Section A-Research paper

ANIMAL MODELS FOR BREAST CANCER STUDIES AND THEIR APPLICATION IN DRUG DEVELOPMENT

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ABSTRACT:

Preclinical models of breast cancers are indispensable in the drug discovery and development process for new cancer drugs, small molecules and biologics. They are however imperfect facsimiles of breast cancers given the genetic and epigenetic heterogeneity of the latter and the multiplicity of dysregulated survival and growth-regulatory pathways that characterize this spectrum of diseases. Although markers for early diagnosis and drugs that limit the spread of breast cancer to other organs have been developed, it is difficult to prevent the relapse of breast cancer. Additionally, evaluating the efficacy of novel therapeutic agents emerging from drug discovery programs in a variety of pre-clinical models can better mimic the heterogeneity of human cancers and also aid in establishing dose levels, dose regimens and drug combinations for use in clinical trials. Nonetheless, despite the sophistication and physiological relevance of these breast cancer models (e.g., genetically engineered tumor models and primary human tumografts), the ultimate proof of concept for efficacy and safety of novel oncology therapeutics lies in humans. The information for the present study was obtained from various internet sources like research articles and paper presentation documents and research book publications. Emerging data have suggested that animal models are a good system to investigate this communication. Therefore, studies with animal models have been developed as a reasonable method for a systemic approach to understand breast cancer metastasis. In this review, we summarize animal models of breast cancer and their

applications to the study of human breast cancers, and discuss limitation of model system and advanced techniques to overcome it.

KEYWORDS: breast cancer, animal models, mice, cell line

INTRODUCTION:

Metastasis is the main cause of death in women with breast cancer. Development of clinical trials for tumor regression and metastasis prevention and the elucidation of their underlying molecular mechanisms help to reduce the death rates of cancer patients. Despite the accumulating knowledge of the underlying mechanisms of metastasis and its clinical application to breast cancer treatment, many patients die from relapse after the removal of the primary tumors because of metastasis of cancer throughout the body. Therefore, many efforts have been made to develop therapeutic drugs that prevent tumor invasion and to identify diagnostic markers to classify each stage of cancer and metastasis for early diagnosis.[1,2]

The roles of oncogenes and tumor suppressor genes have been validated from experimental animal models carrying deletions or mutations of genes initially identified in patient tissue samples. Animal models of human cancer and the in vivo biological, pharmacodynamic/pharmacokinetic (PD/PK) and pharmacological information they can provide remain critical components in: (i) understanding the pathophysiology of cancer including new target identification; (ii) identifying novel therapeutic agents; (iii) exploring the utility of novel therapeutics in combination with, or adjunct to, established chemo- and radio-therapeutic regimens and approved targeted therapeutic agents; and (iv) in studying mechanisms of intrinsic and acquired resistance to cytotoxic and targeted therapies. Despite differences in the types of models discussed below, in general, tumor development is more rapid and homogeneous in murine models as compared to the heterogeneity of human cancers discussed above, while offering considerable practical benefits for drug discovery, development, translational biology and biomarker assessment of anti-cancer therapeutic agents.[3,4,5,6,7,8] A wealth of data generated from animal models has provided insights into the biological functions of genes and signalling pathways involved in cancer and has allowed for the generation of an advanced concept of metastasis.[9,10]

Various in-vitro cell line studies are also available in the market for cancer research. Few of them are: MDA-MB-231 1-7HB2, HMT-3522 T4-2, HMT-3522 S1, BICR/M1Rk, FM3Ats C1.T85, FM3A, Hs 578T, ZR-75-30, ZR-75-1, MDA-MB-231, MDA-MB-435, SUM1315, SUM149, BT-474, MCF7, T47D,MTT, TA3 Hauschka, VP303, VP267, VP229, T47D P1.HTR, P1.HTR.TK-, P1Bb1.1 (DBA/2), P815-1-1, MFM-223, MDA-MB-157, CL-S1, C127I, CNC 127I, MTSV1-7 CE1, HMT-3522 S.

Even though in vitro culture of established breast cancer cell lines is probably the most widely used preclinical model, it is limited by the lack of stromal cells and three-dimensionality. The limitations of this model make it unrepresentative of real cancers[11] . To overcome these limitations, we discuss here how animal models have played an important role in basic and translational breast cancer researches. That could be useful for drug development.

