

REVIEW

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Breast cancer lung metastasis: Molecular biology and therapeutic implications

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ABSTRACT

Distant metastasis accounts for the vast majority of deaths in patients with cancer. Breast cancer exhibits a distinct metastatic pattern commonly involving bone, liver, lung, and brain. Breast cancer can be divided into different subtypes based on gene expression profiles, and different breast cancer subtypes show preference to distinct organ sites of metastasis. Luminal breast tumors tend to metastasize to bone while basal-like breast cancer (BLBC) displays a lung tropism of metastasis. However, the mechanisms underlying this organ-specific pattern of metastasis still remain to be elucidated. In this review, we will summarize the recent advances regarding the molecular signaling pathways as well as the therapeutic strategies for treating breast cancer lung metastasis.

Abbreviations: BLBC, basal-like breast cancer; TNBC, triple-negative breast cancer; CSCs, cancer stem cells; BCSCs, breast cancer stem cells; EMT, epithelial-mesenchymal transition; DKK1, Dickkopf1; TGF- β , transforming growth factor- β ; DTCs, disseminated cancer cells; ECM, extracellular matrix; TAMs, tumor-associated macrophages; CAFs, cancer-associated fibroblasts; TNC, Tenascin-C; POSTN, Periostin; VCAN, Versican; MSCs, mesenchymal stromal cells; SCB, succinobucol; VCAM-1, vascular cell adhesion molecule-1; CXCR4, C-X-C motif chemokine receptor 4; CXCL12, Chemokine (C-X-C motif) ligand 12; GLI1, glioma-associated oncogene homolog 1; TLR4, toll-like receptor 4; NICD, Notch intracellular domain.

ARTICLE HISTORY

Received 13 September 2017

Revised 18 March 2018

Accepted 19 March 2018

KEYWORDS

Breast cancer; cancer stem cell; chemokine; lung metastasis; microenvironment

Introduction

Breast cancer is the most common malignant disease in women worldwide.¹ It is a heterogeneous disease, and its pathogenesis remains unclear in most cases. Much progress has been made in early detection and better treatment of breast cancer, leading to improved survival. However, a considerable number of patients will relapse as a result of organ metastasis, especially those with triple-negative breast cancer (TNBC) which has the worst prognosis. Breast cancer cells are able to spread to distant sites, specifically lung, liver, bone, and brain.^{2–4} There, they proliferate into macroscopic masses that lead to death of most patients.^{5,6} The 5-y survival rate of breast cancer patients who recurred with distant metastasis is less than 20%.^{7,8}

The lung, bone, and liver, are the most common metastatic target sites for breast cancer. In fact, approximately 60% of metastatic breast cancer patients suffer lung or bone metastasis in their life.⁷ BLBC is specifically prone to metastasize to the lung. Life expectancy is low when this occurs, with median survival only 22 months after treatment for lung metastasis.⁹ In particular, 60–70% of metastatic breast cancer patients who eventually died were diagnosed with lung metastasis.¹⁰

Despite a variety of available approaches for the treatment of lung metastasis, such as chemotherapy, radiotherapy, and targeted therapy, the survival rate of breast cancer patients with lung metastasis remains very low. Elucidating and understanding the underlying mechanisms is crucial for developing new

therapeutic strategies. Of note, BLBC markers such as EGFR and FOXC1 have been shown to control and correlate with lung metastasis.^{11–13} In this review, we seek to provide an overview of the recent advances in understanding the molecular basis of lung metastasis of breast cancer with a special emphasis on cancer stem cell pathways and microenvironment. In addition to presenting the clinical characteristics of breast cancer lung metastasis, we discuss the potential therapeutic approaches that may improve the prognosis of breast cancer patients with lung metastasis.

