Breast cancer molecular subtypes and the metastatic microenvironment: Review of literature

Original Article

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ABSTRACT

Breast Cancer is a major public health concern worldwide. Breast cancer is the most common form of cancer and second leading cause of cancer death in women, with >90% of deaths resulting from metastasis. Clinically, human breast cancers are classified into distinct molecular subtypes (luminal A/B, Her2-positive, and triple-negative [TN]) which exhibit organ-specific patterns of metastasis. The lung is one of the most common sites of metastasis in patients with aggressive triple-negative (TN) disease, while less aggressive luminal A/B cancers most often metastasize to bone. Experimentally, it has been suggested that the presence of a primary tumor may serve to induce a "pre-metastatic niche" in the lung in order to make it more hospitable for metastasizing breast cancer cells. This review integrates how the cellular and molecular components of the metastatic niche evolve in the context of molecular subtype. Understanding the cancer-induced components of the metastatic niche will develop opportunities for improved clinical management and new therapeutic strategies.

Key Words: Molecular subtypes of breast cancer, organ metastasis, premetastatic niche

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INTRODUCTION

1.1. Global Incidence of Breast Cancer:

Cancer is a major public health concern worldwide. There were an estimated 14.1 million cancer cases around the world in 2012 and more than 522,000 women died worldwide due to breast cancer. In 2015, an estimated 560,000 individuals died due to breast cancer globally. Over 90% of these cases were directly related to the metastatic spread of the disease throughout the body. The number of breast cancer cases is expected to increase to 24 million by 2035[1]. The number of cases per 100,000 women, is still lower in developing countries overall than in the West, however mortality rates from cancer are on the rise. This may be attributed to later diagnosis and poor access to treatment (Table 1)[2].

Table 1: Global Prevalence of breast cancer (2):

	Percentage of world population	Percentage of new breast cancer cases	Percentage of breast cancer deaths
Asia	59	39	44
Africa	15	8	12
United States & Canada	5	15	9

1.2. Breast cancer and risk factors:

Breast cancer is a complex disease driven primarily by regulatory failure of homeostatic cellular growth mechanisms, and there are many factors that play a role in its development. Breast cancer occurrence is associated with risk factors including unmodifiable factors such as genetic inheritance, as well as modifiable factors such as obesity, stress, environmental factors, use of hormone replacement therapy, or exposure to chemicals that are known to change the breast cellular DNA (deoxyribonucleic acid) resulting in malignant transformation of normal cells[3]. This most often results in disruptions to two different categories of genes inside the nucleus: oncogenes and tumour suppressor genes. Oncogenes promote cell growth and are tumour-promoting factors when over-stimulated. Oncogenes push cells destined for apoptosis or cell death to survive and

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continue to proliferate[4]. In contrast, tumour suppressor genes are those that inhibit cell division and survival and promote apoptosis and cell cycle arrest[5]. Malignant transformation occurs either as a result of over-activation of oncogenes or down-regulation of the tumour suppressor genes and the healthy cell is transformed into a mutated malignant cell[6,7].

The origin of breast cancer is most commonly from the ducts that carry milk or the lobules that supply them. The susceptibility of these structures to form benign and malignant transformations are in part a consequence of cycling hormonal stimulation throughout life leading to increased cell turnover and accumulation of genetic defects[8,9]. Cancer originating from the milk ducts and lobules are referred to as ductal carcinoma or lobular carcinoma, respectively[10]. Ductal carcinoma represents the most commonly diagnosed breast tumour, accounting for approximately 75% of breast cancer cases[11, 12]. Histopathological classification is commonly used when determining the stage, grade and characteristics of breast cancer. Tumours confined within the ducts or lobules that have not spread beyond their margins are referred to as either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS). Both DCIS and LCIS are non-invasive in nature and remain within their structure of origin without invading into surrounding tissues[13, 14]. Therefore, when breast cancer remains in situ, traditional treatments are highly effective and successful. However, breast cancer can also spread beyond the breast area, whereby cells break off from the breast tumour and travel through the bloodstream or lymphatic system to other parts of the body, a process called metastasis. Prognosis worsens for patients with invasive ductal or lobular carcinoma; characterized by tumour that has infiltrated surrounding tissues[15], as well as for those patients whose disease has disseminated throughout the body, leading to distant metastatic disease. Many of the basic methods for treating breast cancer often fail once the cancer has entered the metastatic phase[16].