ANIMAL MODELS FOR BREAST CANCER:

These models can be divided into two categories:

1. Non- mammalian models

2. Mammals models

1. Non- mammalian models

The growth, migration, and metastasis of breast cancer is frequently mimicked by non-mammalian animals such as Caenorhabditis elegans, zebrafish, Drosophila, and chickens. As a result of their short reproductive cycles, these animals offer rapid experimental cycles at low costs. Several studies on tumor angiogenesis have used chickens and zebrafish[12]. To visualize human breast cancer spread, invasion, and metastasis, researchers transplanted fluorescent protein and chemically labelled cancer cells into zebrafish embryos[13] . In contrast, their disadvantage is that they are very different from humans and appear genetically different. Furthermore, most organs of these animals do not have the same physiological structure as those of humans[14].

2. Mammalian models

In comparison with non-mammalian animals, mammals are more like humans. The most commonly used animals for breast cancer research are mice and rats. There are also other types of animals commonly used for breast cancer research, including moles, hamsters, cats, dogs, pigs, tree shrews, and NHPs (non-human primates)[15].

A new animal model for experimental studies, tree shrews are considered advantageous because they are small (100–200 g) and highly productive (2–3 offspring)[16 17] . A number of advantages are associated with tree shrews. They have three pairs of breasts, reach sexual maturity at three to four months, can breed for up to three years, and live for an average of five to seven years. Furthermore, genome sequences have revealed an evolutionary relationship between tree shrews and primates[1819] . Moreover, tree shrews are more likely to develop spontaneous breast cancer with an increased frequency Here some other models are discussed in detail.[20]

Spontaneous models:

A major characteristic of spontaneous breast cancer is that it is not treated artificially; it is therefore similar to the etiology of human breast cancer. Rodents frequently develop spontaneous breast tumors [21]. Despite the fact that inbred mice are most commonly used in research on spontaneous breast cancer, the incidence and frequency of cancer may vary greatly among different strains (TA2, CBA/J, A, and C3H)[22] . Based on Kunming outbred mice, the researcher developed a spontaneous breast tumor animal model, which detected tumors in 25% (89/398) of female breeding mice, whose tumorigenesis took an average of 13.5 months to develop[23] . Large animals, such as dogs, cats, tree shrews, and monkeys, have also been reported to develop spontaneous breast cancer[24]

The natural occurrence of spontaneous breast cancers in genetically heterogeneous populations is similar to humans' tumors. A number of disadvantages of spontaneous breast cancer animal models include their low incidence rates, lengthy experimental periods, and long latency periods.

Induced models:

A variety of chemical, physical, and biological carcinogens can be administered orally, injected, or applied all over to animals to enhance breast tumor incidence rates and

accelerate tumorigenesis (https://doi.org/10.1002%2Fijc.24674). The most common method is to administer DMBA (7,12-dimethylbenz(a) anthracene) or MNU (N-methyl-N-nitrosourea)[2526] . Chemically induced breast tumors in mice are most commonly adenomas and type B adenocarcinomas. The use of DMBA, MNU, MCA(3-methylcholan-threne), 2-acetylamino-fluorene, 3,4-benzopyrene, ethylnitrosourea, and butylnitrosourea to induce breast cancer is widespread in rats. Most of the breast cancers caused by DMBA and MNU in rats are hormone-dependent.[27] DMBA or NMU are most commonly administered intragastrically, subcutaneously, or intravenously to Sprague-Dawley (SD) or Fischer 344 rats to induce breast cancer[2829]

Induction of mammary gland carcinomas by the subcutaneous injection of 1-methyl-1-nitrosourea. Cancer research, 43(4), 1628–1629). There is a similarity between breast cancers induced by NMU in rats and low-grade ER-positive breast cancers in humans . In 47-day-old SD rats injected with 20 mg of DMBA, researchers observed an 8-13 week incubation period and an almost 100% incidence of breast tumors after 13 weeks (https://doi.org/10.1590/s0041-87812004000500006). Additionally, authors found that DMBA and MPA injections could shorten the latency of breast lesions by 56 days in tree shrew[30] . Physical approaches, like ionizing radiation, can also cause breast cancer. Radiation from X-rays or neutrons can cause breast cancer in rats, whether it is whole-body or segmental[31] [32]. Biologically inducing breast cancer relies heavily on lentiviruses to silence tumor suppressor genes and overexpress oncogenes. A disadvantage is low efficiency, long incubation times, different incidence rates, as well as different pathological characteristics. In addition, tumor number, latency, and histological type may be affected by their age, reproductive history, and endocrine environment.

