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Research Progress on Mouse Models of Breast Cancer Metastasis

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Abstract: Breast cancer metastasis is a major cause of treatment failure and patient mortality. Mouse tumor models largely replicate the pathophysiological processes of human tumors. Establishing mouse models of breast cancer metastasis helps to elucidate metastatic mechanisms, and *in vivo* imaging techniques enable dynamic monitoring of tumor cell metastasis in animals. This paper summarizes the mechanisms of breast cancer metastasis, the development, and application of various mouse breast cancer distant metastasis models over the past decade, and evaluates the characteristics and efficacy of each model to provide references for future experimental studies.

Keywords: Breast cancer; Transplanted tumor metastasis model; Spontaneous metastasis model; Experimental metastasis model

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1. Introduction

Breast cancer is the most common malignant tumor among women. According to the latest national cancer statistics released by the National Cancer Center in 2024, breast cancer accounts for approximately 31% of all cancer cases among women, and in 2022, newly diagnosed breast cancer cases among Chinese women surpassed those of lung cancer [1,2]. It is estimated that 20–30% of breast cancer patients may develop metastases after diagnosis and primary tumor treatment, and about 90% of cancer-related deaths are attributed to metastases [3]. For patients without metastasis, the 5-year overall survival rate exceeds 80% [4]; however, distant metastases drastically reduce this rate to about 25% [5]. The recurrence and metastasis of breast cancer severely threaten patients' quality of life and survival rates. Common metastatic sites include the bones, liver, lungs, and brain.

For non-metastatic breast cancer, the primary treatment goal is to prevent recurrence and metastasis through surgical removal of the breast and axillary lymph nodes, along with adjuvant radiotherapy. For metastatic breast cancer, treatment focuses on prolonging life and improving quality of life, typically involving

local therapies such as surgery and radiotherapy to alleviate symptoms [6]. A fundamental barrier to better treatment of metastatic breast cancer is the limited understanding of its mechanisms of metastasis, which restricts therapeutic advancements.

To address this issue, this study aims to outline the mechanisms of breast cancer metastasis, review the development and application of various mouse models of breast cancer distant metastasis over the past decade, and evaluate their characteristics and utility. This aims to provide a reference for future experimental research and offer new perspectives for optimizing breast cancer diagnostic and treatment strategies.

2. Overview of the mechanisms of breast cancer metastasis

2.1. The "seed and soil" hypothesis and organotropism in metastasis

Tumor metastasis refers to the process by which tumor cells spread from the primary site to secondary locations via the bloodstream or lymphatic system, forming new tumors. In 1889, Stephen Paget proposed the "seed and soil" hypothesis, likening tumor cells to seeds and metastatic organs to soil, suggesting that the distribution and colonization of tumor cells must adapt to the microenvironment of the target organ. Organotropism, or organ-specific metastasis, is a hallmark of breast cancer, wherein breast cancer cells preferentially metastasize to specific organs [7].

Organ-specific metastasis is a complex, multi-step process involving the dissemination of tumor cells from the primary site, vascular penetration into circulation, and eventual colonization at distant metastatic sites. This process is influenced by tumor characteristics, the immune microenvironment of the primary and secondary sites, and the metastatic organ itself. For example, molecular subtypes of breast cancer are closely associated with specific organ metastases. Studies have shown that all breast cancer subtypes are prone to bone metastases, particularly Luminal A/B subtypes. HER2-positive breast cancer patients exhibit a higher propensity for liver metastases than HER2-negative patients. Additionally, basal-like (ER^-, PR^-, $HER2^+$, $EGFR^+$, or $CK5/6^+$) and triple-negative (ER^-, PR^-, $HER2^-$) breast cancer patients show a greater likelihood of lung and brain metastases [8].

The microenvironment of distant metastatic sites also plays a significant role in organ-specific metastasis of breast cancer. Factors such as stromal components, intercellular signaling, and the presence of immune cells at the metastatic site affect tumor cell survival and proliferation. For instance, the interaction between osteoblasts and osteoclasts in the bone microenvironment promotes breast cancer cell growth and metastasis. Moreover, tumor-associated fibroblasts (CAFs), immune cells, endothelial cells, and adipocytes within the metastatic microenvironment play critical roles in cancer progression and metastasis [9]. For example, anti-tumor immune cells like cytotoxic $CD8^+$ T lymphocytes and natural killer (NK) cells interact with antigen-presenting cells (APCs) to generate cytotoxic responses against metastatic tumors [9,10]. Conversely, pro-tumor immune cells such as M2 macrophages and myeloid-derived suppressor cells (MDSCs) are recruited by inflammatory cytokines to enhance cell proliferation, angiogenesis, and migration, facilitating metastasis [11,12].