Clinical features of metastasis in breast cancer

With improvements in earlier diagnosis of breast cancer, only 5–10% of patients have distant metastasis at the time of diagnosis.^{14,15} However, the risk of recurrent metastatic disease following standard treatment is still high. More than 30% of breast cancer patients suffer recurrence, and the occurrence of lung or bone metastasis can reach greater than 60% in metastatic breast cancer patients.⁷ More than half a million women worldwide still suffer from metastatic breast cancer annually, and 90% of the deaths can be attributed to metastasis from breast cancer.^{16,17}

Bone, liver, lung, and brain are the most common sites of distant metastasis in breast cancer, which are associated with the patients' poor survival outcome.^{18,19} Furthermore, the

preference of metastatic organ has also shown to differ between subtypes of breast cancer.^{9,17,20,21} Bone metastasis preferentially occurs in luminal breast cancer patients, while lung metastasis is commonly diagnosed in TNBC.^{9,17,20,21} The incidence of lung metastasis can reach up to 40% in TNBC compared with only 20% in non-TNBC.²² Gene expression analysis showed that lung relapse patients were most abundant in the luminal B and basal subtypes, whereas bone relapse was less frequent in BLBC. Strikingly, the absence of lung relapse was observed in the luminal A subtype, while brain metastasis was predominantly found in patients with BLBC and HER2+ breast cancer.⁹ Of note, Yhim et al. analyzed the survival record of patients with lung metastasis and found that hormone receptor-positive breast cancers had the best clinical outcome, while HER2+ cancers and TNBC had the worst prognosis.²³ The HER2+ subtype was also found to display a higher risk of developing liver metastasis.^{21,24}

Most recently, a SEER database analysis indicated that patients with TNBC, especially BLBC, primarily presented with lung metastasis. However, there was no difference in the total probability of lung metastasis across all subtypes.²⁵ Furthermore, the study revealed that all breast cancers regardless of subtype, were prone to metastasize to bone over other locations. Specifically the incidence of bone metastasis is highest in luminal cancers. Although there are discrepancies among reports regarding the preferred metastatic sites of breast cancer subtypes, it is widely accepted that different subtypes exhibit distinctive behavior with regards to the sites of distant metastasis.

In addition to the poor prognosis associated with metastatic breast cancer, the clinical presentations and consequences of lung metastasis are extremely serious. Pain, cough, hemoptysis, pleural effusion, and pulmonary dysfunction are common clinical symptoms which profoundly affect quality of life and survival. The prognosis of breast cancer patients with lung metastasis is still poor despite receiving chemotherapy, targeted therapy, and endocrine therapy based on molecular receptor profiles. Currently the best and only method to prevent breast cancer lung metastasis is an earlier diagnosis. Therefore, we must fully understand the mechanism of lung metastasis of breast cancer to create better treatment strategies. In this review, we summarize the reported cancer cell- and its surrounding microenvironment-based mechanisms of breast cancer lung metastasis and present the challenges we are facing.

Cancer stem cells and associated signaling pathways

Cancer stem cells (CSCs), also named tumor-initiating cells or stem-like cells from solid tumors of different organs (ie. breast, lung, thyroid, etc.), have the ability of self-renewal and differentiation. As such, CSCs can differentiate sufficiently to recapitulate the heterogeneity of tumors.²⁶⁻²⁸ It has now been established that breast cancer stem cells (BCSCs) are responsible for metastatic growth in breast cancer which contributes to the majority of breast cancer related mortality.^{29,30}

A large body of evidence now suggests that the presence of BCSCs is highly associated with specific subtypes.^{31,32}