1.3. Molecular Subtypes of Breast Cancer:

Breast cancer is commonly categorized based on the molecular and genetic information of the tumor cell. However, when certain characteristics are taken into consideration, such as the hormone receptor status, the presence of HER2 (human epidermal growth factor receptor 2) and proliferation rate (Ki67), breast cancer is clinically classified into four major molecular subtypes[17, 18]. The Cancer Genome Atlas (TCGA) also classifies breast cancer into four molecular subtypes[19]. The first classification is Luminal A, which makes up 30-70% of all breast cancers. This subtype originates in the inner (luminal) cells lining the mammary ducts. It is hormone-dependent and tends to be estrogen receptor (ER)- and/or progesterone (PR)-positive, HER2-negative and displays low proliferative activity based on Ki67 expression. The second subtype is Luminal B, which has a prevalence of 10-20% of all breast cancers and is characterized by ER/PR-positivity, high Ki67, and/or HER2-positivity. Patients with Luminal A/B breast cancer have the best prognosis, and if they do progress to metastasis is usually to the bone[17]. The third subtype HER2–enriched, with ER/PR negativity and positive HER2, with a prevalence of 5-15% of all breast cancers. The fourth type is the triple negative/basal-like (ER-/PR-/HER2-), with a prevalence of 15-20% of all breast cancers. The latter two subtypes (TN) are basal-like tumours, due to the tumor cells expressing similar features to those of the outer (basal) cells surrounding the mammary ducts. Triple negative (TN) breast tumors have the highest incidence of metastasis and show a high rate of metastasis to the lungs. The TN tumors are often more aggressive and have a poorer prognosis (at least within the first five years after diagnosis) compared to the ER-positive subtypes (Luminal A and Luminal B tumors)[20].

1.4. The Metastatic Cascade and Breast Cancer:

Metastasis is a complex process by which tumour cells spread to distant sites throughout the body. This occurs by a series of coordinated cellular events: (A) development of a primary tumour; (B) tumour cells from the primary tumour escape, invading (intravasating) into the vasculature; (C) tumour cells disseminate through the blood and/or lymphatics, until they arrest in the capillary beds of distant organs and extravasate into a new tissue where they can ultimately initiate and establish a secondary tumour, distant from the primary tumour[21].

Current therapies are largely non-curative in the metastatic setting[22]. Lung metastasis often occurs within 5 years of initial breast cancer diagnosis and has a significant impact on patient morbidity and mortality[23]. Physiologically, these metastases disrupt normal lung function, and result in coughing, labored breathing, hemoptysis, and eventual death[24,25]. Advanced lung metastases remain difficult to treat, highlighting the need to better understand the cellular and molecular drivers in order to develop new therapeutic strategies.

1.5. Metastatic Organ Tropism and the Pre-Metastatic Niche:

Breast cancer has special preference to metastasize to specific organs, which is termed “organ tropism”[26,27]. Multiple theories have been developed to explain the process of organ-specific tropism, most notable of which are Stephen Paget’s seminal “seed and soil” hypothesis in 1889[28], and Ewing’s mechanical arrest theory[29]. The “seed and soil” theory states that metastasis selectively colonizes in specific organs because of a “match” between the migrating tumor cell and a “suitable” environment. However, Ewing's theory, anticipated thirty years later, that organ tropism can be accounted for by circulatory patterns within the body, when the cancer cells are mechanically arrested in the first capillary bed they meet. Dr. Leonard Weiss, who compared both theories in an autopsy study examining the frequency of metastasis in a number of anatomic sites, leans towards a combination of the two theories being correct[30].