2.2. Epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is the process by which epithelial cells acquire mesenchymal characteristics, a key mechanism in cancer metastasis. EMT is reversible, as mesenchymal cells can redifferentiate into epithelial or other cell types [13]. EMT and its reverse process, mesenchymal-epithelial transition (MET), occur during wound healing, fibrosis, and tumor progression [14]. EMT allows cancer cells to

gain migratory and invasive abilities during tumor progression [15].

EMT transforms epithelial cells with strong intercellular adhesion into migratory mesenchymal cells, enabling cancer cells to travel through blood and lymphatic vessels to distant sites. EMT is essential for most cancer metastases [16]. Transcription factors (TFs) play a critical role in regulating gene expression during EMT by binding to chromatin. For example, E-cadherin, an epithelial cell adhesion protein, is crucial in preventing tumor invasion. Loss of E-cadherin is associated with the upregulation of factors like transforming growth factor-beta (TGF-β) and reactive oxygen species, altering apoptotic signaling pathways [17].

E-cadherin suppression leads to increased mesenchymal markers such as N-cadherin and vimentin [18]. Transcription factors like TWIST1, SNAIL, and ZEB1/2 are known to repress E-cadherin expression and promote EMT, playing significant roles in cancer invasion, progression, metastasis, and therapy resistance [19]. For example, the TWIST1/Mi2/NuRD protein complex suppresses E-cadherin expression, facilitating EMT and breast cancer metastasis. Silencing TWIST1 has shown potential in reducing metastatic breast cancer [20]. Similarly, SNAIL binds to the E-cadherin promoter to suppress its expression, increasing vimentin expression and advancing the EMT process [21].

2.3. Tumor stem cell theory

Recent studies suggest that cells with stem cell-like properties—capable of self-renewal and differentiation—contribute significantly to tumor initiation, progression, and metastasis [22,23]. Breast cancer stem cells (BCSCs) are a subset of cells with self-renewal, functional differentiation, and tumor-initiating abilities, playing a critical role in mediating tumor recurrence, metastasis, and resistance to chemotherapy and radiotherapy [24].

BCSCs are closely linked to distant metastases, including maintaining primary tumor stem cells, invading the circulatory system, and colonizing distant organs [25]. Studies have found that BCSC subpopulation transitions are similar to EMT and play a key role in tumor metastasis [26]. Classical biomarkers of BCSCs include CD24, CD44, and ALDH1. Based on these markers, BCSCs can be classified into three types: $CD24^{-}CD44^{+}$ BCSCs, $ALDH^{+}$ BCSCs, and $CD24^{-}CD44^{+}ALDH^{+}$ BCSCs, each exhibiting distinct biological characteristics. $CD24^{-}CD44^{+}$ BCSCs, located at the tumor invasive edge, are quiescent but highly invasive. $ALDH^{+}$ BCSCs, found in the tumor core, are highly proliferative. $CD24^{-}CD44^{+}ALDH^{+}$ BCSCs are considered the most potent, with strong tumor-initiating capabilities [27].

BCSC heterogeneity correlates with molecular subtypes of breast cancer. Luminal A/B cell lines tend to express CD24highCD44lowALDHlow, while HER2-enriched cell lines exhibit high ALDH1 activity. Basal-like cell lines display $CD24^{low}CD44^{high}$ characteristics [28]. Additionally, Tsukabe *et al.* [29] found partial overlap between $ALDH^{+}$ BCSCs and HER2-positive tumor cells.

Circulating tumor cells (CTCs) in the bloodstream are considered the “seeds” of metastasis [30]. Initial CTC retention in specific organs is often mechanical, but subsequent growth depends on the compatibility between the “seed” and the target organ “soil.” Circulating cancer stem cells (CCSCs), identified within CTC populations, play a role in breast cancer liver metastasis. CCSCs isolated from Luminal A/B breast cancer patients express epithelial adhesion molecules like EpCAM, CD44, CD47, and MET, initiating bone, lung, and liver metastases in mice [30].