Chekhun et al. demonstrated that BCSCs are not significantly associated with breast cancer of luminal and HER2-positive subtypes.³³ Honeth et al. reported that the CSC phenotypes are enriched in BLBC compared with other breast cancer subtypes.³⁴ Studies suggest that CSCs may play a role in breast cancer lung metastasis, although whether this is the primary mechanism underlying the organ tropism of BLBC metastasis is unclear. A subset of CD44+ CSCs in primary breast tumors may possess the ability to promote distant metastasis.^{35,36} Yae et al. found that the lung colonization potential of CD44v+ 4T1 mouse mammary tumor cells is much higher than that of CD44v-cells due to the increased activity of the cystine transporter xCT induced by CD44v. This transporter activity is in turn regulated by the gene epithelial splicing regulatory protein 1.³⁷ In another related study involving clinical samples, Hu et al. further demonstrated the heterogeneity of BCSCs in lung metastasis capacities³⁸ and found that CD44v expression both denotes a subset of BCSCs and promotes lung metastasis by interacting with osteopontin in the lung microenvironment. Of note, CD44 is not sufficient to identify all BCSCs. Whether CD44-negative human BCSCs also dictate lung metastasis awaits to be determined.

In summary, preclinical and clinical studies have shown that enrichment of BCSCs may result in increased invasiveness and a worse prognosis. The development of CSC properties is known to depend on an intricate signaling network. These signaling pathways play an important role in balancing self-renewal with differentiation of cancer stem cells.^{39,40} In the following sections, we summarize the potential roles of common CSC-associated signaling pathways in breast cancer lung metastasis.

Notch signaling pathway

Notch signaling is a pathway that relies on cell-cell contact.⁴¹ The ligands of Notch bind to the receptors on adjacent cells leading to activation of the signaling pathway.⁴² In breast cancer, the activation of the Notch pathway could allow BCSCs to undergo uncontrolled proliferation.⁴³⁻⁴⁵ Studies demonstrated that Notch-1, a Notch signaling pathway receptor, could regulate epithelial-mesenchymal transition (EMT) in breast cancer and BCSCs, where it plays a critical role in self-renewal, proliferation, and apoptosis of BCSCs.^{46,47}

As stated above, the abnormal activation of the Notch signaling pathway participates in breast cancer metastasis by primarily modulating EMT and angiogenesis.⁴⁸ In addition, BCSCs that disseminate from primary sites to distant microenvironments establish lung niches that are associated with Notch.⁴⁸ A study by Chen et al. focusing on the role of Notch in salivary adenoid cystic carcinoma cells, found that knock-down of Notch-1 significantly inhibited the formation of metastatic lung nodules induced by EMT.⁴⁹ While it is unclear how the Notch signaling pathway regulates primary tumor cells disseminating to the lung, we speculate that it may play a critical role in the adaptation of breast cancer cells to metastatic niches. Notch signaling pathway may also interact with other signaling pathways to dictate the function and fate of breast cancer cells during the metastatic process.

Wnt/ β -catenin signaling pathway

Wnt/ β -catenin signaling also plays an important role in embryonic induction and tumorigenesis of the mammary gland.^{50,51} The β -catenin nuclear localization and overexpression is an indicator of Wnt/ β -catenin signaling activation. Many clinical and laboratory studies demonstrated that aberrant activation of Wnt/ β -catenin signaling is associated with poorer prognosis in breast cancer patients and is enriched in the subgroup of TNBC.⁵²⁻⁵⁴ Moreover, the Wnt co-receptor LRP6 is often overexpressed in a subtype of aggressive invasive breast cancer like triple-negative breast cancer.^{55,56}

In addition, BCSCs have increased activation of Wnt/ β -catenin signaling when compared to normal stem-like cells. Greater signaling could maintain BCSCs in a self-renewing state and induce the formation of metastatic niches.⁵⁷ Suppression of GSK3 β (a negative regulator of the Wnt pathway) was sufficient to diminish the stem cell features of breast cancer cells.⁵⁸ Wnt/ β -catenin signaling also contributes to EMT and metastasis in breast cancer. Dey et al. found that the patients identified by the Wnt/ β -catenin classifier had a greater risk of lung metastasis in TNBC.⁵⁹ Studies using xenograft models demonstrated that Wnt signaling may link cancer cell self-renewal and expression of EMT transcription factors with tumor seeding and lung metastasis in BLBC.⁶⁰ Other studies suggested that Wnt/ β -catenin can also regulate breast cancer cell proliferation.^{61,62} The proteins of the Wnt family are functionally separated into two classes: those activating the canonical Wnt/ β -catenin pathway and those activating the planar cell polarity and Wnt/calcium pathways, which do not involve β -catenin.^{63,64} Recently, it was reported that the inhibitor of Wnt Dickkopf-related protein 1 (DKK1) suppresses macrophage and neutrophil recruitment in breast cancer lung metastases in part by antagonizing cancer cell non-canonical Wnt-JNK signaling.⁶⁵ Lung metastasis inhibition by DKK1 is also mediated by reduced Wnt-NF- κ B signaling in breast cancer cells.

