ZOOLOGICAL RESEARCH

# Breast cancer animal models and applications

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## ABSTRACT

Breast cancer is the most common malignancy in women. Basic and translational breast cancer research relies heavily on experimental animal models. Ideally, such models for breast cancer should have commonality with human breast cancer in terms of tumor etiology, biological behavior, pathology, and response to therapeutics. This review introduces current progress in different breast cancer experimental animal models and analyzes their characteristics, advantages, disadvantages, and potential applications. Finally, we propose future research directions for breast cancer animal models.

**Keywords:** Breast cancer; Animal models; Metastasis; Drug development

## INTRODUCTION

Breast cancer is the most common disease in women worldwide both in terms of morbidity and mortality (Fidler et al., 2017). By 2018, there was an estimated 18.1 million new cancer cases and 9.6 million cancer deaths globally, including ~2 million new breast cancer cases, accounting for 11.6% of all cancer cases, and ~626 000 breast cancer deaths, accounting for 6.6% of all deaths (Bray et al., 2018). Among breast cancer patient deaths, many have stemmed from a lack of cost-effective treatment (Anastasiadi et al., 2017). Animal models have played a vital role in the history and development of basic and translational breast cancer research in humans. This review introduces different experimental animal models of breast cancer, from the selection of animals to the establishment of different animal models, and analyzes their characteristics, advantages, disadvantages, and potential applications. Finally, we propose future research directions for breast cancer animal models.

## ANIMAL SPECIES

### Non-mammals

Non-mammalian animals, such as *Caenorhabditis elegans*, *Drosophila*, zebrafish, and chickens, are frequently used to mimic breast cancer cell growth, migration, and metastasis. The benefits of utilizing these animals include rapid experimental cycles and low costs because of their short reproductive cycles. Chickens and zebrafish have been applied to study tumor angiogenesis (Gheorghescu et al., 2015; Jagadeeshan et al., 2017). For example, Mercatali et al. (2016) injected primary cultured bone metastases cells from breast cancer patients into zebrafish embryos to study their metastatic potential. Ren et al. (2017) transplanted fluorescent protein and chemically labeled human breast cancer cells into zebrafish embryos and visualized the spatiotemporal processes of cancer cell spread, invasion, and metastasis.



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However, the disadvantage of these animals is that they are very different from humans and appear to lack many homologous genes. Additionally, the physiological structures of most organs are very different between these animals and humans.

### Mammals

Compared with non-mammalian animals, mammals are more similar to humans. Rodents, especially mice and rats, are the most popular animals for breast cancer research. In addition, tree shrews are increasingly used due to their closer evolutionary relationship to primates than to rodents. However, the disadvantages of using mammals for breast cancer research include long experimental periods and high costs.

Among mammalian animals, mice are the most popular (Jonkers & Derksen, 2007) due to their small size, low cost, short generation time, and mature gene editing technology. Mice are also similar to humans in terms of anatomy, physiology, and genetics. Additionally, there are many inbred strains of mice available. However, breast cancer mouse models also have several drawbacks. For example, mice can tolerate higher doses of drugs than humans, and thus the blood concentration that can be achieved in mice cannot be reached in human patients; as such, 90% of new anticancer drugs fail in human clinical trials even though they were effective in mouse models. Additionally, mouse breast cancer metastasis usually occurs in the lung, but not the lymph node, liver, bone, and brain (Kim & Baek, 2010), where human breast cancers usually metastasize.

Other rodents (e.g., rats, hamsters, moles), dogs, cats, pigs, tree shrews, and non-human primates (NHPs) are also commonly used for breast cancer research. Smith et al. (2003) reported that intrauterine transplantation in cats is a reproducible experimental model for metastatic breast cancer. Spontaneous breast tumors are very common in female dogs, accounting for 50% of all tumors diagnosed. Sahabi et al. (2018) also found dogs to be a suitable large-animal model for human breast cancer. NHPs, such as monkeys, are similar to humans and have been widely used to study human diseases, including breast cancer. However, due to the low incidence of spontaneous tumors, long incubation periods, and high costs, NHPs have not been widely used in cancer research (Xia & Chen, 2011).

Tree shrews are a new experimental animal model and are considered advantageous because they are small in body size (100–200 g) and highly productive (2–3 offspring) (Xia et al., 2012; Xiao et al., 2017). Tree shrews exhibit several advantages. For example, they contain three pairs of breasts, reach sexual maturity at 3–4 months, with pregnancy and lactation lasting only 40–45 and 35–40 days, respectively, can breed for up to three years, and have a lifespan of 5–7 years (Fan et al., 2013). In addition, high-quality genome sequences have revealed that tree shrews are evolutionarily close to primates (Fan et al., 2019; Xiao et al., 2017). Most importantly, tree shrews develop spontaneous breast cancers with high frequency (Xia et al., 2014). Spontaneous tumors in tree shrews were actually reported as early as the 1960s (Elliot et al., 1966). Breast tumors can be induced in tree shrews by chemical carcinogens and oncogenes (details below).

According to different requirements, different animal models have been constructed to simulate the occurrence and development of human breast cancer. These construction methods are listed in Table 1.

## SPONTANEOUS MODELS

Spontaneous tumors occur naturally in experimental animal populations. The most important characteristic of spontaneous breast cancer is that the experimental animals have not been artificially treated, and thus are similar to humans in terms of etiology.

Spontaneous breast tumors have been frequently observed in rodents (Rao et al., 1987). Although inbred mice are mostly used in spontaneous breast cancer research, both the incidence and frequency of such tumors can vary considerably among different mouse strains (C3H, A, CBA/J, and TA2), as shown in Table 2 (Russo & Russo, 1996). For example, SHN inbred mice show a high incidence of breast cancer (Nagasawa et al., 1976). TA2 inbred mice (cultivated by Tianjin Medical University), which exhibit a stable genetic phenotype, develop spontaneous breast cancer similar to human basal breast cancer in terms of biology, morphology, and phenotype, with an average age at the time of

**Table 1 Summary of breast cancer animal models**

| Model | | Method | References |
|---|---|---|---|
| Spontaneous | | No treatment | Rao et al., 1987 |
| Induced | Chemical | DMBA or MNU | Chan et al., 2007 |
| | Physical | Radiation | Russo & Russo, 1996 |
| | Biological | Lentivirus infection | Bu et al., 2009; Fisher et al., 1999 |
| Transplantation | Homeotransplantation | Spontaneous or induced breast cancer cells transplanted into same strain | Paschall & Liu, 2016 |
| | Heterograft | Human breast cancer cells or patient tumor tissues transplanted into immunodeficient animals | Burdall et al., 2003 |
| Genetic engineering mouse model | Transgenic | Oncogene activation | Rashid & Takabe, 2015 |
| | Knockout | Tumor suppressor gene inactivation | Hutchinson, 2000 |

**Table 2 Common spontaneous mouse breast tumors**

| Strain | Latency | Frequency | Pathology | References |
|---|---|---|---|---|
| C3H | 6–10 months | Breeding female mice: 95%; Virgin mice: 88%; Male mice: <1% | AC | Heston & Vlahakis, 1971; Machida et al., 2019 |
| A | | Breeding females: 80%–84% | | Strong, 1936 |
| DBA/2 | | Female mice: 72%; Virgin mice: 48%; Male mice: 1% | | Szymanska et al., 2014 |
| BALB/c | 12 months | Female mice: 5%; Virgin mice: 1% | AC | Heston & Vlahakis, 1971; Machida et al., 2019 |
| SHN | 6.6–8.7 months | Breeding rats: 97.2%; Virgin rats: 88.3% | AC | Nagasawa et al., 1976 |
| TA2 | 329.81±95.3 days | 84.1% | | Sun et al., 2008 |
| Kunming | 13.5 months | 25% | IDC | Zheng et al., 2014 |

AC: Adenocarcinoma; IDC: Invasive ductal carcinoma.

tumorigenesis of 329.81±95.3 days (Sun et al., 2008). Zheng et al. (2014) developed a spontaneous breast tumor animal model from Kunming outbred mice and found breast tumors in 25% (89/398) of female breeding mice, with an average time of tumorigenesis of 13.5 months.