It is possible that these two theories are not equally exclusive, cells arrest due to mechanical obstruction and/or specific chemical signals and then require a suitable microenvironment for initiation and maintenance of secondary tumor growth[27].

More recently, an additional layer of complexity has been uncovered whereby the presence of a primary tumour may actually promote that development of a "pre-metastatic niche" in distant organs before the metastatic cells actually arrive in the secondary site. The pre-metastatic niche is best explained as a supportive and receptive tissue microenvironment undergoing a series of molecular and cellular changes to build the fertile soil for tumour settlement and metastasis in distant organs[31,32].

1.6. Molecular Aspects of the Pre-Metastatic Niche:

Figure 1 illustrates the role and steps of the pre-metastatic niche in the promotion of tumour metastasis, including the molecular and cellular components of pre-metastatic niche formation and the pathological sequence of events. The establishment of a pre-metastatic niche passes through the following phases until it forms the macrometastases in the target organ:

A. Priming:

The primary tumour undergoes uncontrolled proliferation and become hypoxic and inflammatory. This results in release of growth factors including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), transforming growth factors (TGF), and other molecular components. As a result, inflammatory S100 chemokines are upregulated in pre-metastatic sites, leading to clustering of bone marrow-derived hematopoietic progenitor cells (HPCs) which can induce the mobilization of bone marrow-derived cells (BMDCs)[33, 34].

B. Licensing Phase:

This is the key link between the maturation of the pre-metastatic niche and initiation of tumour metastasis. The BMDCs and immune cells are continuously mobilized and recruited into the secondary sites[35].

C. Initiation Phase:

In this phase the pre-metastatic niche contributes to initiation of metastasis by facilitating the extravasation of circulating tumour cells (CTCs) from the vasculature and attracting tumour cell colonization into the niche. Mobilization of platelet-deployed stromal-derived growth factor 1 (SDF-1) from bone marrow to the pre-metastatic niche, also attracts C-X-C chemokine receptor type 4 ($CXCR4^+$) haematopoetic progenitor cells (HPCs) and metastatic tumour cells (MTCs)[36]. HPCs secrete a variety of pre-metastatic factors including TNF-α, matrix metalloproteinase 9 (MMP-9) and TGF-β. Activated fibroblasts secrete fibronectin, an important adhesion protein in the niche. The upregulation of integrins, TNF-α, fibronectin, and MMPs enhances the adhesion of MTCs to the pre-metastatic niche[37]. The pre-metastatic niche also regulates tumour dormancy between the MTCs and reactivates them when the pre-metastatic niche is ready and suitable for the growth of the tumour. The well-established pre-metastatic niche is then competent for the initiation and formation of micrometastases[38].

D. Progression Phase:

In this phase the MTCs proliferate, and expansion of the tumour mass facilitates the change in micometastases to macometastases. The recruitment of endothelial progenitor cells (EPCs) to the early metastatic niche mediates the angiogenic switch and enables progression to macrometastases[31,39].

In summary, tumor-type specific factors are released from the primary tumor to facilitate changes in the microenvironment of distant sites before the cells arrive, initiating a so-called "pre-metastatic niche". Cancer cells and associated stromal cells secrete chemokines that direct the migration, proliferation and differentiation of the vascular cell network to support the tumor and the metastatic microenvironment. It has been demonstrated that bone marrow-derived cells respond to these systemic factors by migrating to the pre-metastatic niche and forming clusters of cells in the tissue parenchyma at the sites of metastasis before evidence of any primary tumor cells[28]. CD117 is an important cell surface marker used to identify certain types of hematopoietic (blood) progenitors in the bone marrow. More specifically, hematopoietic stem cells (HSC), multipotent progenitors (MPP), and common myeloid progenitors (CMP) express high levels of $CD117^+$. Common lymphoid progenitors (CLP) express low surface levels of CD117. Signaling through CD117 plays a role in cell survival, proliferation, and differentiation. CD117 is a proto-oncogene, meaning that overexpression or mutations of this protein can lead to cancer. Primary tumour–derived components, tumour-mobilized bone marrow-derived cells (BMDC) with their surface markers like CD117, and the local stromal microenvironment of the host are the three major factors crucial for the formation of the pre-metastatic niche[40]. These are the three factors which need to be investigated in order to gain a greater understanding of the pre-metastatic niche in the lung during breast cancer progression with the goal of new and improved therapeutic strategies.