It is well-documented that canonical Wnt signaling components are commonly up-regulated in breast cancer cells relative to normal mammary epithelial cells. In contrast, there are conflicting reports regarding the expression and role of non-canonical Wnt.^{66,67} Jiang et al. found that enhanced non-canonical Wnt (Wnt5a) expression in breast cancer cells can inhibit lung metastasis through downregulating multiple cell motility-related pathways by regulating transcription and splicing of some key pathway-associated genes.⁶⁸ Concordantly, other studies showed that Wnt5a may suppress breast cancer progression and loss of its expression is associated with poor prognosis.⁶⁷⁻⁷⁴ On the contrary, reports also demonstrated a positive role of Wnt5a in promoting tumor growth and migration in TNBC.⁷⁵⁻⁷⁷ The observed paradoxical effects of Wnt5a may be dependent upon its signaling context, leading to controversy over its role in breast cancer tumorigenesis and metastasis. In addition, Wnt5a can elicit both canonical and non-canonical Wnt pathways. More work is needed to elucidate the mechanisms of specific Wnt members and pathways in breast cancer development and metastasis.

Hedgehog signaling pathway

Hedgehog (Hh) signaling plays an essential role in ductal development in the mammary gland. It also regulates BCSCs and plays a crucial role in carcinogenesis.⁷⁸⁻⁸¹ Several recent studies have provided evidence that paracrine Hh signaling appears to be an important mechanism in breast cancer growth.^{82,83} Moreover, Hh has been shown to regulate breast cancer cell migration.⁸⁴ We also found such paracrine signaling is associated with poor prognosis and the basal-like phenotype.⁸⁵

Inauma et al. demonstrated that the glioma-associated oncogene homolog 1 (GLI1) transcription factor enhances lung metastasis of breast cancer cells in a mouse model via its interaction of CXCL12-CXCR4 axis.⁸⁶ FOXC1, a transcription factor that is normally overexpressed in BLBC, could directly induce CXCR4 expression by activating its promoter in endothelial cells thereby controlling angiogenesis in breast cancer.⁸⁷ Furthermore, FOXC1 controls the cancer stem cell (CSC) properties enriched in BLBC cells via activation of Smoothened (SMO)-independent Gli2 activation. This activation leads to enhanced lung metastasis.⁸⁸ Zuo et al. used mouse models to demonstrate that FOXC1 overexpression has more tumorigenicity and pulmonary metastatic ability in BLBC.⁸⁹ It is possible the secretion of Hh ligand by breast tumor cells mediates a crosstalk with the lung environment in a paracrine manner.