Compared with spontaneous breast cancer animal models, induced breast cancer animal models have relatively high incidence rates, short latencies, and more reliable predictions. In addition, animals' tumor number, latency, and histological type may be affected by their age, endocrine environment, and reproductive history.[33] .

Allograft model:

Breast cancer cells can be transplanted into same genetic strain with normal immune function from spontaneous or induced sources. There are several transplantable animal breast cancer cell lines, most of which are derived from mice. Cell lines used for allogeneic transplantation have strict germline specificities. Adenocarcinoma Ehrlich ascites (EAC) arises spontaneously from serial intraperitoneal passages in outbred mice. The EAC is an undifferentiated carcinoma with a rapid growth rate in suspension and a sensitivity to chemotherapy

The allograft breast cancer model offers several advantages, including multiple characterized cell lines, rapid growth and metastasis, and an immune-component microenvironment, but there are also limitations. Most importantly, the cancer cells transplanted are not human.

Xenograft transplantation:

Cell-derived xenografts (CDX), patient-derived xenografts (PDX), and a syngeneic model are well-established tools for evaluating therapeutic efficacy and toxicity and for

applying to preclinical assessment. Intravenous, intraperitoneal, subcutaneous or orthotopic injection of human cancer cells into mice is termed xenograft transplantation, and it is a well-defined method to monitor tumor and metastasis processes and to manipulate specific genes related to human cancers. In an immune-compromised mouse, injected human breast cancer cells form a tumor mass and metastasize into other organs, as observed in cancer patients. Invasion of tumor cells into the blood or lymphatic vessels is a critical feature indicating the metastatic ability of tumor cells.

For this reason, intravenous or cardiac injection of human metastatic tumor cells can focus on colonization steps at metastasis without differential ability of primary tumor incidence. After the injection of breast cancer cells in which the target genes are manipulated, the function of these genes in promoting or suppressing metastasis is monitored by comparing the number of metastatic nodules per lung to those observed in the control mouse.[34] Syngeneic mouse models in which murine cancer cells, such as 4T1, are injected into immune-competent mice (e.g. BALB/c) show more effective metastasis, with characteristics similar to those of breast cancer patients. Advantages of these models over CDX transplantation models include the use of immune-competent mice with normal immune cells and immune system, enabling investigation and development of various immune therapies, for example, with anti-PD-1/PD-L1. These models are also useful to investigate the anti-tumor and anti-metastatic effects of multiple drugs due to the high invasiveness of murine cancer cells.

Genetically engineered mice

Genetically engineered mice (GEM) for cancer study use techniques for the genomic deletion of tumor suppressor genes or the transgenic insertion of oncogenes. For breast cancer research, a mammary gland-specific promoter is often used to restrict the expression of oncogenes in specific breast regions. Transgenic mice expressing oncogenes (PyMT, ErbB2, Wnt1, or Ras) under the control of the MMTV (mouse mammary tumor virus) or WAP (Whey Acidic Protein) promoter initiate tumors in the mammary gland, leading to metastasis to other organs during the latter stages of cancer.[35] These mice have been a relevant tool to investigate the spontaneous initiation of breast tumors and to follow each step of metastasis progression.

Gene signature

Bioinformatics analysis of gene expression profiles in tumor cells supports the idea of existence of a so-called ‘‘gene signature” representing global changes in a group of genes (i.e., clusters) between normal cells and cancer cells .[36,37] As mentioned above, microarray analysis using tissue samples of GEM and cancer patient at different stages of cancer development provides evidence that a subset of genes is selectively activated or repressed under certain conditions. For example, analysis of tumor cells infiltrating the brain identified COX2, the EGFR ligand HBEGF, and ST6GALNAC5 as mediators for the passage of cancer cells through the blood–brain barrier.[38] Interestingly, certain cytokines, exemplified by Cxcl12, Igf1, and Pdgfa, are selectively overexpressed in bone metastatic tumor samples

compared to other metastatic tumor samples.[39] A group of genes that are expressed in matrix remodeling or tumor angiogenesis processes are differentially expressed in tumor-associated macrophages versus normal splenic macrophages.[40] The identification and understanding of tissue or cell-specific gene signatures provides a molecular basis for the development of drugs to selective therapeutic targets.