1.7. Criteria for Establishment of a Pre-Metastatic Niche (Tumour Microenvironment):

The tumour microenvironment is composed mainly of endothelial cells, fibroblasts, perivascular cells, and inflammatory cells, which regulate the tumorigenic process. There are six characteristics that are believed to determine whether metastatic tumour cells may die, survive and colonize, or become dormant after arrival in the secondary organ. These are:

1. Immunosuppression: Regulatory immune cells like CD8+ T cells, natural killer cells and non-classical monocytes inhibit local tumour immunity and contribute to

the formation of immunosuppressive pre-metastatic niche. However, some regulatory immunosuppressive cells such as MDSCs, macrophages, and Treg cells within the pre-metastatic niche potentially suppress anti-tumour immune responses[41].

2. Chronic Inflammation triggers many signaling pathways and molecular components which are important drivers of tumour development and metastasis. An example of this is the pro-inflammatory mediators S100A8/A9 become upregulated in the lung pre-metastatic niche. This in turn induces the expression of serum amyloid A that recruits myeloid cells to these sites. These myeloid cells will enhance pre-metastatic niche formation in an inflammatory state and promote migration of the of the primary tumour cells to the secondary lung site. Clara cells of the lungs are also involved in the inflammatory process, and $CD11b^+$ cells in the lung pre-metastatic niche enhances the inflammatory and proliferative processes occurring in the lung and stimulate the expression of MMPs to enhance the adhesion[42].

3. Angiogenesis and Vascular Permeability: Both angiogenesis and vascular permeability become increased in the pre-metastatic niche to promote metastasis. The migrated bone marrow monocytes and the endothelial progenitor cells express high levels of VEGF and other proangiogenic factors, which help facilitate the switch from micrometastasis to macrometastasis[43]. In addition, the increased vascular permeability within the pre-metastatic niche will facilitate the extravasation of circulating tumour cells and the establishment of lung metastasis[44].

4. Lymphangiogenisis: The lymphatic system and lymphangiogenesis often serve as the initial route of tumour dissemination[45], and clinical research has confirmed that tumour-derived VEGF-A and VEGF-D induce pro-metastatic lymphangiogenisis[46].

5. Organ Tropism: Primary breast cancer cell-derived VEGF alters the pre-metastatic lung microenviroment by triggering an inflammatory response and prostaglandin production, which influences the preferential migration of the cancer cells to the lung[47].

6. Metabolic, Stromal and Epigenetic Reprogramming: These processes are involved in pre-metastatic niche-promoted tumour metastasis. Breast cancer cells can suppress glucose uptake by non-tumour cells in the pre-metastatic niche, by secreting vesicles that carry high levels of the miR-122 microRNA. High miR-122 levels in the circulation have been associated with metastasis in breast cancer patients, and Fung et al showed that cancer-cell-secreted miR-122 facilitates metastasis by increasing nutrient availability in the pre-metastatic niche[48].