In breast cancer, dysregulated Hh signaling also exerts its function through its interaction with other signaling pathways. In a hepatocellular carcinoma study, activation of Hh signaling and the transforming growth factor- β (TGF- β) was shown to promote liver cancer lung metastasis in mouse models.⁹⁰ Whether the same mechanism is involved in lung metastasis of breast cancer remains to be determined. It is noted that concomitant dysregulation of Hh, Notch, and Wnt signaling pathways has been observed in cancer, suggesting their potential cooperation in promoting tumor development and metastasis.^{58,91-94} Co-activation of both Hh and Wnt pathways in clinical TNBC samples is associated with shorter recurrence-free and overall survival.⁹³ Consistent with this finding, nuclear β -catenin has been shown to increase Gli1 transcriptional activity in other cancer types.⁹⁵ To date, mechanisms that coordinate Hh, Notch, and Wnt activity in cell function remain poorly understood. Using HEK293T, human embryonic kidney cells, and gene knockout mouse models, Kikuchi et al. demonstrated that parafibromin, a PAF complex component, binds to β -catenin, Gli1, and Notch, thereby enabling concerted activation of Hh-, Wnt-, and Notch-target genes.⁹⁶ How these pathways crosstalk in breast cancer is unclear. In short, further understanding of their role, regulation, and potential interaction in breast cancer metastasis may facilitate the development of more effective therapeutic strategies (Fig. 1).

Chemokines

Nearly every tissue expresses chemokines and chemokine receptors. Chemokines are small proteins that govern the directed migration of leukocytes under homeostatic conditions and during specific immune responses.⁹⁷⁻⁹⁹ They are grouped into four families: C, CC, CXC and CX3C.^{100,101} Currently, more than fifty chemokines and twenty chemokine receptors

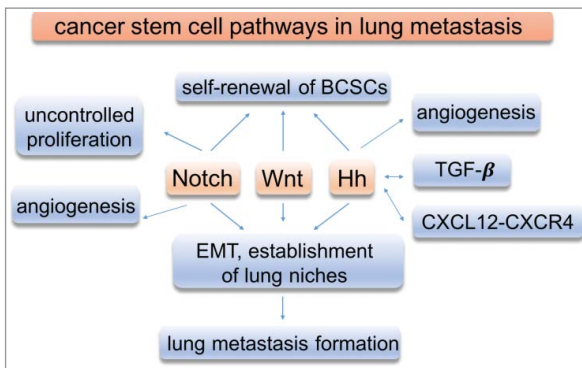


Figure 1. Functions of signaling pathways in breast cancer. Signaling pathways play important roles in breast cancer development and lung metastasis.

have been discovered.¹⁰² Apart from their major function in leukocyte recruitment and inflammation, chemokines have been implicated in the progression of many cancers including breast cancer.

CC and CXC

CC chemokines are important determinants of macrophages and lymphocytes that infiltrate human carcinomas of the breast.^{103,104} In addition, CCL2 mediates the development of the cancer stem cell phenotype.¹⁰⁵ In fact, CCL2 can induce lung overexpression of endogenous toll-like receptor 4 (TLR4) ligands like S100A8 and SAA3, which can enhance cancer cell survival.^{106,107} The endogenous TLR4-dependent innate immune system plays an important role in pre-metastatic niche formation in the lung, which is an essential procedure of process in lung metastasis.¹⁰⁶

The CXCL12-CXCR4 axis is one of the most extensively studied CXC chemokine signals in metastasis. The expression of C-X-C motif chemokine receptor 4 (CXCR4) is higher in malignant breast tumors than in normal breast tissues. Chemokine (C-X-C motif) ligand 12 (CXCL12) is highly expressed in the lung, bone, liver and lymph nodes, locations where breast cancer cells prefer to metastasize.¹⁰⁸⁻¹¹⁰ Chemokine and chemokine receptors have interactions with inflammatory microenvironments in metastatic sites.¹¹¹ In a study using radiation-treatment mouse models of breast cancer, Gong et al. found that pulmonary injury from radiation-treatment induced CXCL12-CXCR4 overexpression, which resulted in increased number of metastatic nodules in the lungs.¹¹² Recently, the expression of CXCR4 was reported to be higher in TNBC, which has a propensity for lung metastasis.¹¹³ Although how the CXCL12-CXCR4 axis induces lung metastasis remains unclear, some studies suggest that this may be due to increased macrophages and micro vessel density.¹¹⁴ In addition, many reports have demonstrated VEGF, estrogen, hypoxia and NF- κ B can upregulate CXCR4.¹¹⁵⁻¹¹⁷ Therefore, CXCR4 may serve as a key downstream effector or mediator for these cancer progression regulators.

