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Role of cancer stem cell ecosystem on breast cancer metastasis and related mouse models

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ABSTRACT

Breast cancer metastasis is responsible for most breast cancer-related deaths and is influenced by many factors within the tumor ecosystem, including tumor cells and microenvironment. Breast cancer stem cells (BCSCs) constitute a small population of cancer cells with unique characteristics, including their capacity for self-renewal and differentiation. Studies have shown that BCSCs not only drive tumorigenesis but also play a crucial role in promoting metastasis in breast cancer. The tumor microenvironment (TME), composed of stromal cells, immune cells, blood vessel cells, fibroblasts, and microbes in proximity to cancer cells, is increasingly recognized for its crosstalk with BCSCs and role in BCSC survival, growth, and dissemination, thereby influencing metastatic ability. Hence, a thorough understanding of BCSCs and the TME is critical for unraveling the mechanisms underlying breast cancer metastasis. In this review, we summarize current knowledge on the roles of BCSCs and the TME in breast cancer metastasis, as well as the underlying regulatory mechanisms. Furthermore, we provide an overview of relevant mouse models used to study breast cancer metastasis, as well as treatment strategies and clinical trials addressing BCSC-TME interactions during metastasis. Overall, this study provides valuable insights for the development of effective therapeutic strategies to reduce breast cancer metastasis.

Keywords: Breast cancer; Metastasis; Cancer stem cell; Ecosystem; Tumor microenvironment; Mouse model

INTRODUCTION

Despite advancements in diagnosis and treatment, breast cancer remains one of the major causes of morbidity and mortality among women, with a high number of new cases and second highest number of deaths (Siegel et al., 2023; Xia et al., 2022). Common treatment strategies for primary breast cancer include chemotherapy, surgical resection, and radiotherapy; however, metastatic breast cancer presents a significant challenge (Park et al., 2022) and is a leading cause of cancer-related death. The inability to precisely predict metastasis risk in individuals results in approximately 40% of patients experiencing relapse and ultimately dying of metastatic breast cancer (Weigelt et al., 2005). The predominant organs affected by breast cancer metastases include the bones (67%), liver (40.8%), lungs (36.9%), and brain (12.6%). Bone metastasis in breast cancer is primarily found in the Luminal A (70%) and Luminal B (79%) molecular subtypes (Harbeck et al., 2019), while the Her2$^+$ and Basal-like subtypes exhibit an increased tendency to metastasize to the liver (45% and 35%, respectively), lungs (45% and 35%, respectively), and brain (30% and 25%, respectively) (Harbeck et al., 2019).

Cancer stem cells (CSCs) possess the distinct capacity for self-renewal and the differentiation into diverse cancer cell types, contributing to the inherent heterogeneity of cancer (Liu et al., 2014). Breast cancer stem cells (BCSCs) play a pivotal role in the initiation, recurrence, metastasis, and drug resistance of breast cancer (Zhang et al., 2022). *CD24*$^-$*CD44*$^+$ and aldehyde dehydrogenase (ALDH)$^+$ are two frequently used markers for the identification of BCSCs (Al-Hajj et al., 2003; Ginestier et al., 2007). Although both *CD24*$^-$*CD44*$^+$ and ALDH$^+$ BCSCs contribute to breast cancer growth, their biological roles in metastasis are distinct (Liu et al., 2014). Specifically, *CD24*$^-$*CD44*$^+$ BCSCs are associated with increased metastatic potential, while ALDH$^+$ BCSCs

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demonstrate greater proliferation (Liu et al., 2014). Furthermore, research has also suggested that ALDH$^+$ BCSCs are more invasive than other breast cancer cells (Chen et al., 2019).

Non-tumor cells within the tumor microenvironment (TME) also markedly influence metastasis. The TME is a complex, multifaceted environment, harboring both tumor-promoting or tumor-suppressing components, such as immune cells, stromal cells, and the extracellular matrix. Studies have indicated that BCSCs and various stromal cells contribute to tumor heterogeneity and progression through both direct and indirect interactions (Tan & Naylor, 2022). Breast cancer metastasis also involves the formation of an immunosuppressive microenvironment and a metastatic niche, facilitated by the dynamic interactions between cancer cells and the TME (Tan & Naylor, 2022). Hence, elucidating the mechanisms underlying BCSC and TME interactions in the context of tumor cell metastasis is crucial.

In the current review, we discuss the complex molecular and cellular processes involved in BCSC and TME interactions during breast cancer metastasis. Furthermore, we introduce mouse models used for breast cancer metastasis research and summarize clinical trials targeting disease management. Overall, this review aims to provide valuable insights into the development of effective treatment strategies for patients with breast cancer.

BCSCs PROMOTE BREAST CANCER METASTASIS

Cancer cell heterogeneity is an important factor in tumor metastasis. Cancer cells migrate directly to distant sites or promote metastasis via various mechanisms, including molecular signaling, metabolic cooperation, and exosome or soluble factor secretion (Zhang et al., 2022). BCSCs exhibit high tumorigenic ability, and their maintenance is attributed to the expression of several epithelial-mesenchymal transition (EMT)-related genes involved in the regulation of breast cancer metastasis (Wada et al., 2017; Zhang et al., 2022). BCSCs can also form vascular mimicry structures, with an increase in vascular mimicry formation strongly associated with metastasis (Li et al., 2021a). Additionally, recent research has highlighted the significance of metabolic reprogramming in regulating metastasis, linking metabolic abnormalities to the self-renewal of BCSCs (El-Sahli & Wang, 2020).

Markers of BCSCs and their functions in breast cancer metastasis

Based on xenotransplantation experiments, Al-Hajj et al. (2003) provided the first evidence of the presence of BCSCs with a *CD44*$^+$*CD24*$^{-/low}$ lineage$^-$ phenotype. In mice, cancer cells with a phenotype similar to that of *CD24*$^+$*CD29*$^+$ cells exhibit BCSC characteristics and a high frequency of metastasis (Vassilopoulos et al., 2014). Previous studies have demonstrated that *CD24*, *CD44*, and ALDHs not only act as markers of BCSCs but also play roles in cell-cell interactions and signal transduction (Jaggupilli & Elkord, 2012; Xia et al., 2023). *CD24* is a highly glycosylated glycophosphatidylinositol-anchored protein involved in cell-cell interactions, particularly cell adhesion and migration. It also serves as an immune checkpoint for tumor cell resistance against macrophage phagocytosis in breast cancer (Barkal et al., 2019; Jaggupilli & Elkord, 2012). Furthermore, *CD44*, a transmembrane glycoprotein associated with cell migration, acts as a receptor for hyaluronic acid and binds to other ligands such as osteopontin, collagens, and matrix metalloproteinases (Ouhtit et al., 2018).