In addition to rodents, spontaneous breast cancer has been reported in large animals, such as dogs, cats, tree shrews, and monkeys. Canines are of great value in studies as they are outbred, large in size, exhibit a high incidence of spontaneous breast cancer, live in similar environments as humans, and have an intact immune system. Dogs and humans also show more than 80% genetic similarity. Furthermore, canine breast cancers are frequently observed in pet hospitals and are often treated with therapies or under clinical trials (Mottolese et al., 1994). Peña et al. (2003) analyzed the histopathological morphology and clinical characteristics of spontaneous inflammatory breast cancer in 21 dogs and revealed that such cancer can be used as a spontaneous model of human inflammatory breast cancer. Spontaneous breast tumors have been reported in tree shrews as early as the 1960s (Elliot et al., 1966). Xia et al. (2012) also reported the occurrence of intraductal papillary tumors in tree shrews. Furthermore, Xia et al. (2014) analyzed 18 spontaneous tree shrew breast tumors with hematoxylin and eosin staining and immunohistochemistry (IHC), and identified four cases of intraductal papilloma (4/18, 22.2%), 10 cases of papillary carcinoma (10/18, 55.5%), and four cases of invasive ductal carcinoma (4/18, 22.2%). Among them, five cases of spontaneous breast tumors carried PIK3CA/PTEN mutations, which activate AKT (Xia et al., 2014). Interestingly, the well-known tumor suppressor gene *TP53* showed no mutation in these tumors, suggesting that the PI3K-AKT pathway may play an important role in tree shrew breast tumor initiation. Consistently, human breast tumors also show a high frequency of PIK3CA and PTEN mutations (Koboldt et al., 2012). These findings suggest that tree shrew spontaneous breast tumor models may well mimic a subset of human breast tumors. Currently, however, there are no tree shrew strains with a pure genetic background to establish orthotopic tumor transplantation models (Xiao et al., 2017).

Spontaneous breast tumors are similar to human tumors because they occur naturally in genetically heterogeneous populations. The disadvantages of spontaneous breast cancer animal models include low incidence rates, long latency, lengthy experimental periods, and non-synchronization. Therefore, spontaneous breast cancer animal models are usually used for studying cancer etiology and treatment.

## INDUCED MODELS

To increase breast tumor incidence rates and accelerate tumorigenesis, scientists can artificially treat animals with chemical, physical, and biological carcinogens through oral administration, injection, and whole-body treatment (Su et al., 2010). The most common method is administration of 7,12-dimethylbenz(a) anthracene (DMBA) or N-methyl-N-nitrosourea (MNU) (Russo & Russo, 1996; Thompson & Singh, 2000).

In mice, DMBA, 3,4-benzopyrene, 3-methylcholan-threne (MCA), 1,2,5,6 dibenzanthracene, and urethane have been used to induce breast cancer. Most chemically induced breast tumors in mice are adenomas and type B adenocarcinomas (Russo & Russo, 1996). Fabris et al. (2014) used DMBA to induce breast tumors in female (BALB/c×DBA/2) F1 mice with an incubation period of seven months, but found that progesterone or a combination of medroxyprogesterone acetate (MPA) and DMBA can shorten the incubation period to three months and increase the incidence of breast cancer. Lanari et al. (1986) continuously administered MPA in BALB/c mice and induced mammary ductal carcinoma in 79% of mice with an incubation period of one year.

In rats, DMBA, MNU, MCA, 2-acetylamino-fluorene, 3,4-benzopyrene, ethylnitrosourea, and butylnitrosourea are widely used to induce breast cancer (Russo & Russo, 1996; Welsch, 1987). Common inducible rat breast cancer models are shown in Table 3. DMBA and MNU-induced rat breast cancers are mostly hormone dependent (Russo & Russo, 1996). The most common method for generating induced rat breast cancer models is to treat Sprague-Dawley (SD) or Fischer 344 rats with DMBA or NMU, usually by intravenous, subcutaneous, or intragastric administration (Gullino et al., 1975; Thompson & Meeker, 1983). NMU-induced primary rat tumors are similar to ERα-positive low-grade human breast cancer (Chan et al., 2007). Following gavage administration of 20 mg of DMBA in 47-day-old SD rats, Barros et al. (2004) reported a tumor induction incubation of 8–13 weeks and an incidence of breast tumors close to 100% at 13 weeks.

**Table 3 DMBA or MNU-induced mammary tumors in female rats**

| Strain | Age (d) | Carcinogen | Dose | Route | Primary tumor | | References |
|---|---|---|---|---|---|---|---|
| | | | | | Incidence (%) | Latency | |
| SD | 47 | DMBA | 20 mg/kg | ig | 100 | 8–13 w | Barros et al., 2004 |
| | 50 | NMU | 50 mg/kg | iv | 73 | 86 d | Gullino et al., 1975 |
| NSD | | DMBA | 5 mg/animal | ip | 100 | | Russo et al., 1990 |
| | | NMU | 50 mg/kg | iv | 100 | | Russo et al., 1990 |
| BUF/N | 50 | NMU | 50 mg/kg | iv | 89 | 77 d | Gullino et al., 1975 |
| F344 | 50 | NMU | 50 mg/kg | iv | 89 | 94 d | Gullino et al., 1975 |

SD: Sprague-Dawley; F344: Fischer 344; NSD: Inbred S -D; BUF/N: Buffalo; iv: Intravenous; ig: Intragastric; ip: Intraperitoneal injection; d: Day; w: Week.

Furthermore, Gullino et al. (1975) found that a single intraperitoneal injection of 50 mg/kg MNU in 50-day-old SD rats induced mammary tumors in more than 73% after 86 days. This model has several advantages, such as low cost, high specificity, high reproducibility, and the induction of ERα-positive tumors.

Xia et al. (2014) demonstrated that intragastric administration of DMBA can induce breast tumors in 3–4-month-old female tree shrews with a low frequency (12%), with the addition of MPA increasing the incidence to 50% after a seven month incubation period. They identified three intraductal papillary carcinomas and one invasive ductal carcinoma, with *PIK3CA* gene mutations detected in all tumors induced by DMBA and MPA. Consistently, these mutations were positively correlated with the activation of AKT. These genetic alterations in induced mammary tumors are similar to those in spontaneous mammary tumors in tree shrews (Xia et al., 2014). Chen et al. (2019) also found that an injection of DMBA and MPA can shorten the latency of breast lesions to 56 days in this species.

Breast cancer can also be induced by physical approaches, such as ionizing radiation. In rats, X-ray or neutron radiation can induce breast cancer by whole-body or segmental irradiation (Broerse et al., 1985; Welsch, 1987). Among rat strains, SD and Lewis rats are most susceptible to radiation-induced tumorigenesis, whereas AxC, Fisher, Long-Evans, and Wistar/Furth rats are less susceptible. Mammary tumors from irradiated rats are usually hormone-dependent adenocarcinomas or fibroadenomas (Russo & Russo, 1996).