In summary, the heterogeneity of BCSCs aligns with the molecular subtypes of breast cancer and their organ-specific metastatic tendencies. Therefore, BCSCs may play a crucial role in mediating organ-specific metastasis in breast cancer.

3. Common methods for constructing mouse models of breast cancer metastasis

To better investigate the complex process of breast cancer metastasis, various types of breast cancer metastasis models have been established in laboratories worldwide.

The primary types of breast cancer metastasis models include spontaneous tumor metastasis models, induced tumor metastasis models, transgenic tumor metastasis models, and transplantable tumor metastasis models. Spontaneous and induced tumor metastasis models have a low metastasis rate and are less frequently used in experimental studies on breast cancer metastasis. Although transgenic models offer advantages such as short disease progression, some predictability, high metastasis incidence, and good reproducibility, their high cost limits their current application. Transplantable tumor metastasis models, on the other hand, are widely used due to their simplicity, good reproducibility, and stable biological properties.

Based on the steps involved in the metastatic process, metastasis models can be categorized into spontaneous metastasis models and experimental metastasis models. Experimental metastasis models can further be divided into syngeneic and xenogeneic graft models depending on whether the donor and recipient are of the same species. According to the type of graft, they can be classified into tumor tissue inoculation, tumor fragment suspension injection, or cell suspension injection. Based on the source of the graft, they can be divided into syngeneic transplantation and xenogeneic transplantation, with xenografts requiring implantation into immunodeficient mice, such as T-cell-deficient nude mice or severely immunocompromised SCID (severe combined immunodeficiency) mice with T- and B-cell deficiencies.

Transplantation models can also be categorized by implantation site, such as orthotopic transplantation or ectopic transplantation. Ectopic transplantation can involve methods such as subcutaneous transplantation, tail vein injection, or left ventricular injection, depending on the experimental goals. Researchers need to select the most appropriate method based on the specific aims of their study. For instance, portal vein inoculation and splenectomy are primarily used to induce liver metastasis [31], iliac artery injection facilitates bone metastasis, and carotid artery injection promotes brain metastasis [32].

The following sections will provide a detailed discussion of spontaneous metastasis models and experimental metastasis models.

3.1. Selection of cell lines for breast cancer metastasis experimental models

Cell lines commonly used for breast cancer metastasis modeling can be categorized into human-derived breast cancer cell lines and animal-derived breast cancer cell lines.

3.1.1. Human-derived breast cancer cell lines

Human-derived breast cancer cell lines frequently used in animal experiments include MDA-MB-231, MDA-MB-435, MCF-7, ZR-75-1, and SUM1315. Most of these lines are derived from exudates at metastatic sites of breast cancer and exhibit characteristics of malignant tumors.

3.1.2. Animal-derived breast cancer cell lines

Animal-derived breast cancer cell lines currently in use are mostly derived from mice or rats. These include the mouse breast cancer cell line 4T1, the MRMT-I rat breast cancer cell line, the MADB-106 rat breast cancer cell line, and the Walker256 rat breast cancer cell line.

3.2.1. Spontaneous metastasis models

Spontaneous metastasis models involve directly inoculating tumor cells into the orthotopic mammary gland of immunocompetent mice, closely simulating the complete process of metastasis [33]. This model closely resembles the progression of human breast cancer and is theoretically considered an ideal animal model. For example, Amy et al. established a mouse model capable of developing lung and bone metastases by injecting 4T1-Luc breast cancer cells into the mammary fat pad of BALB/c mice [34].

Peng and colleagues used the 4T1 mouse breast cancer metastasis model to investigate the potential of the polymer P-DOX-iRGD in inhibiting breast cancer and its metastases. Flow cytometry and confocal microscopy were employed to evaluate the uptake of different forms of doxorubicin (DOX) by tumor cells. The results showed that the uptake of free DOX was significantly higher than that of P-DOX-iRGD, and the latter exhibited lower cytotoxicity than free DOX. In experiments using mouse models, P-DOX-iRGD effectively suppressed the growth of primary tumors and lung metastases while nearly completely preventing the increase in hepatic hematopoiesis. This suggests its positive impact on the anti-tumor immune response, providing robust experimental evidence for drug-targeted therapy. The significant anti-tumor effects of P-DOX-iRGD, combined with its mechanism of interaction with free DOX, highlight the potential of utilizing the EPR effect and iRGD strategies to reduce cancer metastases and improve therapeutic outcomes [35].