ALDHs are a group of nicotinamide adenine dinucleotide (phosphate) (NAD(P))-dependent enzymes that catalyze the conversion of retinol to retinoic acids. In total, 19 ALDH isoforms have been identified in the human genome; however, only nine specific ALDH isoforms have enzymatic activity in breast cancer cells, as reported by Zhou et al. (2019) using the Aldefluor assay, potentially contributing to the characterization of ALDH$^+$ BCSCs. The involvement of ALDHs in tumor progression includes maintaining a stem cell-like phenotype, leading to rapid and aggressive clinical advancements (Xia et al., 2023). In addition, ALDH$^+$ BCSCs exhibit a propensity to rapidly form metastatic foci in the lungs via *NAS1*/*NR2F1* and EMT (Liu et al., 2021b).

Evidence also suggests heterogeneity of BCSCs, especially between *CD24*$^-$*CD44*$^+$ and ALDH$^+$ BCSCs (Liu et al., 2014). *CD24*$^-$*CD44*$^+$ breast cancer cells are mesenchymal-like BCSCs, predominantly quiescent, and localized at the front of tumor invasion, whereas BCSCs expressing ALDH are epithelial-like BCSCs, more proliferative, and more centrally localized (Liu et al., 2014). A rare population of BCSCs (ALDH$^+$*CD24*$^-$*CD44*$^+$ BCSCs) that overlaps the ALDH$^+$ and *CD24*$^-$*CD44*$^+$ phenotypes is considered the most tumorigenic (Ginestier et al., 2007). Compared with the population of ALDH$^-$*CD24*$^-$*CD44*$^+$ breast cancer cells and ALDH$^+$non-*CD24*$^-$*CD44*$^+$ BCSCs, ALDH$^+$*CD24*$^-$*CD44*$^+$ BCSCs are known to enrich the *p53* signaling pathway, stem cell pluripotency regulating pathways, and central carbon metabolism-related pathways in cancer (Liu et al., 2018). Notably, in the ALDH$^+$*CD24*$^-$*CD44*$^+$ population, *IL1R2* overexpression promotes BCSC self-renewal and breast cancer lung metastasis (Zhang et al., 2020).

EMT of BCSCs enhances breast cancer metastasis

The role of EMT in metastasis has been a topic of debate among researchers. Currently, the consensus is that tumor cells undergo EMT under specific conditions, losing their epithelial characteristics and gaining mesenchymal traits. This transformation facilitates their detachment from the primary site and entry into the circulatory system, enabling them to reach and colonize distant sites (Bakir et al., 2020). At these metastatic sites, cells typically revert to an epithelial phenotype through mesenchymal-to-epithelial transition (MET) to establish new tumors. EMT is closely regulated by transcriptional factors like zinc finger protein *SNAI1*, zinc finger protein *SNAI2*, and zinc finger E-box-binding homeobox 1/2, and various signaling pathways, including the TGF-β, Wnt, and Notch pathways (Bakir et al., 2020). Vimentin, N-cadherin, and E-cadherin are the main markers of the EMT process, with high expression of vimentin or N-cadherin or loss of E-cadherin promoting cancer metastasis.

The transition from epithelial to mesenchymal properties in tumor cells, particularly in BCSCs, plays important roles in tumor occurrence and development (Liu et al., 2014). Activation of EMT may be necessary for CSC formation (Shibue & Weinberg, 2017) and triggers various stemness-associated pathways, such as the *TGFβ*-*SMAD* and Wnt-β-catenin pathways linked to the maintenance of *CD24*$^-$*CD44*$^+$ BCSCs (Blick et al., 2010). Nitric oxide synthase 2 can increase tumor aggressiveness, and nitric oxide synthase 2 inhibitors can suppress CSC self-renewal and cell migration *in vitro*, inhibit EMT, and reduce lung metastases and tumor self-renewal *in vivo* (Granados-Principal et al., 2015).

CD24^{-}*CD44*$^{+}$ BCSCs, displaying a *vimentin*$^{+}$, epithelial cellular adhesion molecule^{-} (*EpCAM*$^{-}$), and *E-cadherin*$^{-}$ phenotype, are located at the invasive tumor edge and play a role in breast cancer metastasis (Liu et al., 2014). Additionally, yes-associated protein (*YAP*) shows a positive correlation with *CD24*$^{-}$*CD44*$^{+}$ BCSC enrichment and promotes tumor cell EMT and breast cancer metastasis (El-Sahli & Wang, 2020). ALDH^{+} BCSCs in SUM149, an inflammatory breast cancer cell line, exhibit a higher invasive potential than other tumor cells, potentially due to the activation of the *YAP*/tafazzin signaling pathway (Chen et al., 2019).

Vascular mimicry formation of BCSCs enhances breast cancer metastasis

Tumors necessitate an extensive blood supply to fulfill their metabolic requirements for nutrients, oxygen, and waste removal. However, the normal vasculature adjacent to tumors is inadequate for these needs, leading to the development of a TME characterized by hypoxia, acidosis, and elevated interstitial pressure in tumor tissue. This environment triggers the release of abundant growth factors and cytokines, stimulating angiogenesis and lymphangiogenesis to meet the needs of tumor growth and metabolism (Liu et al., 2023). Maniotis et al. (1999) discovered that tumor cells within metastatic cutaneous melanomas can form blood vessel-like channels, known as vascular mimicry, independently of endothelial cells and fibroblasts. Similar observations have been made in various other cancers, including breast, colorectal, liver, and gastric cancers (Li et al., 2021a; Wei et al., 2021). To facilitate the formation of vascular mimicry, thereby aiding tumor proliferation, tumor cells release signaling proteins such as VEGF, platelet-derived growth factor, vascular endothelial cadherin (VE-cadherin), and matrix metalloproteinases (Wei et al., 2021; Zhang et al., 2016).

While early research suggested that vascular mimicry primarily functions as a way for tumors to receive nutrients, recent studies have indicated that vascular mimicry also plays a role in the spread of breast cancer (Zhang et al., 2016). This may be due to the unique ability of malignant cells to form networks similar to blood vessels, providing the necessary resources and routes for tumor growth and metastasis (Zhang et al., 2016). The formation of vascular-like networks has also been linked to the stemness of tumor cells (Li et al., 2021a). ALDH1 is strongly associated with vascular mimicry in aggressive triple-negative breast cancer. ALDH^{+} BCSCs form mesh-like structures in Matrigel culture, a phenotype of vascular mimicry, whereas ALDH^{-} breast cancer cells do not, suggesting an association among ALDH^{+} BCSCs, vascular mimicry, and breast cancer metastasis (Izawa et al., 2018). The stem cell marker *CD44* has also been linked to enhanced vascular mimicry formation and metastasis (Wei et al., 2021). Analysis of the transcriptomes of aggressive (vascular mimicry-forming) and non-aggressive (non-vascular mimicry-forming) cells has revealed that the *CD44*/mesenchymal-epithelial transition factor (*c-MET*) signaling pathway plays a significant role in regulating vascular mimicry formation. Tumor endothelial marker 8, another BCSC marker, also promotes vascular mimicry formation and lung metastasis in triple-negative breast cancer through the Ras homolog gene family, member C (*RhoC*)/Rho-associated, coiled-coil containing protein kinase 1 (*ROCK1*)/*SMAD5* signaling pathway (Li et al., 2021a).