Biological induction of breast cancer mainly relies on lentiviruses to overexpress oncogenes or silence tumor suppressor genes in experimental animals. This technology was originally developed by Professor Yi Li from the Baylor College of Medicine. His team developed two new retrovirus-based systems to study the role of specific genes in tumorigenesis. The principle is shown in Figure 1. The first method replies on RCAS (replication competent ALV-LTR splice acceptor) and TVA (TVA tumor virus A) to stably introduce oncogenes into somatic cells *in vivo.* Specifically, chicken DF-1 cells are transfected with a plasmid encoding a replication-competent subgroup of avian virus vector RCAS (vector contains viral genes *gag, pol, and env* and the gene of interest (gene X)) to produce a high titer virus, whose surface glycoprotein attaches to the extracellular domain of the TVA receptor on the cell surface of avian cells (Du & Li, 2007). Recombinant avian retroviruses are generated to infect TVA transgenic mice to produce various cancer models, including breast cancer (Fisher et al., 1999). The RCAS virus containing foreign genes of interest can be directly injected into the glands of MMTV-TVA or WAP-TVA transgenic mice to induce breast tumors. The MMTV-TVA transgenic mice are injected with $10^7$ RCAS viral particles encoding the *PyMT* (T-antigen in mouse mammary tumor virus-polyomavirus) gene in mammary ducts, resulting in a median tumor latency of 12.5 days. Infected mice develop tumors in all infected glands within three weeks (Du et al., 2006). In addition to *PyMT*, several other oncogenes, including *Neu* and *Wnt-1*, can also induce breast tumors. It takes about seven months for MMTV-TVA transgenic mice infected with RCAS-Neu to induce tumors in half of infected mice (Du et al., 2006).

The second method is based on the FUCGW lentiviral vector, as shown in Figure 1B. The gene is stably introduced into the mouse mammary gland by injecting a FUCGW lentiviral vector with the gene of interest to construct a breast cancer model. Compared with the RCAS-TVA system, lentiviruses can infect any cells and can accommodate larger inserts (Bu et al., 2009). Dong et al. (2016) constructed a lentivirus expressing *PyMT* to induce breast tumors, with all mice developing tumors within 30 days. The median incubation period is reported to be nine days and all tumors are ERα-/PR- (Bu et al., 2009; Dong et al., 2016; Mukherjee et al., 2006). Ge et al (2016) induced breast tumors in tree shrews by injecting a lentivirus expressing the *PyMT* oncogene into the ducts of 22 tree shrews. They reported an incubation period of three weeks and development of breast tumors in all injected animals at seven weeks, including papillary carcinomas (main tumor type) and lymph node metastasis and lung metastasis in one case (Ge et al., 2016).

The advantages of induced breast cancer animal models include relatively high incidence rates, short latencies, and more reliable predicted results compared with spontaneous breast cancer animal models. The disadvantages are low efficiencies, long incubation times, different incidence times, and different pathological characteristics. Breast tumors induced by carcinogens are usually hormone-dependent adenocarcinomas. In addition, number of tumors, latency, and

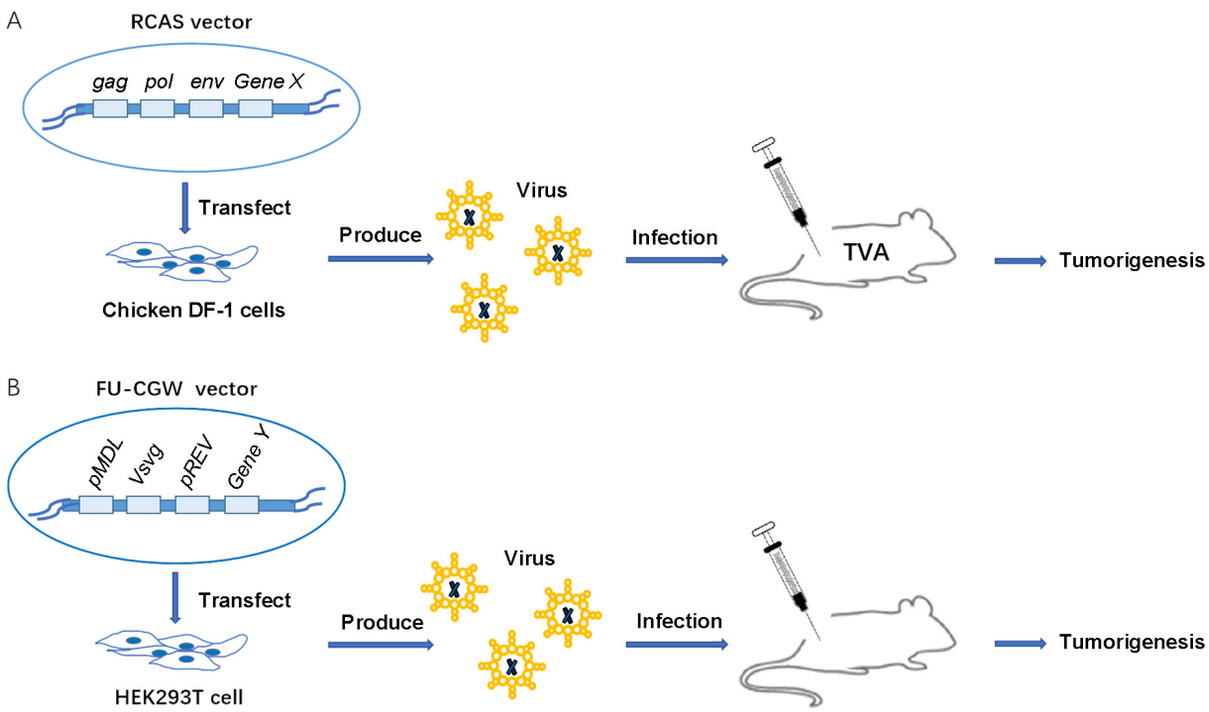


**Figure 1 Biological approach to induce breast tumors**

A: RCAS-TVA system induces breast tumors in TVA transgenic mice under control of MMTV or WAP promoters. B: FU-CGW lentivirus system induces breast tumors in normal mice or tree shrews.

histological type in animals can be affected by age, reproductive history, and the host's endocrine environment when exposed to carcinogens (Russo & Russo, 1996). Overall, induced breast cancer animal models are used for studies on etiology and prevention (Mollard et al., 2011).

## TRANSPLANTED MODELS

Transplantation models involve the transplantation of spontaneous or induced breast cancer tissues or cells into experimental animals (DeRose et al., 2011). According to the source of the transplant, models can be divided into allograft and xenograft, with the latter requiring immunodeficient mice. Transplantation sites can be divided into orthotopic and ectopic transplantations, and the latter can be divided into subcutaneous transplantation, tail vein injection to generate lung metastasis, and left ventricular injection to generate bone and brain metastasis (Mollard et al., 2011). For orthotopic transplantation, an intraductal route of transplantation is considered a better alternative to mammary fat pad transplantation. Indeed, intraductal transplantation can generate a better pathological microenvironment for breast cancer cells; however, fewer cancer cells can be injected, and it is technically challenging. Currently, the most popular animal model for testing new therapies is the transplantation model, especially the human xenograft model (Lacroix & Leclercq, 2004). The transplantation model has the advantages of short cycles, low costs, small variations, and high tumor formation rates.

