *FcγRII*–/– BALB/c nude mice 2–4 months old may be were injected subcutaneously with standard / test drug 24 h before tumor cell injection. Therapeutic test drug may be injected intravenously beginning on day 1 at a loading dose of 4 µg/mg, with weekly injections of 2 µg/mg for BALB/c nude and *FcRγ* /– BALB/c nude. The so formed tumor measurements are obtained weekly[20].

CONCLUSION

Chemoprevention is an important approach in order to minimize cancer related mortality by the use of natural or synthetic moieties to reverse the processes of initiation, promotion, and progression of cancer cells. Preclinical animal screening models have been used extensively in testing of potential chemo preventive agents for their possible efficacy and potency. Since there is a lack of standard animal model, there are chances for improving currently available screening animal models to reflect the exact etiology and comparable progression of the human cancer. Also there is a significant need to improve upon the existing models for specific target organ chemoprevention.

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM: Estimates of worldwide burden of cancer in 2008, Globocan 2008. International Journal of Cancer 12, 2010, 2893-2917.
2. Lee MJ, Wang CJ, Tsai YY, Hwang JM, Lin WL, Tseng TH and Chu CY: Inhibitory effect of12-O-tetradecanoylphorbol- 13-acetate-caused tumor promotion in benzo[a]pyrene- initiated CD-1 mouse skin by baicalein. Nutrition and cancer, 34(2), 1999, 185–191.
3. Davis TW, Zweifel BS, O'Neal JM, Heuvelman DM, A.L. Abegg, T.O. Hendrich and J.L. Masferrer," Inhibition of cyclooxygenase-2 by celecoxib reverses tumor-induced wasting. Journal of Pharmacology and Experimental Therapeutics, 308(3), 2004, 929–934.
4. Ma GZ, Liu CH, Wei B, Qiao J, Lu T, Wei HC, Chen HD and He CD: Baicalein Inhibits DMBA/TPA-Induced Skin Tumorigenesis in Mice by Modulating Proliferation, Apoptosis and Inflammation. Inflammation, 36 (2), 2012.
5. Currier N, Solomon SE, Demicco EG, Chang DL, Farago M, Ying H, Dominguez I, Sonenshein GE, Cardiff RD, Xiao ZX, Sherr DH and Seldin DC: Oncogenic Signaling Pathways Activated in DMBA-Induced Mouse Mammary Tumors. Toxicologic Pathology, 33(6), 2005, 726-737.
6. Shimkin MB and Stoner GD: Lung tumors in mice: application to carcinogenesis bioassay. Advances in cancer research, 21, 1975, 1-58.
7. Hecht SS: Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nature reviews. Cancer, 3(10), 2003, 733-44.
8. Malkinson AM: The genetic basis of susceptibility to lung tumors in mice. Toxicology, 54(3), 1989, 241-71.
9. Malkinson AM: Primary lung tumors in mice: an experimentally manipulable model of human adenocarcinoma. Cancer Research, 52(922), 1992, 2670-2676.
10. Chaudhary A, Sutaria DK, Huang Y, Wang J and Prabhu S: Chemoprevention of Colon Cancer in a Rat Carcinogenesis Model Using a Novel Nanotechnology-based Combined Treatment System. Cancer Prevention Research (Philadelphia,Pa), 4(10), 2011, 1655–1664.
11. Morton CL and Houghton PJ: Establishment of human tumor xenografts in immunodeficient mice. Nature Protocols, 2, 2007, 247–250.
12. Becher OJ and Holland EC: Genetically engineered models have advantages over xenografts for preclinical studies. Cancer Research, 66, 2006, 3355–3358.
13. Huynh AS, Abrahams DF, Torres MS, Baldwin MK, Gillies RJ and Morse DL: Development of an orthotropic human pancreatic cancer Xenograft model using ultrasound guided injection of cells. PUBLIC LIBRARY OF SCIENCE One, 6(5), 2011, 20330.
14. Reynolds CP, Sun BC, DeClerck YA and Moats RA: Assessing growth and response to therapy in murine

tumor models. Methods in Molecular Medicine, 111, 2005, 335–350.

15. Garrido-Laguna I, Uson M, Rajeshkumar NV, Tan AC, de Oliveira E, Karikari C, Villaroel MC, Salomon A, Taylor G and Sharma R: Tumor engraftment in nude mice and enrichment in stroma- related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. Clinical Cancer Research, 17, 2011, 5793–5800.

16. Feldmann G, Mishra A, Bisht S, Karikari C, Garrido-Laguna I, Rasheed Z, Ottenhof NA, Dadon T, Alvarez H and Fendrich V: Cyclin-dependent kinase inhibitor Dinaciclib (SCH727965) inhibits pancreatic cancer growth and progression in murine xenograft models. Cancer Biology & Therapy, 12, 2011, 598–609.

17. Niedergethmann M , Alves F, Neff JK, Heidrich B, Aramin N, Li L, Pilarsky C, Grützmann R, Allgayer H and Post S: Gene expression profiling of liver metastases and tumour invasion in pancreatic cancer using an orthotopic SCID mouse model", British Journal of Cancer, 97, 2007, 1432–1440.

18. Killion JJ, Radinsky R and Fidler IJ: Orthotopic models are necessary to predict therapy of transplantable tumors in mice. Cancer and Metastasis Review, 17, 1998- 1999, 279–284.

19. Hoffman RM: Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. Investigational New Drugs, 17, 1999, 343–359.

20. Clynes RA, Towers TL, Presta LG and Ravetch JV: Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. Nature Medicine, 6(4), 2000, 443-446.

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