Despite these findings, much remains to be learned about the mechanisms underlying BCSC-mediated metastasis through vascular mimicry.

Metabolic reprogramming of BCSCs regulates breast cancer metastasis

Cancer is a metabolic disease characterized by metabolic abnormalities that occur early during tumorigenesis. Unlike normal somatic cells, which metabolize glucose through oxidative phosphorylation, cancer cells rely primarily on aerobic glycolysis, a phenomenon known as the Warburg effect (El-Sahli & Wang, 2020). Through metabolic reprogramming, cancer cells acquire energy and substances that promote cell proliferation, microenvironmental adaptation, and metastasis in various tumors, including breast cancer, colon cancer, and melanoma (El-Sahli & Wang, 2020). Breast cancer cells that metastasize to different sites exhibit metabolic differences, with liver metastatic cells demonstrating a stronger demand for glycolytic phenotypes (Dupuy et al., 2015). Additionally, breast cancer cells reprogram their lipid and cholesterol metabolism, with invasive cells exhibiting increased activated fatty acid oxidation (El-Sahli & Wang, 2020).

Metabolic differences are also apparent between CSCs and non-CSCs. For instance, BCSCs exhibit stronger glycolytic metabolism than differentiated breast cancer cells (Ciavardelli et al., 2014). *YAP* signaling is activated in triple-negative breast cancer with *CD24*$^{-}$*CD44*$^{+}$ BCSC population enrichment and is a driving factor of metastasis (El-Sahli & Wang, 2020). Further studies have reported that the shift of cancer cells to aerobic glycolysis is regulated by YAP, which promotes the transition of cancer cells to fatty acid oxidation and lymph node metastasis (Enzo et al., 2015), confirming that BCSCs can promote metastasis through metabolic reprogramming. Epidermal growth factor-like 9 promotes a Warburg-like metabolic phenotype in triple-negative breast cancer via *c-MET*/cytochrome C oxidase assembly factor 3, which increases lymph node and lung metastases (El-Sahli & Wang, 2020). The combination of *c-MET* inhibitor and glycolysis inhibition reverses the epidermal growth factor-like 9-promoted increase in ALDH^{+} BCSCs, validating the association between ALDH^{+} BCSCs and Warburg-like metabolic phenotypes.

In addition to glucose and lipid metabolism, amino acid metabolism is an important factor in breast cancer metastasis. Phosphoglycerate dehydrogenase catalyzes the first step of the serine synthesis pathway and is commonly found in estrogen receptor-negative breast cancers (Possemato et al., 2011). Previous research has shown that phosphoglycerate dehydrogenase promotes cancer cell proliferation, increases lung and bone metastasis, and enhances BCSC enrichment, with even small amounts found to lead to early lymph node and lung metastases in breast cancer (Rossi et al., 2022; Samanta et al., 2016).

Despite increasing evidence of the role of BCSCs in breast cancer metastasis, further investigations are needed to clarify the relationship between metastasis and EMT, vascular mimicry, and metabolic flow, particularly in site-specific metastasis.

TME OF BCSCs PLAYS A ROLE IN BREAST CANCER METASTASIS

BCSC-TME interactions mediate breast cancer stemness and metastasis (Mehraj et al., 2021; Zhang et al., 2022, 2023a) (Figure 1).

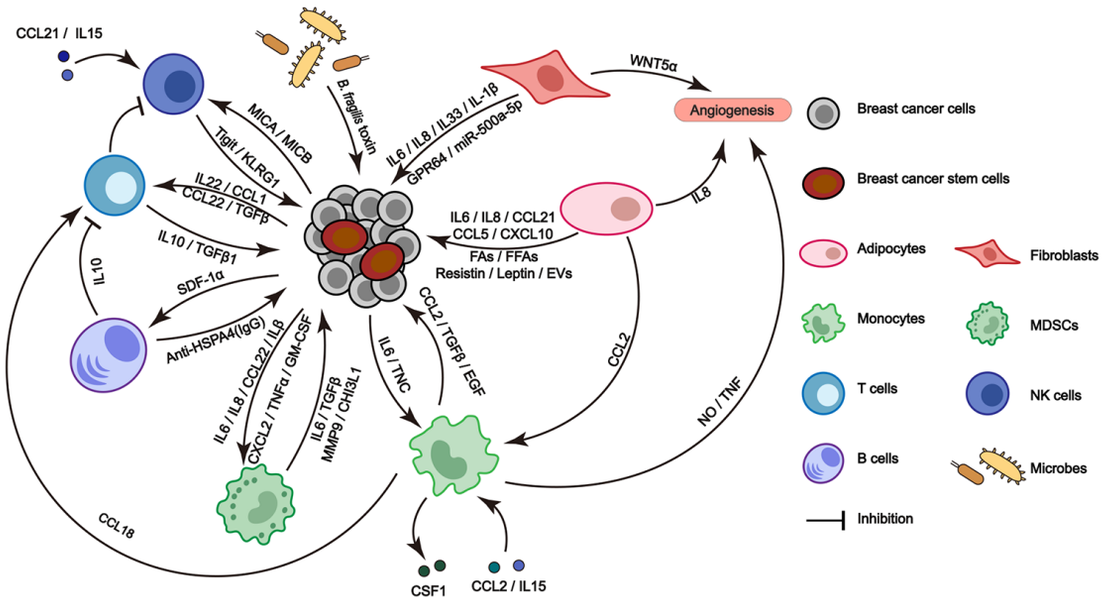

Figure 1 Factors from BCSCs and cellular components in tumor microenvironment (TME) regulate breast cancer stemness and metastasis

Impact of different cellular components on breast cancer metastasis. Breast cancer cells survive immune-killing by regulating T cells, B cells, NK cells, and MDSCs, which, in turn, promote breast cancer cell metastasis. Conversely, breast cancer cells induce the formation of tumor-associated macrophages and fibroblasts. These induced cell components assist breast cancer metastasis by increasing breast cancer cell invasiveness or by forming a metastatic niche. Cell components in the TME also interact with each other. *CD4*$^+$ T cells can be regulated by B cells and macrophages can be converted into M2-like macrophages by T cells or adipocytes, increasing the risk of breast cancer metastasis. Finally, fibroblasts, monocytes, and adipocytes can promote angiogenesis and facilitate breast cancer cell metastasis. *βGBP*, β-galactoside-binding protein; *MICA*, MHC class I polypeptide-related sequence a; *MICB*, MHC class I polypeptide-related sequence B; *TIGIT*, T cell immunoreceptor with Ig and ITIM domains; *KLRG1*, killer cell lectin-like receptor subfamily G member 1; *HSPA4*, heat shock protein family a member 4; FAs, fatty acids; FFAs, free fatty acids; EVs, extracellular vesicles; BFT, *B. fragilis* toxin.