Recently, many consortiums including national and clinical research centres have constructed databases of gene expression profiles from cancer cell lines, tissues, and patient samples. These databases support cancer research by contributing clinical data, and their applications go beyond in vitro experiments. Analysis of ‘‘new gene signatures” will shed light on solving the complex nature of the underlying mechanisms of cancers including breast cancer.

In vivo imaging

Imaging technology has been developed to understand the complexity and dynamics of tumor cells in vivo. Indeed, fluorescence imaging technology can be used to investigate networks in malignant cells and normal cells with the dynamic behaviours of invasive tumor cells in real time. For breast cancer research, single cell movement in mammary tumors was demonstrated with MMTV-GFP or MMTV-Cre/CAG-CAT-EGFP transgenic mice.[41] In these mice, carcinoma cells were labelled with green fluorescent protein (GFP) and monitored by fluorescence microscopy. MMTV-PyMT transgenic mice crossed with ACTB-enhanced cyan fluorescent protein (ECFP) or c-fms-enhanced GFP (EGFP) showed macrophage infiltration more precisely.[42] In another strategy, MMTV-PyMT/c-fms-GFP or MMTV-PyMT/lys-GFP mice made it possible to track GFP-tagged macrophages during mammary tumor development.[43] These experiments suggested that tumor cell intravasation occurs in association with macrophages in mammary tumors.

During the chemotactic migration of carcinoma cells via blood vessels in tumor metastasis, differences in cell polarity of metastatic tumor cells (MTLn3-GFP) and non-metastatic tumor cells (MTC-GFP) were confirmed by the examination of intravital images.[44] In addition, cancer imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single photon emission computed tomography (SPECT) are used for basic research and clinical trials.[45]

Application of breast animal models in drug development:

For the development of new therapies based on the biological understanding of breast cancer, animal models can be used. Prior to human testing, preclinical animal models are used to predict drug safety and efficacy[46] . Animal models of breast cancer can be used in a wide range of contexts and will continue to provide insights into disease progression, treatment response, and resistance mechanisms[47] To study the underlying mechanisms of resistance to drugs, pathogenesis of breast cancer and metastasis, and drug efficacy and toxicity, xenograft models and genetically engineered mice are widely used, among others [48].

Breast cancer treatments are currently based on receptor status[49]. A considerable amount of success has been achieved in the treatment of breast cancers using personalized medicine. The GEMM has been successfully utilized in "preclinical breast cancer trials. In

this regard, a Brca1 and p53 conditional double knockout mouse model is a good drug development model[50]

Animal models can be applied for studies on the biological understanding of breast cancer to the development of new therapies. Preclinical animal models are primarily used to predict the safety and efficacy of candidate drugs prior to use in humans. Breast cancer animal models are useful in many different contexts and will continue to contribute to our understanding of disease progression, treatment response, and resistance mechanisms. Spontaneous and induced breast cancer models are rarely used in routine screening of anti-tumor drugs. Currently, transplantation and transgenic models are the most common. Xenograft models and GEMMs are widely used to elucidate the underlying mechanisms of drug resistance, pathogenesis of breast cancer and metastasis, and drug efficacy and toxicity.

Current treatments of breast cancer are based on receptor status. Personalized medicine has achieved considerable success in the treatment of breast cancers. Commonly used targeted drugs for ERα-positive metastatic breast cancer include anti-estrogens (e.g., tamoxifen and fulvestrant), aromatase inhibitors (e.g., letrozole and anastrozole), CDK4/6 inhibitors (e.g., palbociclib, ribociclib, and abemaciclib), and PI3Kα inhibitors. For HER2-positive breast cancer patients, trastuzumab and pertuzumab are the most effective agents. TNBC patients are usually treated with chemotherapy, including anthracyclines, taxanes, and platinum, and targeted therapies, including PARP inhibitors (e.g., olaparib and talazoparib) for BRCA1/2 mutation carriers and anti-PD-L1 mAb (e.g., atezolizumab) for PD-L1-positive patients. Different breast cancer animal models have been used for drug efficacy evaluation, biomarker identification, and resistance research.[51]

CONCLUSION:

Breast cancer is the most common type of cancer in females. Despite recent advances in its diagnosis and effective therapeutic strategies, further investigations into tumorigenesis, metastasis, and resistance are urgently required. In this review, we provide an overview of animal models available for breast cancer. Nonetheless, despite the sophistication and physiological relevance of these human cancer models and animal models (e.g., genetically engineered tumor models and primary human tumografts), the ultimate proof of concept for efficacy and safety of novel oncology therapeutics lies in humans.

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