Atypical chemokine

Atypical chemokine receptor proteins are predominantly expressed on non-leukocytic cell types and are unlikely to be

directly involved in leukocyte migration. D6 and DARC are atypical receptors for most inflammatory CC chemokines including CCL2.^{118, 119} The overexpression of D6 or DARC in breast cancer was reported to downregulate CCL2 levels and to subsequently inhibit the proliferation and metastasis of breast cancer.¹¹⁹⁻¹²² However, there is limited research regarding atypical chemokines, and the invasive effects of D6 and DARC combined expression in breast cancer cells has not been demonstrated.

Microenvironment factors

It is well-established that the metastatic cascade is composed of numerous barriers that must be overcome in order for cancer cells to form distant metastasis. As discussed, when breast cancer cells spread from the primary tumor, they prefer to metastasize to specific tissues such as bone, lung, liver and brain. The communication between disseminated tumor cells (DTCs) and resident stromal cells in those colonized tissues is diverse. There are diverse components that create the microenvironment of tumors such as growth factors, immune cells, cytokines, chemokines, extracellular matrix (ECM), tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs) as well as other components that have not yet been confirmed.^{123,124} The metastatic microenvironment can be influenced by both organ-specific factors and the infiltration of different stromal cells.¹²⁵

Extracellular matrix (ECM) proteins

ECM proteins like Tenascin-C (TNC), Periostin (POSTN) and Versican (VCAN) are important for the formation of metastasis and play a critical role during the earliest stage of breast cancer colonization of a metastatic site such as the lung.¹²⁶ TNC, normally produced by fibroblasts, can also be expressed by BCSCs.¹²⁷ This aberrant expression of TNC by BCSCs exerts a metastasis-initiating effect for niche formation for lung colonization.^{127,128} POSTN is also a stromal-derived factor capable of binding to Wnt ligands. It has been shown to promote cancer stem cell expansion in lung metastasis development.¹²⁹ Similarly, the infiltrating bone marrow-derived CD11b⁺/Ly6C^{high} myeloid cells secrete VCAN within metastatic niches in the lung to potentiate lung metastasis.¹³⁰ In addition, ECM components may facilitate metastatic growth by providing a milieu for disseminated tumor cells to interact with other cells. Vascular cell adhesion molecule-1 (VCAM-1) is aberrantly expressed in breast cancer cells and binds to α 4 β 1 integrin, which also interacts with fibronectin and is expressed in natural killer cells, monocytes, and other immune cells.¹³¹ It was shown that the pulmonary parenchyma containing collagen and elastin fibers acts as a preferable soil for the homing of VCAM-1-expressing breast cancer.¹³²

Transforming growth factor β (TGF- β)

Numerous studies have demonstrated that abnormal expression of TGF- β promotes breast cancer progression by altering the microenvironment.¹³³⁻¹³⁶ Ye et al. used the 4T1 syngeneic mouse model to demonstrate that TGF- β participates in creating a lung pre-metastatic microenvironment

by modulating certain inflammatory cytokines (S100A8/A9) and growth factors (VEGF, Angpt2).¹³⁷ Park et al. found that IN-1130, a novel TGF β -1 receptor kinase (ALK5) inhibitor, could suppress lung metastasis in the 4T1 breast cancer orthotopic xenograft mouse model.¹³⁸ Another ALK-5 inhibitor, EW-7197, also blocked breast cancer metastasis to the lung.¹³⁹ According to the results of these studies, we could presume that inhibition of TGF- β signaling alone or combined with immunotherapy may act as a promising therapy for breast cancer lung metastasis.