Interactions between BCSCs and immune cells regulate breast cancer metastasis

Immune cells in the TME encompass various cell types, including lymphocytes, tumor-associated macrophages, and myeloid-derived suppressor cells (MDSCs).

Lymphocytes

Lymphocytes, including T lymphocytes (T cells), B lymphocytes (B cells), and natural killer (NK) cells, are thought to have a positive impact on breast cancer prognosis (Slaney et al., 2013). However, studies have shown that T cells can act as double agents. For example, targeting knockout of the *CCL18* receptor, membrane-associated phosphatidylinositol transfer protein 3, in *CD4*$^+$ T cells reduces lymph node and lung metastases in mice, suggesting that T cells contribute to breast cancer metastasis (Su et al., 2017). BCSCs also interact with T cells and evade *CD8*$^+$ effector T cells by increasing *PD-L1* expression, thereby promoting cancer progression (Wu et al., 2023a). *PD-L1*, in turn, promotes the expression of octamer-binding transcription factor 4 and Nanog homeobox in BCSCs through the *PI3K*/*Akt* signaling pathway (Wu et al., 2023a), suggesting that immune checkpoints may influence the metastatic process by promoting BCSCs. Furthermore, overexpression of the pluripotency factor *Sox2* in breast cancer cells can activate the nuclear factor kappa B (*NFκB*)/*CCL1* signaling pathway, recruiting regulatory T cells (Tregs) and promoting ALDH$^+$ BCSC enrichment, thus leading to breast cancer lung metastasis (Mehraj et al., 2021).

While there is no conclusive evidence of a direct interaction between BCSCs and B cells and the spread of breast cancer, tumor-educated B cells are known to activate the integrin subunit beta 5/*Src*/*NFκB* pathway, which promotes hypoxia-inducible factor 1α (*HIF1α*)/*CXCR4*/stromal cell-derived factor 1 (*SDF1*)-mediated lymph node metastasis in breast cancer (Gu et al., 2019). The *SDF1*/*CXCR4* axis also plays a critical role in regulating cancer stem cell metastasis (Kucia et al., 2005).

In general, NK cells act as suppressors of breast cancer. BCSCs with up-regulated PVR proteins can bind to the DNAX accessory molecule 1 ligand expressed in NK cells, stimulating NK cell activation and inhibiting BCSC metastatic spread (Tallerico et al., 2017). However, NK cells, when reprogrammed by *K14*$^+$ breast cancer cells, can facilitate the spread of breast cancer to the lungs by impacting certain protein receptors, such as T cell immunoreceptor with Ig and ITIM domains and killer cell lectin-like receptor subfamily G member 1 (Correia et al., 2021; Slaney et al., 2013). Additionally, *K5*$^+$*K14*$^+$ cells serve as markers of breast epithelial stem cells (Boecker et al., 2018).

Tumor-associated macrophages

Macrophages are the predominant immune cells infiltrating tumors and can be broadly categorized as either pro-inflammatory M1-like or anti-inflammatory M2-like macrophages. However, in breast cancer, tumor-associated macrophages activate both pro-inflammatory and anti-inflammatory signaling pathways, paving the way for bone and lung metastases (Allavena et al., 2021). Tumor-associated macrophages linked with bone metastasis express *CD204* and *IL4R*, enhancing the invasive capacity of tumor cells by inducing EMT (Ma et al., 2020). Additionally, tumor-associated

macrophages reprogram cell metabolism toward glycolysis and promote tumor angiogenesis by inducing HIF1 expression, which is associated with cancer metastasis (Capece et al., 2013).

Recent studies have identified the *EGFR*/*STAT3*/*Sox2* signaling pathway as a crucial mediator of the interaction between BCSCs and tumor-associated macrophages, affecting both BCSC maintenance and the progression of breast cancer lung metastases (Allavena et al., 2021). *IL-6*, another key factor involved in the interplay between BCSCs and macrophages, promotes the enrichment of tumor-promoting M2 macrophages in the TME, increasing breast cancer cell invasiveness, stemness, and EMT (Weng et al., 2019). *Jagged1/2*, released by macrophages, has been shown to activate Notch signaling and promote breast cancer cell stemness in metastatic tumors, ultimately driving lung metastases (Sharma et al., 2021).

MDSCs

MDSCs are a group of cells that suppress the anti-tumor innate immunity and adaptive immune responses. These cells are derived from common myeloid progenitors and progress from immature myeloid cells into neutrophils, macrophages, and dendritic cells. However, certain pathological conditions, such as inflammation and cancer, can cause immature myeloid cells to differentiate into MDSCs (Talmadge & Gabrilovich, 2013). MDSCs are further classified into monocytic-MDSCs (M-MDSCs) and granulocytic-MDSCs (G-MDSCs). Both types of MDSCs show increased intracellular arginase 1 expression, leading to the suppression of T cell activity. Monocytic-MDSCs are characterized by activated *STAT1* and up-regulated endogenous nitric oxide synthase 2 expression, whereas granulocytic MDSCs are characterized by activated *STAT3* and increased *NADPH* expression, which affect endogenous reactive oxygen species levels (Talmadge & Gabrilovich, 2013). In breast cancer, MDSCs promote tumor cell invasion by inducing the EMT pathway and enhancing tumor angiogenesis (Smith & Kang, 2013).

MDSCs promote breast cancer metastasis by forming an immunosuppressive environment at metastatic sites through interactions with BCSCs. Studies have identified various factors, such as *IL20RA* and *IL8*, which are associated with $ALDH^+$ BCSCs, responsible for the recruitment of MDSCs to breast cancer, thus enhancing cancer cell stemness and metastatic potential (Gao et al., 2021; Zhang et al., 2023c). *ΔNp63* regulates stem cells in normal mammary and breast cancer tissues (Chakrabarti et al., 2014; Kumar et al., 2018). Breast cancer cells with high levels of *ΔNp63* recruit MDSCs via *CXCL2* and *CCL22*, which, in turn, secrete pro-metastatic factors that promote angiogenesis, metastasis (liver, lung, and lymph nodes), and cancer cell stemness in triple-negative breast cancer (Kumar et al., 2018).