### Allograft models

Spontaneous or induced breast cancer cell lines can be transplanted into the same genetic strain with normal immune function (Figure 2A). Several transplantable animal breast cancer cell lines have been established, most of which are derived from mice. Breast cancer cell lines used for allogeneic transplantation have strict germline specificity. Commonly used mouse-derived breast cancer cell lines include 4T1, EMT6, TM40, and D2A1 derived from BALB/c mice; E0771 from C57BL/6 mice; and MVT1, 6DT1, and M6 from FVB mice (Yang et al., 2017), as shown in Table 4. Most mouse cell lines are derived from spontaneous breast tumors in inbred and genetically engineered mice (Yang et al., 2017). Among them, the BALB/c-derived 4T1 model is a major transplantable mouse breast cancer model for screening anti-cancer drugs and studying tumor and host-derived factors associated with spontaneous metastasis to the lung, brain, bone, and other organs (Aslakson & Miller, 1992; Kusuma et al., 2012). EMT6 is another BALB/c-derived hormone receptor-negative murine breast tumor cell line (Rockwell et al., 1972). This model has the advantage of short latency and is often used to screen and evaluate pre-clinical anti-tumor drugs (Duan et al., 2013). Additionally, Ehrlich ascites carcinoma (EAC) is a spontaneous murine mammary adenocarcinoma carried in outbred mice by serial intraperitoneal passages. EAC is an undifferentiated carcinoma, which exhibits a rapid growth rate in suspension and sensitivity to chemotherapies (Ozaslan et al., 2011).

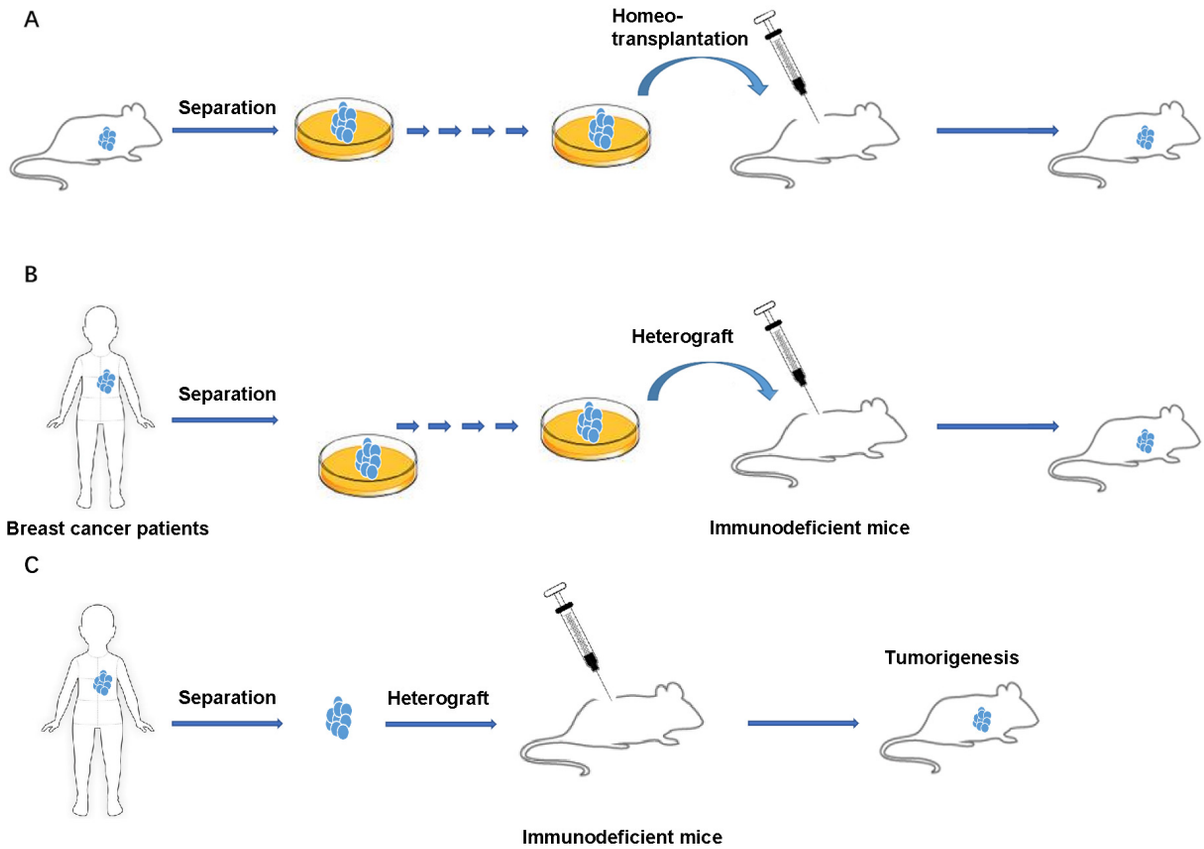


**Figure 2 Transplanted breast cancer animal models**

A: Allograft breast cancer animal model: Mouse or rat-derived breast tumor cells are transplanted into the same genetic background animals. B: Cell line-derived xenotransplantation breast cancer animal model: human-derived breast cancer cell lines are transplanted into immunodeficient mice. C: PDX model of breast cancer: tumor tissues from human breast cancer patients are transplanted into immunodeficient mice.

**Table 4 Basic characteristics of commonly used mouse breast cancer cell lines**

| Cell line | Origin | Latency | Pathology | Metastasis | Transfer site | References |
|---|---|---|---|---|---|---|
| 4T1 67NR 4T1.2 | BALB/C | 8–17 d | Luminal<br>Basal | Yes<br>No | Lung | Johnstone et al., 2015 |
| TM40D | BALB/C | 1 w | | Yes | Lung | Shi et al., 2001 |
| D2A1 | BALB/c | 14–18 d | | Yes | Lung, heart | Morris et al., 1993 |
| EMT6 | BALB/c | 3–5 d | | Yes | Lung | Duan et al., 2013; Rockwell et al., 1972 |
| E0771 | C57BL/6 | | Basal | Yes | Lung | Johnstone et al., 2015 |
| MVT1 | FVB/N | | Luminal | Yes | Lung | Pei et al., 2004 |
| 6DT1 | FVB/N | | Luminal | | | Yang et al., 2017 |
| M6 | FVB/N | 44 d | Luminal | Yes | Lung | Holzer et al., 2003 |
| CST | FVB/N | 20 d | Basal | | | Hámori et al., 2020 |
| EAC | Outbred | | | Yes | Lung, liver, heart, bone | Mishra et al., 2018; Ozaslan et al., 2011 |

d: Day; w: Week.

Recently, Hámori et al. (2020) introduced a newly established *Brca1*-/-, *p53*-/- mouse breast tumor cell line (CST). CST shows significant features of triple negative breast cancer (TNBC) with *BRCA1* mutations, as well as sensitivity to platinum-based chemotherapy and PARP inhibitors. Following the transplantation of $1.5\times10^6$ of CST cells into the fat pads of wild-type female FVB mice, Hámori et al. (2020) reported a tumor incidence rate of 100% and an incubation period of 20 days.

Additionally, several rat breast cancer cell lines, such as

UHKBR-01 and RM22-F5, are available for allograft. The UHKBR-01 cell line was initially established from DMBA-induced female SD rat breast tumors. UHKBR-01 cells show a slow growth rate in culture and are highly tumorigenic in nude mice. UHKBR-01 cells are positive for both ERα and PR (Chow et al., 2003; Mollard et al., 2011). The RM22-F5 cell line was first derived from a spontaneous malignant breast tumor in an old female Wistar rat (Nakanishi et al., 1995).