Immune cells

The immune system can both suppress tumor growth and facilitate tumor progression. In addition to their role in response to infection, leukocytes are also involved in cancer progression and metastasis. Studies have shown that subclinical changes in leukocyte composition at distant sites of the primary tumor can induce metastasis.¹⁴⁰⁻¹⁴² Neutrophils, one type of leukocyte, could act as mediators of metastatic initiation.¹⁴³⁻¹⁴⁵ However, how neutrophils affect metastasis is poorly understood and remains controversial. A lung metastasis model of murine breast cancer demonstrated that some special neutrophils like CXCR2+ neutrophils are responsible for the pro-metastatic effect of mesenchymal stromal cells (MSCs).¹⁴⁶ Recently Wculek et al. defined the role of neutrophils as mediators of metastatic initiation by modifying the pre-metastatic lung microenvironment in breast cancer mouse models,^{147,148} suggesting that immune cells regulate the formation of metastatic niches.

Poolard et al. demonstrated that TAMs are essential for the formation of lung metastasis in breast cancer.^{149,150} This is partially due to CCL18, secreted by TAMs, which induces EMT in breast cancer cells.^{151,152} Several clinical studies demonstrated that macrophage infiltration could increase metastatic potential and correlates with poor prognosis in cancer.¹⁵³ Some pre-clinical studies found that pulmonary macrophages play an important role in initiation of lung metastasis.¹⁵⁴⁻¹⁵⁶ One study demonstrated that binding of TAMs to receptor VCAM-1 could provide a survival advantage to breast cancer cells in the lung microenvironment.¹⁵³

CAFs likely also play a major role in breast cancer metastasis. A report showed that CAFs express Tiam1 and osteopontin in human breast cancer and regulate metastasis of breast cancer.¹⁵⁷ Moreover, expression of platelet-derived growth factor receptor β (PDGFR β), a CAFs associated protein is significantly associated with lung metastasis in breast cancer.¹⁵⁸ CAFs could also regulate TGF- β ligands, thereby promoting primary tumor growth. CAFs may also regulate the accumulation of fibrosis, which is associated with distant lung metastasis in breast cancer.¹⁵⁹ Takai et al. demonstrated that Pirfenidone (PFD), a TGF- β inhibitor, could inhibit the tumor-fibrosis and TGF- β signaling. Its combination with doxorubicin could inhibit tumor growth and lung metastasis in TNBC patients.¹⁶⁰

In conclusion, diverse resident and infiltrating cell types, along with secreted growth factors, chemokines, cytokines and the deposition of ECM components in the metastatic microenvironment (Fig. 2), create a fertile soil for the

formation of organ-specific niches. Targeting the microenvironment may be an effective strategy to improve the outcome of breast cancer metastasis.

Therapeutic strategies

Patients' overall survival has improved dramatically secondary to early diagnosis and improved treatments in breast cancer. However, the 5-y overall survival rate of metastatic breast cancer is less than 30%.¹⁶¹ Despite available therapies for metastatic breast cancer, such as cytotoxic chemotherapies, endocrine therapies, and targeted therapies, survival rate is still low. This may be secondary to the lower response rate to systemic chemotherapy and stronger therapeutic resistance of metastatic breast cancer.¹⁶²

Furthermore, we need more targeted treatments which build on the mechanism of metastatic breast cancer in addition to standard therapies. To date, cytotoxic chemotherapy is the only standard of care systemic treatment for TNBC. Given BCSCs are enriched in TNBC, targeting CSC-associated pathways may be an effective therapeutic approach. Inhibitors against Wnt and Hh signaling are under preclinical and clinical tests for TNBC treatment.¹⁶³⁻¹⁶⁵

Some genomics-based studies continue to shed light on the molecular understanding of TNBC tumorigenesis and heterogeneity and may provide implications for developing TNBC-targeted therapies.¹⁶⁶⁻¹⁶⁹ Of note, Bartholomeusz et al. found that the MEK inhibitor selumetinib inhibits and prevents lung metastasis of TNBC in xenograft models,¹⁷⁰ suggesting that MAPK pathway could be a potential therapeutic target for preventing TNBC lung metastasis. Similarly, Cao et al. used succinobucol (SCB), a selective vascular cell adhesion molecule-1 (VCAM-1) inhibitor, to suppress lung metastasis in breast cancer.⁴ In addition, Citterio et al. reported that Rho GEFs could