Cancer-associated fibroblasts

Cancer-associated fibroblasts regulate metabolism in themselves and surrounding cancer cells. They also play a role in remodeling the immune microenvironment (Kalluri, 2016). Studies have found that high expression of FOS-like 2 in fibroblasts can increase breast cancer cell invasion and promote endothelial cell angiogenesis independent of *VEGF* (Zhang et al., 2023c). Furthermore, exosomal miR-500a-5p from cancer-associated fibroblasts binds to and reduces universal stress protein 28 mRNA levels in breast cancer cells, thereby promoting cell proliferation and lung metastasis (Zhang et al., 2023c). Cancer-associated fibroblasts also promote *MMP9* and *IL-8* expression in breast cancer cells, thereby enhancing their invasive abilities (Zhang et al., 2023c). Inhibitors of histone deacetylases, which are chromosome-modified proteins, can effectively target BCSCs and suppress human breast cancer-associated fibroblast activity (Hii et al., 2020; Kim et al., 2018).

The role of cancer-associated fibroblasts in promoting breast cancer metastasis is attributed to the formation of a niche conducive to metastasis. $CD10^+GPR77^+$ cancer-associated fibroblasts provide a survival niche for BCSCs (Su et al., 2018). *IL-33*-expressing cancer-associated fibroblasts can tip the immune microenvironment in the metastatic niche toward type 2 inflammation, thus promoting breast cancer lung metastasis. Furthermore, endogenous *IL-33* is a potent driver of BCSCs (Hu et al., 2017; Zhang et al., 2023c).

Tumor-associated adipocytes

Tumor-associated adipocytes may influence breast cancer cells by releasing factors, such as *IL-6*, *IL1β-s*, *CCL2*, and *CCL5*, that promote cancer progression (Wu et al., 2019). Furthermore, tumor-associated adipocytes enhance invasive capabilities and protect cancer cells from ferroptosis via tafazzin-dependent protein secretion (Gao et al., 2020; Wu et al., 2023b). Tumor-associated adipocytes secreting *CCL2* recruit immunosuppressive cells to the TME, leading to the remodeling of tumor immunity and impairment of *PD-L1*-blocking immunotherapy (Liu et al., 2021a). Elevated expression of *leptin*, *IL-6*, or *IL-8* in adipocytes also promotes invasion, particularly in *IL-8*-dependent angiogenesis (Al-Khalaf et al., 2019; Khaledian et al., 2023; Wu et al., 2019).

The leptin receptor stimulates the expression of EMT and stemness genes in MDA-MB-231 cells and is associated with an increase in $CD24^-CD44^+$ BCSCs (Zheng et al., 2013). Leptin can also promote breast cancer lymph node metastasis and enrichment of BCSCs ($CD24^-CD44^+$ BCSCs and $ALDH^+$ BCSCs) via the Toll-like receptor4/nuclear factor kappa B and *STAT3* signaling pathways (Khaledian et al., 2023). Adipocytes can activate *Src* in breast cancer cells, induce the expression of pro-inflammatory factors via the *Src*/*Sox2*/miR-302b signaling pathway, and promote tumor cell stemness and lung metastasis (Khaledian et al., 2023).

Microbes

Breast tissue contains microorganisms associated with both benign and malignant diseases, showing differences in microbial composition (Hieken et al., 2016). Fu et al. (2022) found that some microbes residing in breast cancer cells promoted lung metastasis without primary tumor growth. Blood pressure in the blood vessels can damage cancer cells as they spread. Fu et al. (2022) showed that intracellular microorganisms help tumor cells resist pressure by remodeling the cytoskeleton during metastasis.

Enterotoxigenic Bacteroides fragilis, a microbe that secretes the *B. fragilis* toxin, was recently discovered in the TME of patients with breast cancer (Parida et al., 2021). Although this microbe usually colonizes the gut, it can also abnormally colonize the mammary glands and promote mammary epithelial hyperplasia, inducing breast cancer proliferation and lung metastasis. A correlation has also been observed between $CD24^-CD49f^+$ BCSCs and *Enterotoxigenic Bacteroides fragilis* in mouse tumor tissues (Parida et al., 2021). However, further studies are required to elucidate the interactions between microbes and BCSCs.

MOUSE MODELS FOR BREAST CANCER METASTASIS STUDIES

Mouse models have been widely used to study breast cancer metastasis due to their genetic, physiological, and pathological similarities to humans (Bosenberg et al., 2023; Chuprin et al., 2023; De La Rochere et al., 2018). Breast cancer metastasis models include tumor cell transplantation mouse models, genetically engineered mouse models (GEMMs), and humanized mouse models (Wilson et al., 2022; Zeng et al., 2020).

Tumor cell transplantation mouse models for breast cancer metastasis

The widely used tumor-transplanted mouse model involves the implantation of tissues/cells into mice to examine tumor growth, spread, and treatment responses. *In situ* tumor transplantation mouse models are commonly applied in breast cancer research and can be categorized into syngeneic and heterologous types. Syngeneic models simulate breast cancer growth, invasion, and metastasis within a complete TME by transplanting cancer cells into the fat pad of mice, offering advantages over subcutaneous transplantation. To accommodate the limited tumor size in mice, the observation period for metastasis can be extended by removing orthotopic allografts. For example, Correia et al. (2021) developed an *in situ* breast cancer mouse model and excised the orthotopic allografts after 4 weeks, revealing that NK cells in the liver promote breast cancer dormancy and prevent breast cancer liver metastasis.

To overcome the limitations associated with the size and experimental duration of transplanted tumors in mouse models, researchers have developed streamlined approaches for studying breast cancer metastasis. These models utilize various injection techniques tailored to the metastatic site under investigation (Table 1), including tail vein injections for lung metastases, left ventricular injections for bone and brain metastases, intraperitoneal injections for intra-abdominal metastases, splenic injections for liver metastases, and subcutaneous transplantation into the fat pad for lymphatic diffusion (Rashid & Takabe, 2015).

Methods for detecting metastatic sites include hematoxylin and eosin staining, X-ray radiography, and *in vivo* magnetic resonance imaging. Luciferase-labeled cell lines facilitate the study of metastasis by *in situ* injection, followed by detection of circulating tumor cells with an *in vivo* flow cytometer or breast cancer cell metastasis with an *in vivo* spectrum imaging system.

Targeted gene expression can be conditionally induced or inhibited by tetracycline and its derivatives (doxycycline) in tetracycline-off (Tet-off) or tetracycline-on (Tet-on) systems. For example, the Tet-off system has been used to transiently reduce osteopontin expression in MDA-MB-231 cells and delay breast cancer bone metastasis in a xenograft model (Kovacheva et al., 2019). Tet-off systems have also been used to decrease high-molecular-weight tropomyosins in MDA-MB-231 cells, negatively affecting TGFβ-mediated breast cancer lung metastasis (Zheng et al., 2008). The infusion of regulatory B cells, co-injected with cancer-associated fibroblasts or cancer-associated adipocytes via the tail vein, has also been applied to investigate the role of the TME in breast cancer metastasis (Al-Khalaf et al., 2019; Olkhanud et al., 2011; Su et al., 2018).