Although allograft breast cancer models have several advantages, such as multiple characterized cell lines, rapid growth and metastasis, and immune-component microenvironment, these models also have limitations. Above all, the transplanted cancer cells are not from humans.

**Xenograft models**

Cell-derived xenografts (CDX) can be transplanted and grown in immunodeficient mice, such as nude mice (lacking T cells), NOD-SCID mice (lacking T and B cells), and NSG mice (lacking T, B, and natural killer (NK) cells and macrophages) (Chakrabarti & Kang, 2015)(Figure 2B). Methods of xenograft include subcutaneous, intravenous, cardiac, and orthotopic inoculation. The orthotopic CDX transplantation model involves the transplanting of tumor cells into the mammary fat pads of mice to study growth and metastasis (Fantozzi & Christofori, 2006b; Hoffman, 1999). The tail vein injection is suitable for monitoring experimental lung metastases (Jiang et al., 2014).

The characteristics of human breast cancer cell lines commonly used for xenograft transplantation are shown in Table 5. ERα-positive luminal A cell lines, such as MCF-7 and T47D, only grow in the presence of estrogen in mice. Several HER2 subtypes, including SK-BR-3 and MDA-MB-453, have weak tumorigenic potential. TNBC cell lines, such as MDA-MB-468, HCC1806, HCC1937, and MDA MB-231, are highly tumorigenic (Holliday & Speirs, 2011).

Due to long-term *in vitro* culture, human breast cancer cell lines differ from primary tumors in terms of genetic aberrations, gene expression patterns, pathological characteristics, drug responses, and tumor microenvironments. Patient-derived xenograft (PDX) models (Figure 2C) are becoming increasingly popular as they are directly derived from human tumor specimens and have never been cultured *in vitro*. These xenografts are very close to patients in terms of genetic abnormalities, gene expression profiles, pathological parameters, metastatic potential, and drug response (DeRose et al., 2011). PDX models are used to identify biomarkers for personalized drug selection and to overcome the limitations of CDX transplantation in clinical treatment (Cho et al., 2016; Pillai et al., 2018). Many institutions now build their own PDX model libraries. For example, the Novartis Pharmaceuticals drug screening tool released in 2015 contains more than 1 000 PDX models; NCI contains more than 300 PDX models; and EurO PDX consists of 16 European research institutions and 1 500 models (Hidalgo et al., 2014). These libraries provide great convenience for the screening of preclinical drugs and basic research.

PDX models can predict clinical outcomes and have been used in preclinical drug assessment, biomarker identification, biological research, and personalized medicine (Hidalgo et al., 2014). However, PDX models are also expensive, difficult, and time-consuming to prepare because NSG mice and humanized matrix components are usually required (DeRose et al., 2011).

Patient-derived organoids (PDOs) are derived from primary human tumors and cultured *in vitro*, which preserves the complex histological architecture and heterogeneity of tumor tissue. PDOs can solve the long cycle and high cost of PDX model establishment and are suitable for mass anti-tumor drug screening (Neal et al., 2018). Duarte et al. (2018) successfully combined a well-defined genetic model of BRCA1 and BRCA2 breast cancer with organoid culture technology to develop a three-dimensional cancer organoid, with the orthotopically transplanted organoids producing breast tumors that retained the epithelial morphology and drug response of the original tumor.

Tumors in immunodeficient mice cannot faithfully copy the microenvironment of human tumors, which makes these models unsuitable for immunotherapy. Therefore, a humanized PDX (Hu-PDX) model, which recapitulates the human immune system, has been developed recently. Most humanized mice are injected intravenously with peripheral monocytes (PBMCs) or $CD34^+$ hematopoietic stem cells (HSCs) before or after tumor transplantation (Meraz et al., 2019). The Hu-PDX model can be used for tumor immunotherapy research, such as evaluating the efficacy of anti-PD-L1/PD-1 antibodies in the treatment of breast cancer. However, humanized mouse models are expensive, time consuming, and require integration of multidisciplinary expertise (Meraz et al., 2019).

## GENETICALLY ENGINEERED ANIMAL MODELS (GEMMs)

**Transgenic breast cancer animal models**

GEMMs of breast cancer are created using transgenic technology (Hanahan et al., 2007). Conventional transgenic mice are constructed by implementing tissue-specific expression of transgenes through tissue-specific promoters (Borowsky, 2011; Park et al., 2018). Multiple copies of oncogenes are then randomly integrated into the mouse genome.

Promoters commonly used in transgenic animal models of breast cancer include mouse mammary tumor virus long terminal repeat (MMTV-LTR) and whey acidic protein (WAP) promoters (Taneja et al., 2009). The MMTV is an important virus causing mammary tumors in mice. The MMTV promoter drives transgene expression in ducts and alveolar cells at all developmental stages of the mammary gland. The MMTV promoter is hormone-activated and its activity is significantly enhanced during pregnancy (Pattengale et al., 1989). Drawbacks of this promoter include uneven mosaic pattern activation (Stamp et al., 1992) and leakage (Liu et al., 2018).

The WAP promoter is only active in the breast during mid-pregnancy. It is activated by lactogenic hormones in mouse breast tumors, and preferentially drives the expression of transgenes in alveolar cells during small alveolar differentiation (Hutchinson & Muller, 2000). Both promoters can be used to achieve specific expression of foreign genes in breast epithelial cells to avoid tumor induction in other organs. The phenotype exhibited by WAP and MMTV transgenes may depend on the developmental stage of the individual mouse. Other less common promoters include the C3(1) promoter (5' flanking region of the C(3)1 component of the rat prostate steroid binding protein) (Maroulakou et al., 1994) and the metallothionein (MT) promoter (Törnell et al., 1991).

Overexpression of breast-specific oncogenes, such as *HER2*/*ERBB2* (erythroblast leukemia viral oncogene homolog 2), *PyMT*, *Wnt*, *Myc*, *Ras*, and *PIK3CA*, has been the primary approach for studying breast cancer in transgenic mice. The most commonly used single transgenic mouse models are