Liu *et al.* examined the effects of commonly used inhalation anesthetics (isoflurane, sevoflurane, and desflurane) on the viability, migration, growth, and lung metastasis of 4T1 breast cancer cells in a spontaneous breast cancer metastasis mouse model. Cell viability was assessed via MTT assays, migration ability was analyzed using scratch assays, and tumors were implanted and removed under anesthesia. Post-mortem lung bioluminescence imaging and histological analysis demonstrated that sevoflurane significantly increased 4T1 cell migration *in vitro*. However, none of the anesthetics had a significant impact on the growth of primary tumors or lung metastases *in vivo* [36].

Nevertheless, these models utilize tumor cell lines with high metastatic potential, limiting their ability to reflect the organ-specific metastasis characteristic of breast cancer, thereby restricting their application in studying organotropism in breast cancer metastasis.

3.2.2. Xenograft metastasis models

Xenograft models involve transplanting human breast cancer cells or tissues into immunodeficient animals for research. For instance, Du and colleagues used GFP-labeled MDA-MB-231-HM cells (control group) and FOXC1-MDA-MB-231-HM cells (experimental group) to inoculate the left second mammary fat pad of nude mice. The control group exhibited significant metastatic foci in the lungs, whereas the experimental group showed a marked inhibition of lung metastasis, indicating that FOXC1 has the potential to suppress breast cancer lung metastasis [37].

Ghajar *et al.* investigated dormant disseminated tumor cells in bone by injecting GFP-expressing MDA-MB-231 cells into the mammary fat pads of non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice. The study revealed that GFP-positive, Ki67-negative cell clusters localized along the microvascular endothelium in the bone marrow of the femur and tibia. Some researchers have also transplanted fresh patient-derived tumor cells and tissues orthotopically. However, successful replication of spontaneous bone metastasis models remains rare [38].

Although these models successfully replicate the histopathological characteristics of primary tumors and

their growth and metastatic processes, the use of human breast cancer cells in mouse stromal environments limits their applicability in studying interactions between breast cancer cells and the stroma during metastasis. Additionally, xenograft metastasis models require immunodeficient animals, such as T-cell-deficient or T- and B-cell-deficient mice. These animals must be housed under pathogen-free, temperature- and humidity-controlled conditions, with sterilized food and water, significantly increasing the complexity, time requirements, and costs of experimental operations.

3.3. Other mouse models of breast cancer metastasis

A key challenge in establishing animal models is providing tumor cells with a metastatic microenvironment that more closely resembles the human body while improving experimental efficiency. The integration of multiple models is considered the optimal strategy for studying breast cancer metastasis, and various attempts have been made in this regard.

For example, Wang *et al.* [39] aimed to establish a lung-specific metastatic cell subline of mouse breast cancer. First, an experimental lung metastasis model was created by injecting the 4T07 breast cancer cell line via the tail vein. Lung metastatic cells were then recovered, expanded *in vitro*, and reinjected, with the process repeated twice. Subsequently, a spontaneous lung metastasis model was constructed by orthotopically inoculating the mammary fat pad of mice. By combining the *in vitro* expansion of lung metastases with iterative cycles of experimental and spontaneous metastasis screening, they successfully obtained a highly lung-metastatic breast cancer cell subline.

To improve the reliability and efficiency of brain metastasis models for triple-negative breast cancer (TNBCBM), Liu [40] developed an enhanced internal carotid artery injection protocol. By employing an implanted constant-rate internal carotid artery injection device, they addressed the issue of excessive tumor cell concentration within blood vessels, which often leads to vascular embolism and subsequent mouse mortality. Using this approach, they successfully established a patient-derived xenograft (PDX) mouse model that better mimics human conditions. Based on this model, they identified optimized treatment strategies for TNBCBM.

4. Conclusion and outlook

Mouse models are indispensable tools for studying breast cancer metastasis. By simulating the pathophysiological processes of breast cancer cell metastasis, it is possible to develop models that better align with clinical realities. Despite significant progress in recent years, researchers can combine various models to create more comprehensive animal models tailored to specific research objectives.

However, substantial differences between mouse models and real-world clinical conditions remain. Reducing these discrepancies, improving the clinical relevance of models, and providing a more effective platform for developing novel therapeutic strategies require further exploration and validation.

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