Genetically engineered mouse models for breast cancer metastasis

Genetically engineered mouse models efficiently simulate human cancer occurrence and development and reflect the real situation in the TME. Up-regulation of the expression levels of oncogenes (*Her2*, *Tp53*, *PyMT*, and *Wnt1*) and mutant tumor suppressor genes (*BRCA1* and *BRCA2*) stimulates breast cancer development. Breast-specific expression can be achieved using various promoters, such as long-terminal repeat of the murine mammary tumor virus, β-lactoglobulin, whey acidic protein, and C3(1). Examples of genetically engineered mouse models of breast cancer include the MMTV-*Neu* (MMTV-*Erbb2*), MMTV-polyomavirus middle T antigen (MMTV-*PyMT*), C3(1)-SV40 large T-antigen, and MMTV-*Wnt1* mouse models.

Exposure to 12-dimethylbenz[a]anthracene accelerates spontaneous breast carcinogenesis in MMTV-*PyMT* and MMTV-*Her2* mice, increases invasive *CD8*$^+$ T cell infiltration, and reduces liver and lung metastases (Li et al., 2021b). Gu et al. (2019) used MMTV-*PyMT*/μMT-deficient mice, which are unable to generate mature B cells, to reveal that tumor-educated B cells can promote lymph node metastasis in breast cancer. MMTV-*PyMT*/*p38δ*$^{-/-}$ mice show delayed primary tumor formation and reduced lung metastases (Wada et al., 2017).

Targeting tumor suppressor genes such as *Tp53*, *PTEN*, and *BRCA* can also induce breast cancer, although such modifications often result in embryonic fatality. To circumvent this, researchers have developed breast cancer-specific or conditional knockout techniques. Notably, breast-specific promoters combined with site-specific recombinases permit target gene knockout in mouse mammary glands. The Cre-loxp system, widely recognized for its precision, utilizes a recombinase-bound mutant estrogen receptor to form a Cre^{ERT}-loxp framework, enabling time-specific gene modification. Breast-specific *p53* knockout mice have been developed using whey acidic protein/MMTV promoters to target *p53* in breast cancer lung and liver metastases (Lin et al., 2004). *FAK* knockout in MMTV-Cre/*FAK*^{flox}/MMTV-*Wnt1* and MMTV-Cre/*FAK*^{flox}/MMTV-*PyMT* mouse models has been shown to slow the formation of breast cancer lung metastases (Paul et al., 2020). Complex models of breast cancer metastasis have also been developed using spontaneous breast cancer mouse models and site-specific recombinases.

Table 1 Metastatic sites in mice according to different injection methods

Injection method	Common metastatic site
In situ tumor transplantation	Lung, Liver
Tail vein injection	Lung
Left ventricular injection	Bone, Brain
Intraperitoneal injection	Intra-abdominal
Splenic injection	Liver
Subcutaneous transplantation in fat pad	Lymphatic

For instance, researchers developed the triple-transgenic mouse model (Tri-*PyMT*, MMTV-*PyMT*/Rosa26-RFP-GFP/*Fsp1*-Cre) to facilitate the direct tracking of EMT in breast cancer metastasis by converting cells expressing the EMT marker *Fsp1* from RFP+ to GFP+ (Fischer et al., 2015). Tri-*PyMT*/*Vim* (MMTV-*PyMT*/Rosa26-RFP-GFP/*vimentin*-Cre^{ERT}) and Tri-*neu* (MMTV-*neu*/Rosa26-RFP-GFP/*Fsp1*-Cre) mice have also been developed applying similar principles to control for external variables. Dre-rox has also been applied to construct a more flexible *Kit*-Cre^{ER}/EMT-gene-LSL-Dre/*NR1*/MMTV-P*yMT* mouse model to track the correlation between EMT and metastasis of breast cancer (Li et al., 2020). These models can be monitored using *in vivo* imaging systems to provide accurate insights into cancer development.

Other genetically engineered mouse models have been used to study breast cancer metastasis, with the exception of spontaneous mammary tumor models. For example, ascorbic acid has been found to inhibit lung metastasis of breast cancer in gulonolactone oxidase knockout 4T1-transplanted mouse model (Cha et al., 2013). Researchers have also used site-specific recombinase technology to pursue spatiotemporal-specific genome modifications. Using specific promoters, such as adipocyte-specific tafazzin knockout mice (*Adiponectin*-cre/tafazzin$^{flox/flox}$), tafazzin-expressing adipocytes have been reported to promote breast cancer proliferation and BCSC enrichment (Gao et al., 2020).

Humanized mouse models of immuno-oncology

At present, there is a dearth of mouse models that accurately mimic the human immune system. Humanized immunodeficient mouse models are more relevant to tumor immunity research (Chuprin et al., 2023). Current humanized mouse models include humanized-peripheral blood mononuclear cell (Hu-PBMC), humanized-hematopoietic stem cell, and humanized bone marrow, liver, and thymus (Hu-BLT) models (Chuprin et al., 2023; De La Rochere et al., 2018) (Table 2).

Injection of human PBMCs into immunodeficient mice leads to rapid immune cell reconstruction, enabling the study of immune system function and drug response (Chuprin et al., 2023). PBMCs comprise various cell types, including lymphocytes, monocytes, phagocytes, dendritic cells, and a small number of other cells. For instance, the effects of human B7 homolog 4 (*B7-H4*, *VTCN1*)/*CD3*-bispecific antibody in NSG-MHC-DKO mice have been explored via the transplantation of PBMCs, with the antibody found to promote the infiltration of activated *CD8*$^+$ T cells into breast cancer tumors, indicating its potential anti-tumor effects in patients with *B7-H4*-positive breast cancers (Iizuka et al., 2019).

While the construction of Hu-PBMC models is relatively quick, they cannot be used for long-term studies or immunological research due to their susceptibility to lethal graft-versus-host diseases (De La Rochere et al., 2018). To overcome these limitations, Hu-hematopoietic stem cell (HSC) mouse models have been developed. Hu-HSC mice, also known as humanized scid-repopulating cell mice, eliminate mouse HSCs using sublethal doses of radiation, then inject *CD34*$^+$ HSCs derived from human umbilical cord blood, bone marrow, granulocyte-colony stimulating factor-activated peripheral blood, or fetal liver (Chuprin et al., 2023). The source of HSCs is important for establishing these mouse models. For instance, fetal-derived HSCs have greater immunotolerance to T cells than adult-derived HSCs (Mold et al., 2010). Hu-HSCs have been used to verify the regulatory effects of BCSCs on the differentiation and function of PMN-MDSCs via *CCL20* (Zhang et al., 2023b). Using a Hu-HSC mouse model, Su et al. (2014) found that breast cancer cells activate macrophages to acquire a tumor-associated macrophage-like phenotype via granulocyte macrophage colony-stimulating factor, facilitating lung and liver metastasis.