**Table 5 Characteristics of commonly used human breast cancer cell lines**

| Name | Origin | Subtype | Pathology | Transplant site | Number of tumor cells | Mouse strain | Latency | Metastasis | Metastasis site | References |
|---|---|---|---|---|---|---|---|---|---|---|
| BT20 | Breast | Basal | IDC | Subcutaneous | $6.25\times10^6$ | Nude mice | 3 w | No | | Ozzello et al., 1974 |
| BT474 | Breast | Luminal B | IDC | Left ventricle | $1\times10^6$ | Nude mice | | Yes | Bone | Lu et al., 2009; Neve et al., 2006 |
| MCF-7 | Pleural effusion | Luminal A | IDC | Mammary gland fat pad | $1\times10^6$ | Ovariectomized female athymic nude mice[b] | 1 w | Yes | Lymph nodes, lymph vessels | Harrell et al., 2006 |
| MDA-MB-231 | Pleural effusion | Basal | AC | Tail vein<br>Mammary gland fat pad<br>Left ventricle | $2\times10^5$<br>$0.5\sim1\times10^6$<br>$0.1\sim1\times10^5$ | Immunodeficient mice | 8–15 w<br>5–9 w<br>4 w | Yes | Lung, liver<br>Lung, liver, brain<br>Brain, bone | Bos et al., 2009; Cailleau et al., 1974; Minn et al., 2005; |
| MDA-MB-453 | Pleural effusion | HER2+ | AC | Mammary gland fat pad | $1\times10^5$ | NOD/SCID | 4 w | Yes | Bone | Charafe-Jauffret et al., 2009; Neve et al., 2006 |
| MDA-MB-435 | Pleural effusion | Basal | IDC | Mammary gland fat pad | $5\times10^6$ | NCr-nu/nu nude mice | | Yes | Lung | Liby et al., 2003 |
| MDA-MB-361 | Breast | Luminal B | AC | | | | | | | Neve et al., 2006 |
| MDA-MB-468 | Pleural effusion | Basal | AC | | | | | | | Neve et al., 2006 |
| SUM149 | Breast | Basal | DC | Mammary gland fat pad | | NOD/SCID | (6–8 w)[a] | Yes | Lung | Kuperwasser et al., 2005 |
| SUM185 | Pleural effusion | Luminal A | DC | | | | | | | Neve et al., 2006 |
| SUM190 | Breast | Basal | C | Mammary gland fat pad | | NOD/SCID | (6–8 w)[a] | | | Kuperwasser et al., 2005 |
| SUM1315 | Skin | Basal | IDC | Mammary gland fat pad | | NOD/SCID | (8–12 w)[a] | Yes | Lung, bone | Kuperwasser et al., 2005 |
| SUM52 | Pleural effusion | Luminal | C | | | | | | | Ethier et al., 1996 |
| T47D[c] | Pleural effusion | Luminal A | IDC | Mammary gland fat pad | $1\times10^6$ | NOD/SCID | | | | Cerliani et al., 2011 |
| SKBR3 | Pleural effusion | HER2+ | AC | | | | | | | Trempe, 1976 |
| ZR-75-1 | Ascites | Luminal B | IDC | | | | | | | Engel et al., 1978 |
| HCC1806 | | Basal | | Subcutaneously | $1.7\times10^6$ | Nude mice | 5 d | | | Wang et al., 2015 |
| HCC1937 | Breast | Basal | DC | Mammary gland | $5\times10^6$ | NOD/SCID | 10 d | | | Jia et al., 2016; Neve et al., 2006 |

AC: Adenocarcinoma; IDC: Invasive ductal carcinoma; C: Carcinoma; DC: Ductal carcinoma; a: Incubation period required for tumor to grow to about 1 cm; b: Mice were implanted with silastic pellets containing cellulose (10 mg) or 17 h-estradiol (2 mg+8 mg cellulose); c: T47D cells stably transfected with constitutively active fibroblast growth factor 2; d: Day; w: Week; m: Month.

listed in Table 6. These models can develop breast carcinoma *in situ*, and even distant metastasis at later stages (Jonkers & Derksen, 2007).

At present, the *PyMT* transgenic mouse model is commonly used because mammary tumors develop rapidly (Rashid & Takabe, 2015). MMTV-PyMT transgenic mice show obvious tumors at 4–8 weeks of age, and 84%–90% of mice develop lung metastases at 14 weeks of age (Guy et al., 1992a, 1992b; Lin et al., 2003). The pathology of these tumors is very similar to that of human breast cancers, involving hyperplasia, adenoma, and early or advanced cancer. MMTV-Wnt-1 transgenic mice are another common model used for studying TNBC and screening drugs (Li et al., 2000). This model includes extensive ductal hyperplasia in the early stage, and about 50% of female transgenic mice develop breast adenocarcinoma by the age of six months. When the tumor is first detected, there is little metastasis to the lung or proximal lymph node, but metastasis often occurs after the primary tumor is removed (Li et al., 2000). *ErbB2*/*HER2*/*Neu* is another well-known oncogene in human breast cancers. The *HER2* gene is amplified or over-expressed in about 20% of human primary breast cancers (Allred et al., 1992; Park et al., 2008). Guy et al. (1992b) constructed a MMTV/wild-type-neu transgenic mouse model and reported focal breast tumors after an incubation period of 205 days, a tumor incidence rate of 100%, and the development of secondary metastatic tumors in the lungs of 72% of transgenic mice after eight months. Bouchard et al. (1989) constructed a MMTV-c-neu transgenic mouse model that carried activated c-neu under the control of the MMTV promoter, which resulted in the asynchronous appearance of poorly differentiated breast adenocarcinomas at 5–10 months.

Davies et al. (1999) constructed MMTV-Neu and MMTV-TGFα transgenic SD rat breast cancer models. When these MMTV-Neu transgenic rats underwent repeated pregnancy and lactation cycles, they developed multiple focal hyperplasia and benign lesions, including lobular and ductal hyperplasia, fibroadenomas, cystic dilatations, and papillary adenomas. In addition to these lesions, ductal carcinoma *in situ* (DCIS) and other malignant lesions also developed with a low frequency. Similar phenotypes have also been observed in MMTV-TGFα transgenic rats (Davies et al., 1999). Moreover, some transgenic rats are highly susceptible to carcinogens, e.g., Hras128 transgenic rats. Hras128 carries a human *c-Ha-ras* proto-oncogene, including its own promoter region. Twenty-two Hras128 transgenic female rats treated with 50 mg/kg MNU rapidly developed multiple and large mammary carcinomas within eight weeks; in contrast, non-transgenic rats developed no or only small tumors within this period (Nohmi et al., 2017).

### Gene knockout breast cancer animal models

In addition to transgenic animal models, there are also tumor suppressor knockout breast cancer animal models. Knockout of tumor susceptibility genes, such as *p53*, *BRCA1/2*, and *pTEN*, in the genome of experimental animals can create breast cancer models. Breast tissue-specific gene knockout can be achieved using the Cre/loxP recombinase system (Ding & Gan, 2012) and MMTV-Cre or WAP-Cre tool mice.

*P53* is a classic tumor suppressor gene, and *P53* knockout (p53−/−) mice can spontaneously develop breast cancers and a variety of other malignant tumors (Liu et al., 2012). Breast-specific *p53* knockout mice show spontaneous tumors after

**Table 6 Basic situation of common breast cancer transgenic mouse models**

| Promoter | Transgene | Primary tumor | | Metastasis | | | Pathology | References |
|---|---|---|---|---|---|---|---|---|
| | | Latency | Incidence (%) | Incidence (%) | Latency | Metastatic site | | |
| MMTV-LTR | TGFα | 6–13 m | 40 | | | | AC | Halter et al., 1992; Matsui et al., 1990 |
| | Wild-type-ErbB-2 (HER2,Neu) | 7 m | 100 | 72 | 8 m | Lung | AC | Guy et al., 1992b |
| | H-ras | 5 w–6 m | | | | | AC | Sinn et al., 1987 |
| | c-rel | 19.9 m | 31.6 | | | Lung | AC | Romieu-Mourez et al., 2003 |
| | PyMT | 4–8 w | 100 | 84–90 | 14 w | Lymph node, lung | IDC | Almholt et al., 2005 |
| | c-Myc | 4–8 m | | | | | AC | Stewart et al., 1984 |
| | Cyclin D1 | 22 m | 40 | | | | | Wang et al., 1994 |
| | Wnt-1 | 6 m | 50 | | | Lymph node, lung | AC | Li et al., 2000; Tsukamoto et al., 1988 |
| WAP | TGFα | 6–12 m | 100 | | | | AC | Rose-Hellekant & Sandgren, 2000b; Sandgren et al., 1995 |
| | Ras | 24 w | 100 | 14 | | Lung | AC | Nielsen et al., 1991 |
| | c-Myc | 5–10 m | 100 | 20 | | Lung | AC | Rose-Hellekant & Sandgren, 2000a |
| | SV40 | 8–9 m | | | | | AC | Li et al., 1996; Santarelli et al., 1996 |
| C(3)1 | SV40 | 16 w | 100 | 15 | | Lung | IDC | Green et al., 2000 |

AC: Adenocarcinoma; IDC: Invasive ductal carcinoma; d: Day; w: Week; m: Month.

about 10 weeks and rapid tumor development after 15–20 weeks (Clarke, 2000). *PTEN* is a common mutated tumor suppressor gene in human cancers. When *Pten*+/− mice are older than six months, they develop a series of tumors, 50% of which are breast cancers (Stambolic et al., 2000).