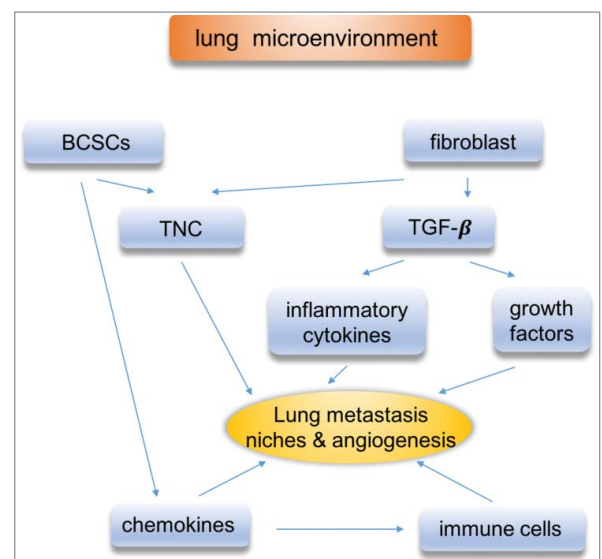


Figure 2. Lung microenvironment. The communication between disseminated cancer cells and resident stromal cells plays a critical role in lung metastasis of breast cancer. Microenvironment components are involved, such as TAMs, CAFs, TGF- β .

be a potential target for breast cancer lung metastasis therapy.¹⁷¹

Recently, immunotherapy has become a hotly pursued therapeutic option for breast cancer, especially metastatic breast cancer. Combining cancer vaccines with standard cancer treatments could increase therapeutic efficiency.¹⁷²⁻¹⁷⁴ To date, the most efficient immunotherapy relies on the infusion of antibodies that directly mediates anti-tumor effector activity, without directly impacting the patients' own immune response.¹⁷⁵ A study using a breast cancer mouse model found that combining phosphatidylserine-targeting antibody with anti-PD-1 therapy could significantly enhance anti-tumor activity.¹⁷⁶

Although the results from these animal *in vivo* studies could provide some basic information to clinical therapy, we still face daunting challenges in treating metastatic breast cancer. Can we use markers to screen for a higher likelihood of developing metastases? Can we screen for drug resistance? There are still many problems to be solved, and having a full understanding of the genetic, environmental, and immune pathways may lead to improved care.

Conclusions

In our review, we summarize the studies related to the biology of lung metastasis in breast cancer. Critical regulators for breast cancer dissemination to the lung include CSCs and related signaling pathways, chemokines, and microenvironmental cues. Our knowledge of breast cancer progression has grown exponentially in recent y. However, it is not well understood whether these regulators connect and cooperate with each other to control breast cancer metastasis or whether some play a more dominant role. In addition, there remains a daunting challenge to develop biomarkers to predict and prognosticate lung metastasis at initial diagnosis in patients with early stage disease. Some markers and mechanisms identified in cell and mouse models need to be validated in clinical studies. This may require matched primary breast cancer and lung metastasis samples, a key barrier in establishing the clinical relevance of research results from preclinical models. Undoubtedly, further understanding of the underlying mechanism for breast cancer migration to and colonization of distant sites will create the foundation to develop more effective therapies for metastatic breast cancer.

Disclosure of potential conflict of interests

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the National Institutes of Health (2R01CA151610) and the Avon Foundation for Women (02-2014-063) to Xiaojiang Cui, and the Fashion Footwear Charitable Foundation of New York, Inc., the Entertainment Industry Foundation, the Margie and Robert E. Petersen Foundation, and the Linda and Jim Lippman Research Fund to Armando Giuliano.

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