Compared to Hu-PBMC and Hu-HSC mouse models, Hu-BLT mouse models demonstrate stronger T cell function and a near-complete replication of the human immune system (De La Rochere et al., 2018). These models are constructed by transplanting human fetal liver and thymus tissues into immunodeficient mice subjected to sublethal doses of radiation, followed by injection of *CD34*$^+$ HSCs from the same fetal source. The resulting Hu-BLT mouse models are well-suited for research on tumor immunotherapy (Chuprin et al., 2023; De La Rochere et al., 2018). In addition to T cells, Hu-BLT mouse models can be used to study cancer-related NK cells, which are part of the innate immune system (Chuprin et al., 2023; Kaur & Jewett, 2023). Notably, studies using Hu-BLT mouse models have demonstrated that human NK cells exhibit anti-tumor activity against poorly differentiated human oral squamous cells (Kaur & Jewett, 2023; Kaur et al., 2018).

However, while humanized mouse studies have focused on tumor immunity, research on tumor metastasis requires further exploration.

THERAPEUTIC STRATEGIES TARGETING BCSC ECOSYSTEM FOR BREAST CANCER METASTASIS

Therapeutic strategies for breast cancer metastasis encompass interventions targeting the interplay between BCSCs and the TME, including direct action on BCSCs, modulation of BCSC-associated signaling pathways, and alterations of the TME, individually or collaboratively. The

Table 2 Comparison between different humanized mouse models

Model	Construction method	Advantages	Disadvantages
Hu-PBMC	Injection of PBMCs into immunodeficient mice	Rapid construction; High efficiency of T cell transplantation	GvHD risk; Limited development of mature activated T cells, B cells, NK cells, and myeloid cells
Hu-HSC/ Hu-SRC	Inactivation of HSCs followed by injection of *CD34*$^+$ HSCs (from human umbilical cord blood, bone marrow, G-CSF-activated peripheral blood, or fetal liver)	Development of multilineage hematopoietic cells, including T cells (HLA-restricted), B cells, NK cells, and myeloid cells	Limited function and number of T cells; Complicated model construction
Hu-BLT	Injection of human fetal liver and thymus tissue with *CD34*$^+$ HSCs into immunodeficient mice	Complete immune system with human thymic education of functional T cells	GvHD risk; Limited availability of donor tissues; Complicated model construction

G-CSF, granulocyte-colony stimulating factor; GvHD, graft-versus-host disease; HLA, human leukocyte antigen; HSC, hematopoietic stem cell; Hu-BLT, humanized-bone marrow, liver, and thymus; NK, natural killer; Hu-SRC, humanized scid-repopulating cell; PBMC, peripheral blood mononuclear cell.

Table 3 Clinical trials for treating breast cancer metastasis

Clinical trial	Target	Intervention/Treatment	Subtype	Phase	Status
NCT01118975	MBC, BCSCs	Lapatinib (*EGFR* inh), Vorinostat (HDAC inh)	*HER2*$^+$	Phase II	Terminated
NCT03323346	MBC	Disulfiram	N/A	Phase II	Recruiting
NCT01281163	MBC, BCSCs	Lapatinib Ditosylate (*EGFR* inh), MK2206(*Akt* inh)	*HER2*$^+$	Phase I	Terminated
NCT04460430	MBC	Neratinib (*EGFR* inh), Exemestane (*HR* inh), Fulvestrant (*HR* inh), Tamoxifen (*HR* inh)	*HR*$^+$ /*HER2*$^-$	Phase II	Terminated
NCT01732276	MBC	Gefitinib (*EGFR* inh)	TNBC	Phase II	Unknown
NCT02020291	MBC	Foxy-5	N/A	Phase I	Completed
NCT02865304	MBC	Endostar (Angiogenesis inh), Taxane	*HER2*$^-$	Phase III	Unknown
NCT02061085	MBC	Eribulin (Microtubule inh)	*HER2*$^-$	Phase II	Completed
NCT05033769	MBC	Eribulin (Microtubule inh)	N/A	Phase IV	Recruiting
NCT02834403	MBC	L-NMMA (*iNOS* inh), Amlodipine (LTCCs inh)	TNBC	Phase I Phase II	Completed
NCT05660083	MBC	L-NMMA (*iNOS* inh), Alpelisib (*PI3K* inh)	*HER2*$^-$, TNBC	Phase II	Recruiting
NCT03291938	Solid tumors	IACS-010759 (*OXPHOS* inh)	N/A	Phase I	Completed
NCT02806817	EBC	ME-344 (*OXPHOS* inh), Bevacizumab (*VEGF* inh)	*HER2*$^-$	Phase I	Completed
NCT01548534	MBC	Dietary supplementation with fish oil and vegetable oil	*PR*$^+$, *ER*$^+$, *HER2*$^-$	Phase III	Terminated
NCT05198843	MBC	Dasatinib (Tyrosine Kinase inh), Icosapent Ethyl	TNBC	Phase I Phase II	Recruiting
NCT05402722	MBC	Eribulin (Microtubule inh), Sintilimab (*PD-1* Antibody)	TNBC	Phase II	Recruiting
NCT03430479	MBC	Nivolumab (*PD-1* Antibody)	*HER2*$^-$	Phase I Phase II	Completed
NCT03696030	MBC	*HER2*(EQ)BBzeta/*CD19t*+ T cells (*HER2*-CAR-T)	*HER2*$^+$	Phase I	Recruiting
NCT04102436	Solid tumors	TCR-T (Unknown)	N/A	Phase II	Recruiting

CAR-T, chimeric antigen receptor-T-cell therapy; EBC, early breast cancer; *EGFR*, epidermal growth factor receptor; *ER*, estrogen receptor; HDAC, histone deacetylase; *HER2*, human epidermal growth factor receptor 2; *HR*, hormone receptor; inh, inhibitor; *iNOS*, nitric oxide synthase 2; LTCCs, L-type calcium channels; MBC, metastatic breast cancer; N/A, not available; *OXPHOS*, oxidative phosphorylation; *PD-1*, programmed cell death protein 1; *PI3K*, phosphoinositide 3-kinase; *PR*, progesterone receptor; TCR-T, T cell receptor-T cells; TNBC, triple-negative breast cancer; *VEGF*, vascular endothelial growth factor. (Source: clinicaltrials.gov website).

following sections outline various studies and clinical trials that have focused on these approaches for the treatment of breast cancer metastasis (Table 3).

Targeting BCSCs to inhibit breast cancer metastases

Previous studies have suggested that BCSCs are viable therapeutic targets, prompting the initiation of clinical trials. A range of inhibitors targeting ALDH$^+$ BCSCs, including disulfiram, diethylaminobenzaldehyde, 4-dimethylamino-4-methyl-pent-2-ynthioic acid-S-methylester, dyclonine, citral, aldehyde dehydrogenase inhibitors 1–4 and 6, and dyclonine, have been documented (Xia et al., 2023). While diethylaminobenzaldehyde, a typically used negative control in the Aldefluor assay, acts on ALDH$^+$ BCSCs with anti-tumor and TME-regulating effects (Xia et al., 2023), its clinical application in treating breast cancer metastasis remains underexplored. Disulfiram, another ALDH inhibitor, has shown inhibitory effects on *CD24*$^-$*CD44*$^+$ BCSCs and is currently undergoing clinical trials for the treatment of breast cancer metastasis (NCT03323346) (Xia et al., 2023; Zhang et al., 2023a).