With the development of technology, spatiotemporal specific knockout and transgene expression can be achieved (Kim et al., 2018). The most frequently used promoters are Tet-off/Tet-on and tamoxifen systems (McLellan et al., 2017; Nagy, 2000). Construction of inducible conditional gene knockout animals can solve the problem of lethality of embryos caused by conventional gene knockout and can achieve gene knockout at a specific time. For example, Xu et al. (1999) used the Cre-loxP system to construct *Brca1* conditional knockout mouse models Brca1$^{Ko/Co}$Wap-Cre and Brca1$^{Ko/Co}$MMTV-Cre. About 30% of Brca1$^{Ko/Co}$MMTV-Cre mice and 15% of Brca1$^{Ko/Co}$Wap-Cre mice developed multiple types of breast tumors at 10–13 months of age (Xu et al., 1999). Liu et al. (2011) used the Tet-off/Tet-on system to construct a mouse model of breast cancer with conditional expression of human *PIK3CA*$^{H1047R}$, in which transgene expression is under the control of the tetracycline-inducible promoter TetO and the specific construction method is to hybridize TetO *PIK3CA*$^{H1047R}$ mice with MMTV-rtTA mice. After this, doxycycline (2 mg/mL) is administered to the drinking water of the double transgenic mice to induce the expression of PIK3CA$^{H1047R}$. Results showed a breast tumor incidence of 95% and average incubation period of seven months, with pathological adenocarcinoma and adenosquamous carcinoma phenotypes of primary tumors (Liu et al., 2011).

Knockout animal models can also be efficiently constructed using the CRISPR-Cas9 system (Shao et al., 2016). CRISPR-Cas is a new genetic engineering technology with RNA-guided endonucleases. It has been used to generate genetically modified mouse models, including knockout (KO) and knockin (KI) animal models and somatic genome editing models (Dow, 2015; Flynn et al., 2015). Large-scale genome-modified mice have been successfully generated using the CRISPR-Cas9 system (Shao et al., 2016). Compared with traditional gene targeting strategies, CRISPR-Cas9 greatly improves efficiency and can knock out multiple genes at the same time (Shao et al., 2016). Li et al. (2013) used the CRISPR-Cas9 system to simultaneously edit the *Tet1*, *Tet2*, and *Tet3* genes in rats, and obtained three-gene mutant rats with an efficiency of 59.1%.

### Compound transgenic breast cancer mouse models

Several genetically engineered mice have been generated, including transgenic mice expressing high levels of specific oncogenes and knockout mice in which specific tumor suppressor genes have been depleted or mutated via homologous recombination. In combination, complex transgenic breast cancer mouse models can be created (Hutchinson & Muller, 2000). Study of these models not only highlights specific genetic events in disease progression but also the complex, multi-step nature of breast cancer progression.

Double transgenic mice developed from crossing MMTV-c-Myc and MMTV-TGFα mice exhibit breast tumors with 100% incidence at an average age of 66 days (Amundadottir et al., 1995). Similarly, MMTV-Neu and WAP-p53$^{172\ R\text{-}H}$ double transgenic mice develop multifocal breast tumors with a shorter latency (154 days) than MMTV-Neu single transgenic mice (234 days) (Li et al., 1997). p53-deficient mice have been crossed with a variety of transgenic mice, including *Wnt1* and *Ras*. Hundley et al. (1997) reported that *p53* knockout and MMTV-Ras transgenic mice develop tumors with a shorter average incubation period (2.2 months vs 8.5 months); however, *p53* deficiency alters the distribution of tumor types. Similar results have been observed in PTEN$^{+/-}$/Wnt-1 transgenic female mice (Stambolic et al., 2000).

The advantages of GEMMs are that the target is clear, the animal's immune function is usually intact, and the genetic alterations are similar to breast cancer patients. Therefore, GEMMs are widely used for etiology and preventive studies. However, there are several disadvantages of GEMMs. First, breast tumors developed from GEMMs are different from human breast tumors in histology. Second, GEMMs are usually expensive and time consuming. Additionally, MMTV promoter activity is not strictly limited to the breast and the WAP promoter requires pregnancy for activation. Finally, gene editing occurs in almost all mammary ductal epithelial cells, which does not reflect the actual situation of cancer initiation.

## BREAST CANCER METASTASIS ANIMAL MODELS

Metastasis is the leading cause of death in breast cancer patients. Priority sites for human breast metastasis include the lymph node, bone, lung, liver, and brain (Fantozzi & Christofori, 2006). After human breast cancer cells are injected into blood circulation of immunodeficient mice, distant metastases may develop. For example, intravenous tail vein injection primarily causes lung metastasis, whereas intracardiac injection results in bone metastases (Chakrabarti & Kang, 2015). This approach bypasses the early steps of migration and invasion and can generate distant metastasis more efficiently.

### Lung metastasis

An experimental animal model of breast cancer metastasis can be established by inoculating breast cancer cells subcutaneously, orthotopically, or through tail vein injection (Vargo-Gogola & Rosen, 2007). Mouse 4T1 cancer cells will easily develop lung metastasis after inoculation in the breast of BALB/c mice (Kij et al., 2018; Qin et al., 2015). EO771.LMB is a spontaneous breast tumor derived from a female C57BL/6 mouse isolated from a rare spontaneous lung metastasis of an EO771 tumor-bearing mouse. EO771.LMB cells spontaneously metastasize to the lung and show increased invasiveness compared to parental EO771 (Johnstone et al., 2015). MDA-MB-231-LM2 cells, derived from the MDA-MB-231 human breast cancer cell line, are prone to lung metastasis when injected into the mammary fat pad of

immunodeficient mice (Aceto et al., 2014). The MMTV-PyMT transgenic mouse model develops breast tumors with lung metastasis (Kaya et al., 2019). Studies have shown that *in situ* inoculation of MDA-MB-435 cells into SCID mice can successfully establish a spontaneous lung metastasis model (Bagheri-Yarmand et al., 1999).

**Bone metastasis**

Breast cancer has the highest incidence of bone metastases and has a long survival time relative to visceral metastases. There are three types of bone metastasis models: local injection to replicate bone metastasis, *in situ* implantation of breast cancer, and blood flow (intravenous and left ventricle) injections. A commonly used model of bone metastasis is via left ventricular injection (Minn et al., 2005). Injection of human MDA-MB-231 or MCF7 cell lines or mouse 4T1-2, Py8119, or E0771 cells into the left ventricle or carotid artery in a suitable mouse strain results in breast tumor cells primarily located in the long bone spine and jaw (Ottewell et al., 2014; Suva et al., 2011; Wright et al., 2016).

After 4T1 cells are inoculated into the mammary fat pads of BALB/c mice, they can spontaneously migrate to the bone, leading to 40%–60% of animals with bone metastases (Lee et al., 2014). Injection of 4T1, Py8119, or E0771 cells into the tibia or femur of BALB/c, FVB/N, or C57BL/6 mice, respectively, results in the development of osteolytic tumors in the corresponding bone (Tulotta et al., 2019; Wright et al., 2016).