1.8. The Soluble Lung Microenvironment:

Previous research studies have previously employed novel *ex vivo* model systems of the lung and has demonstrated that soluble and matrix factors in the normal (healthy) lung microenvironment can interact with aggressive breast cancer cells to promote metastatic behavior in the lung[49]. Organs representing common sites of breast cancer metastasis (lung, bone, liver, brain, LN) are isolated from healthy mice and used to generate organ-conditioned media (CM). Using this model, Chu *et al* (2014) demonstrated that different human breast cancer cell lines show specific chemotactic and proliferative behaviours in response to various organ-CM, reflective of their metastatic behaviours *in vivo*[50]. Specifically, the most aggressive of the cell lines, MDA-MB-231, showed increased migration patterns towards bone, lymph node, and lung-CM. The second most aggressive cell line, SUM159, displayed enhanced migration towards the bone, brain, LN, and while the two least aggressive cells lines (SUM149 and MDA-MB-468) demonstrated increased migration to lung- CM only. In addition to increased migratory patterns, MDA-MB-231 and MDA-MB-468 cell lines demonstrated cell line specific patterns of proliferation in response to organ-CM. MDA-MB-231 cells showed increased proliferation in the presence of liver and lung-CM and MDA- MB-468 cells showed increased proliferation in the presence of lung-CM[50]. These results indicate the potential of the lung microenvironment for promoting metastatic progression of breast cancer cells.

Protein array analysis of organ-CM identified several secreted metastasis-promoting factors from the lung and the innovative 3D *ex vivo* pulmonary metastasis assay (PuMA) confirmed the lung as an effective metastatic microenvironment[51].

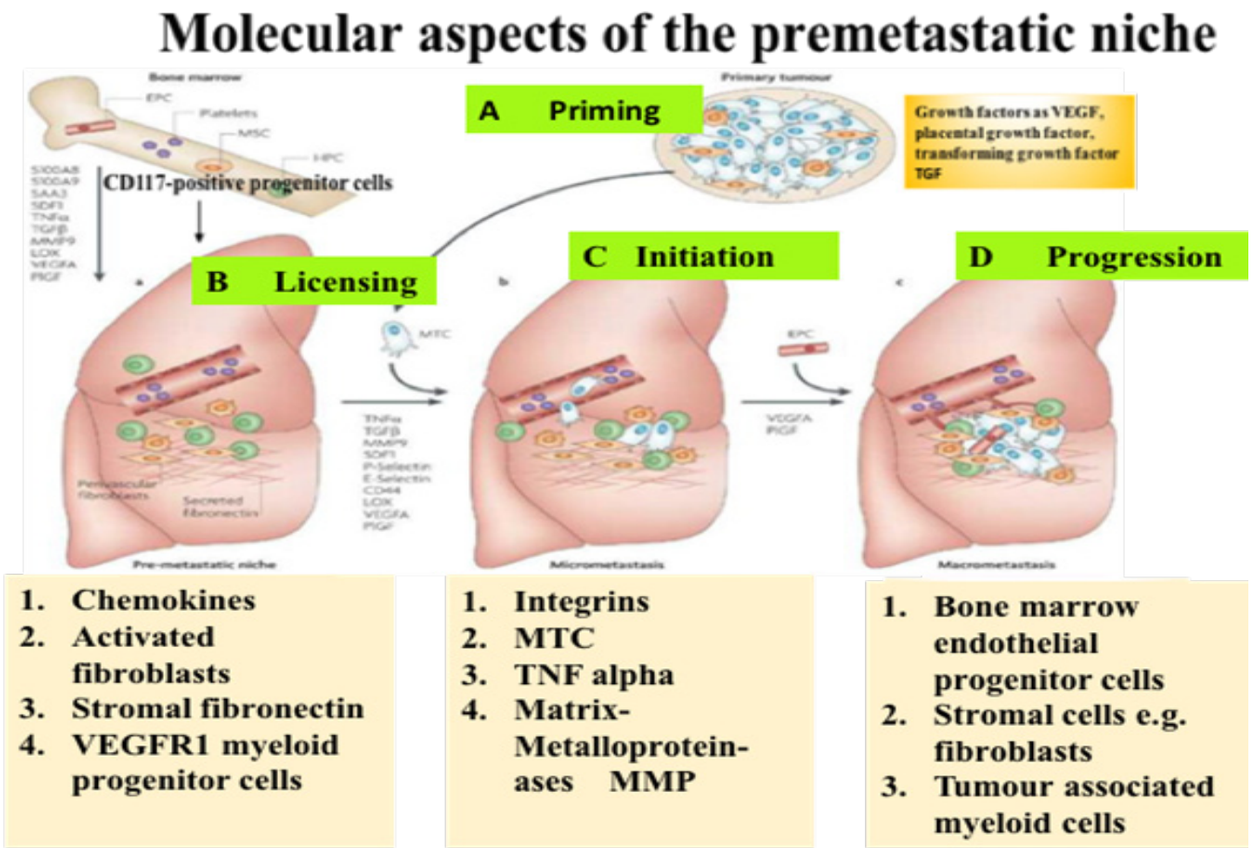
1.9. The Insoluble Lung Microenvironment:

The cellular part of the lung is composed of over 60 different cell types involved in various functions including sensory, mechanical, secretory and transport. The non-cellular part of the lung or the extracellular matrix (ECM) constitute about 15% of alveolar tissue and 50% of non-alveolar tissue[52]. The extracellular matrix is a dynamic 3D structure composed of a myriad of structural proteins including collagen and elastin, specialized proteins such as fibronectin and laminin, as well as high- molecular weight proteoglycans, which function to support surrounding parenchymal cells[53]. Other extracellular matrix components include Tenascin C, which is an oligomeric glycoprotein composed of individual polypeptides with molecular weights ranging from 180 to ~300kDa. These protein modules are lined up like beads on a string and give rise to long and extended molecules[54]. Tenascin-C has been shown to interact with fibronectin in order to modify cell adhesion[55]. A solid-state interaction between fibronectin and TN-C results in cellular up-regulation of matrix metalloproteinase expression[56].

Periostin is a secreted extracellular matrix protein that was originally identified in cells from the mesenchymal lineage. It binds to several integrin receptors. These receptors have been demonstrated to have function in cell adhesion, migration, and survival in these cells. In many cancers, periostin binds to integrins on cancer cells, activating the signaling pathways. This leads to increased

cell survival, invasion, angiogenesis, metastasis, and the epithelial-mesenchymal transition[57]. Many types of epithelial and endothelial cells are dependent upon adhesion to the ECM for their continued survival and often undergo apoptosis when this adhesion is disrupted. Although cancer cells are characterized by their unique ability to progress and grow in the absence of ECM adhesion, solid tumours often exist in a dynamic relationship between anchorage-dependence and independence[53].

Fig. 1: Figure is modified from Kaplan *et al*., 2005[31] and Lyden *et al*., 2009[35].



(A) In the priming phase, primary tumor cells produce various soluble factors molecular components, to trigger the formation of an immature pre-metastatic niche in the secondary organ site by up-regulation of specific chemokines. (B) In the licensing phase, sequestration of bone marrow-derived cells like the VEGFR1, CD-117 progenitor cells and other regulatory/suppressive immune cells are mobilized and recruited into the secondary sites (lungs) in response to tumor-derived molecular components. Also activation of fibroblasts and secretion of fibronectin starts in the premetastatic niche. (C) In the initiation phase, metastatic tumour cells (MTA) arrive and colonize at the fertile pre-metastatic niche, increased expression of integrins, TNF alpha and Matrix-Metalloproteins (MMP) resulting in micrometastases. (D) In the progression phase, the pre-metastatic niche can host more migrated tumor cells, more stroma cells and the migrated Bone marrow endothelial progenitor cells express new blood vessels, leading to macrometastases.

CONCLUSION

As metastasis is the major cause of death from cancer, investigating the sources of metastatic disease and investigating the components of the metastatic microenvironment will lead to develop therapies to be used clinically. As the lung is one of the major deadly sites of breast cancer metastasis, especially in patients with aggressive "triple negative" Breast cancer, so understanding the cancer-induced components of the metastatic niche will develop opportunities for improved clinical management and new therapeutic strategies.

CONFLICT OF INTEREST

There are no conflicts of interest.

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