Targeting BCSC-related signaling pathways to inhibit breast cancer metastases

The inhibition of BCSC-related signaling pathways such as Hedgehog, *Tp53*, Notch, Wnt, *EGFR*/*Her2*, *PI3K*/*Akt*, EMT, vasculogenic mimicry, and *OXPHOS* with specific agents presents an alternative therapeutic option (Zhang et al., 2022). The efficacy of N-lapatinib ditosylate and Akt inhibitor MK2206 on breast cancer metastasis and BCSC dynamics (NCT01281163), as well as the combination of MK-0752, a gamma-secretase inhibitor, with docetaxel targeting Notch signaling to reduce the BCSC population (NCT00645333), has been investigated, demonstrating the potential of these treatments in managing metastasis (Schott et al., 2013). Clinical trials targeting *EGFR*/*Her2*, Wnt signaling, and angiogenesis have also been pivotal in advancing breast cancer metastasis treatment (NCT04460430, NCT01732276, NCT02020291, NCT02865304, NCT01503697). Notably, trial NCT04137406 revealed the role of silent information regulator 2 homolog 1 in breast cancer metastasis through EMT regulation. Currently, eribulin mesilate is under investigation (NCT02061085 and NCT05033769) for its ability to inhibit the EMT process in metastatic breast cancer. Other studies have identified that inhibitors of nitric oxide synthase 2 (1400 W) and pan-NOS (L-NMMA and L-NAME) can reduce breast cancer metastasis through EMT suppression, suggesting a targeted therapeutic regimen involving L-NMMA (Granados-Principal et al., 2015). Furthermore, several recent phase 2 clinical trials have examined the combination of L-NMMA and taxane-based chemotherapy for metastatic breast cancer (NCT02834403 and NCT05660083).

Various substances/drugs, such as salinomycin, endostatin, vitamin D, apatinib, melatonin, and thymoquinone, have been shown to inhibit breast cancer vascular mimicry, several of which are also being tested in clinical trials targeting breast cancer metastasis, such as endostar (endostatin) in *Her2* negative metastatic breast cancer (NCT02865304). IACS-010759, targeting tumor metabolism, is under study for its effect on solid tumor metastasis (NCT03291938). ME-344, aimed at antiangiogenic-induced mitochondrial metabolism, is being tested in breast cancer patients (NCT02806817). Docosahexaenoic acid as a dietary supplement during chemotherapy (NCT01548534) and icosapent ethyl combined with dasatinib for EPA-based epha2 targeted therapy in

patients with metastatic breast cancer (NCT05198843) are also currently being investigated in clinical trials.

Immunotherapies for breast cancer metastasis

In metastatic breast cancer, immunotherapy is preferred over endocrine and targeted therapies because it has more targets, fewer side effects, and less therapeutic resistance (Esteva et al., 2019). Currently, immunotherapy strategies include, but are not limited to, tumor vaccines, chimeric antigen receptor-T cells, T cell receptor-T cells, and chimeric antigen receptor-NK cells (Walcher et al., 2020). Among these, immune checkpoint *PD-1*/*PD-L1* inhibitors are the most frequently used for metastatic breast cancer (NCT05742269, NCT05402722, and NCT03430479).

Chimeric antigen receptor-T cells, T cell receptor-T cells, and chimeric antigen receptor-NK cells have also been tested in breast cancer. For example, *Her2*-targeted chimeric antigen receptor-T cells therapy is used to treat breast cancer brain metastases (NCT03696030), while T cell receptor-T cell therapy targeting multiple cancers, including breast cancer, is currently under evaluation (NCT04102436). Fibroblast activation protein is a highly expressed membrane protease in cancer-associated fibroblasts. Immunotherapy anti-fibroblast activation protein α has successfully been applied in mice to restrict breast cancer growth (Jin et al., 2023). Fibroblast activation protein-chimeric antigen receptor-T cells, another novel fibroblast activation protein-based treatment paradigm, have also successfully been applied in mice to limit breast cancer growth (Das et al., 2023). Furthermore, the combination of *EGFR*-specific chimeric antigen receptor-NK cell technology and oncolytic herpes simplex virus has been shown to alleviate intracranial growth of breast cancer cells in mice (Wu et al., 2023a), indirectly confirming the effectiveness of chimeric antigen receptor-NK cell therapy in the treatment of breast cancer metastasis. Thus, immunotherapies hold great promise for the treatment of metastatic breast cancer.

CONCLUSIONS

Breast cancer cell diversity is influenced by genetic variations and gene expression, resulting in different phenotypes (Li et al., 2021a; Zhang et al., 2022, 2023a). BCSCs have a heightened ability to invade, grow, adapt, and evade immune surveillance, thereby creating a more hospitable environment for regular tumor cells and increasing the risk of metastasis. However, the factors that regulate breast cancer metastasis remain to be explored.

The TME plays a significant role in breast cancer metastasis, with a vast network of blood vessels and support from stromal cells and specific microbes (Mehraj et al., 2021). Additionally, cytokines and other signaling molecules from TME cells promote tumor cell growth, proliferation, vascular mimicry, and BCSC self-renewal, crucial factors in breast cancer metastasis. Therefore, understanding the interactions between TME and BCSCs is essential for studying breast cancer metastasis, particularly organ-specific metastasis.

Given their physiological similarity, reproducibility, low cost, and short cycles, mice are considered the preferred models for studying breast cancer metastasis (Zeng et al., 2020). To better simulate cancer progression, researchers have begun using spontaneous breast cancer genetically engineered mouse models. These models provide more realistic responses to targeted therapies and facilitate the development of new drugs (Bosenberg et al., 2023; Chuprin et al., 2023; De La Rochere et al., 2018; Park et al., 2022). However, mouse models have certain limitations, such as differences in cancer incidence, tumor size, and cancer cell characteristics (Rashid & Takabe, 2015). Furthermore, spontaneous metastasis of breast cancer in mice primarily occurs in the lungs, whereas the main route of human breast cancer metastasis is the lymphatic system (Rashid & Takabe, 2015; Zeng et al., 2020). Advanced transgenic technologies are rapidly developing, which may provide novel mouse models that more closely replicate the physiological conditions of human breast cancer metastasis, thereby accelerating the development of new drugs and therapeutic strategies for breast cancer metastasis.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.L.P. wrote the manuscript and constructed the tables. H.N.D. contributed to the diagrams. S.L.L. and L.X.Z. instructed the writing and editing of the manuscript. All authors read and approved the final version of the manuscript.

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