Bone-targeted subcloning technology can achieve a higher rate of bone metastasis. After MDA-MB-231 cells are injected into the left ventricles of nude mice, MDA-MB-231-B subclones are obtained after repeated cycles. MDA-MB-231-B can cause bone metastasis in all experimental nude mice in only four weeks (Wetterwald et al., 2002). Nutter et al. (2014) developed a new MDA-MB-231 breast cancer bone-seeking clone (MDA-IV). After injection of $1x10^5$ MDA-IV cells into the tail vein of nude mice, tumors only formed in the long bones of mice and large tumors were clearly visible in 83% of mice (Nutter et al., 2014).

**Liver metastasis**

An experimental animal model of liver metastasis of breast cancer can be established by portal vein inoculation and intrahepatic inoculation of tumor tissue (Price, 1996). Goddard et al. (2016) developed a mouse portal vein injection method that delivers tumor cells directly to the liver. This model can cause concurrent metastases to other organs or complications of splenectomy. Three different metastatic breast cancer cell lines (high metastatic 4T1 cells, medium metastatic D2A1 cells, and low metastatic D2.OR cells) are injected into BALB/c mice via portal vein injection to establish liver metastasis models of breast cancer, which could be an important tool for studying breast cancer liver metastasis (Goddard et al., 2016).

**Brain metastasis**

Intracarotid artery injection mainly produces brain metastasis (Kim et al., 2004). Breast cancer brain metastasis models are usually modeled using TNBC cell lines. Yoneda et al. (2001) applied MDA-MB-231 for peripheral inoculation in nude mice, and repeated the sequential passage of metastatic cells obtained from the mouse brain to produce the breast cancer cell line MDA-MB-231BR, which showed preferential transfer to the brain. This cell line only produces brain metastases after intracardiac injection in nude mice (Yoneda et al., 2001). Other human breast cancer cell lines are rarely used due to the long incubation period and low specificity of brain metastases. In addition to ventricular inoculation, 4T1 subline 4TBM has been used to prepare an *in situ* model of breast cancer brain metastasis (Erin et al., 2013).

## APPLICATION OF ANIMAL MODELS IN DRUG DEVELOPMENT

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 (Clarke, 2009). 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 (Holen et al., 2017). 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 (Park et al., 2018).

Current treatments of breast cancer are based on receptor status (Cardiff & Kenney, 2011). 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) (Palmieri et al., 2013), and PI3Kα inhibitors (Keegan et al., 2018). For HER2-positive breast cancer patients, trastuzumab and pertuzumab are the most effective agents (Luque-Cabal et al., 2016; Swain et al., 2015). 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 (Lebert et al., 2018). Different breast cancer animal models have been used for drug efficacy evaluation, biomarker identification, and resistance research (Table 7).

GEMMs have been successfully used in "preclinical breast cancer trials". For example, the *Brca1* and *p53* conditional double knockout mouse model of hereditary breast cancer is a good model for drug development (Liu et al., 2007). Based on this mouse model, Rottenberg et al. (2007, 2008) revealed

**Table 7 Breast cancer animal models for drug research and development**

| Breast Cancer Typing | Therapy | Drug | Model | References |
|---|---|---|---|---|
| Hormone receptor positive breast cancer (HR+) (ERα+/PR+HER2-) | Antiestrogens | Tamoxifen | CDX model (MCF-7) | Osborne et al., 1985 |
| | | Fulvestrant | CDX model (MCF-7) | Lee et al., 1995 |
| | Aromatase inhibitor | Letrozole | CDX model (MCF-7) | Brodie et al., 1998; Wakeling et al., 1991 |
| | | Anastrozole | CDX model (MCF-7) | Brodie et al., 1998 |
| | CDK4 / 6 inhibitor | Palbociclib | CDX model (MDA-MB-435, ZR-75-1) | Fry et al., 2004 |
| | | Ribociclib | CDX model (MDA-MB-435) | Vora et al., 2014 |
| | | Abemaciclib | CDX model (MDA-MB-231) | Knudsen et al., 2017 |
| HER2-positive breast cancer (ERα-PR-HER2+) | Monoclonal antibodies | Trastuzumab | CDX model (BT474) | Baselga et al., 1998 |
| | Epidermal growth factor tyrosine kinase inhibitor | Lapatinib | CDX model (BT474) | Rusnak et al., 2001 |
| Triple negative breast cancer (ERα-PR-HER2-) | Chemotherapy drugs | Cisplatin | GEMM (Brca1 mutant breast cancer mice) | Shafee et al., 2008 |
| | ADP ribose polymerase (PARP) inhibitors | Olaparib | GEMM (BRCA1Co/Co - MMTV-Cre-p53+/− mice) | To et al., 2014 |
| PD-L1 positive patients | Immune checkpoint inhibitor | Pembrolizumab | Hu-PDX model | Wang et al., 2018 |

that breast tumors lacking BRCA1 are highly sensitive to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. These results have accelerated the clinical application of PARP inhibitors in patients with *BRCA1* gene deletion or mutation (Rottenberg et al., 2007, 2008). Jaspers et al. (2013) and Gogola et al. (2018) used the same model to illustrate how tumors become resistant to PARP inhibitors. The efficiency and resistance of topoisomerase I inhibitors topotecan and SN-38 (active metabolite of irinotecan) have also been studied using the above mouse model (Zander et al., 2012). Furedi et al. (2017a) used this model to demonstrate that pegylated liposomal doxorubicin increases the recurrence-free survival rate (by six times) and overall survival rate (by three times) compared with traditional doxorubicin. The same research group also showed that primary cancer cells from these tumors can be used to test new drug candidates (Füredi et al., 2017b). Therefore, disease-specific genetic and syngeneic models (especially transplantation of GEMM tissue into recipient strain-matched mice), along with PDX models, have great potential for assessing monotherapy versus combination therapy or neoadjuvant therapy versus adjuvant therapy after surgical resection.

## CONCLUSIONS AND PERSPECTIVES

In summary, no breast cancer animal model is perfect, with each showing its own advantages and disadvantages. Breast cancer is highly heterogeneous, and even within the same tumor heterogeneity can be marked. Therefore, no single model can fully reflect the heterogeneity and drug reactivity of all breast cancers. Each animal model can only imitate certain aspects of human breast cancer. Thus, it is necessary to combine different models to understand breast cancer biology and develop prevention and therapy methods. The xenograft model remains the primary tool for therapeutic drug discovery and evaluation, and combined PDX and GEM models will work better.

For animal species, mice are still the most widely used animal. The establishment of mouse models of breast cancer has greatly assisted in the research, prognosis, clinical drug screening, and development of new therapeutic methods for breast cancer, especially for studies on breast cancer metastasis mechanisms and the discovery of targeted drugs. In the future, it will be necessary and worthwhile to utilize other animals. In this respect, tree shrews exhibit considerable potential given their close relationship to primates. Thus, the development of gene editing techniques in tree shrews is critical.

Immunotherapy is gaining more and more attention. Mouse allograft models, humanized PDX models, and GEMMs will play increasingly important roles. However, the establishment of ERα-positive breast cancer mouse models and of tissue-specific metastasis models remain a challenge.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

L. Z. prepared the draft. C.S.C. and W.L. revised the manuscript. All authors read and approved the final version of the manuscript.

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