Book Chapter

Breast Cancer Stem Cells: A Key for Breast Cancer Treatment

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Abstract

Breast cancer, a predominant health concern for women worldwide, is frequently distinguished by its stubborn resistance to traditional treatments and a high likelihood of recurrence. Breast Cancer Stem Cells (BCSC), a specific subset of cells within the disease, are thought to be responsible for these challenges. These cells possess distinct abilities for self-renewal and differentiation, enabling them to elude standard therapeutic approaches. Researchers are increasingly acknowledging the crucial role BCSC play in the persistence and recurrence of breast cancer, making them a promising target for more effective treatment strategies. However, successfully neutralizing BCSC requires a comprehensive understanding of their biological nature and proliferation mechanisms. In this review, we explore the intricacies of breast cancer and its various subtypes before focusing on BCSC, offering a detailed examination of their defining characteristics and their involvement in drug resistance, recurrence, and metastasis. By illuminating the elusive nature of these cells, we aspire to pave the way for innovative strategies to eliminate them, ultimately improving the efficacy of breast cancer treatment.

Keywords

Breast Cancer; Breast Cancer Stem Cells (BCSC); Resistance; Recurrence; Treatment Strategies

Introduction

Female breast cancer is the most commonly diagnosed cancer worldwide, according to GLOBOCAN 2020 global estimates of cancer incidence and mortality by the International Agency for Research on Cancer. In 2020 alone, there were 2.3 million new cases, representing 11.7% of all cancer cases. Despite its prevalence, it is the fifth leading cause of cancer mortality worldwide, with 685,000 deaths accounting for 6.9% of all cancer deaths [1].

Interestingly, incidence rates are higher in transitioned countries than in transitioning countries. However, transitioning countries face higher mortality rates. By 2040, breast cancer is projected to cause more than 3 million new cases and 1 million deaths [2].

Focusing on the United States, the American Cancer Society estimates that around 287,850 new cases of invasive breast cancer will be diagnosed in women in 2022, with 43,250 women expected to succumb to the disease [3].

Breast Cancer Risk Factors

Some risk factors for breast cancer are unchangeable, such as age or inherited gene changes. These increase the chance of developing breast cancer. Other risk factors are lifestyle-related and are changeable, and some have unclear effects on the likelihood of developing the disease or are disproven or debatable risk factors [4].

Ageing is a main risk factor for breast cancer. The American Cancer Society reports that 1 in 8 invasive breast tumors occur in women under the age of 45 and invasive breast cancer affects women over the age of 55 in about 2 out of 3 cases [4]. Ageing increases genetic mutations and decreases the ability to repair damage.

Family history is another risk factor occurring in over a quarter of all breast cancer cases. Women with two or more first-degree family members are 2.5 times more likely to develop the disease

[5]. The mutations in breast cancer-related genes, such as BRCA1 and BRCA2, are mainly responsible for this hereditary risk [6]. Additionally, the female's reproductive history is a risk factor. Beginning menstruation before the age of 12 and menopause after the age of 55 expose women to hormones for a longer length of time, increasing their risk of breast cancer [7].

Lifestyle, such as diet and physical activity, are linked to certain breast cancer risk factors. Other lifestyle-related risk factors include having children and taking hormone-containing medications. Women who have just given birth have a short-term increase in breast cancer risk, which disappears after roughly ten years. The cause of this transient rise is unknown, however some experts believe it may be linked to the influence of high hormone levels on cancer development or to the fast expansion of breast cells during pregnancy [8]. Moreover, women who used diethylstilbestrol (DES) during their pregnancy may be at a slightly increased risk of getting breast cancer than women who did not use DES during their pregnancy. DES is an artificial form of estrogen that was used to prevent miscarriages and other pregnancy complications between the early 1940s and 1971 [9].

The risk of breast cancer can be raised by variables associated with modern lifestyles, such as excessive alcohol intake, smoking, and dietary fat consumption [10]. Alcohol consumption is linked to a slight increase in the risk of breast cancer. The influence of the ADH1C genotype on this link, along with the relationship between alcohol consumption and increased breast density, provide additional evidence supporting a causal effect [11]. Moreover, the relative risk of breast cancer linked with smoking was much higher for women with a family history of the disease. Smoking is related to a moderate but significantly elevated risk of breast cancer, particularly among those who started at teenage or peri-menarcheal ages [12]. Additionally, a link likely exists between the prevalence of breast cancer and total dietary fat consumption. Breast cancer incidence rises over time in correlation with increasing fat content, according to research by Bjarnason et al. among the Icelandic population [13]. So, a woman's chance of developing breast cancer is increased if she is physically inactive. Furthermore, Breast cancer risk is

linked to estrogens, including both endogenous and exogenous estrogens. In women before menopause, the ovary normally generates endogenous estrogen, therefore ovarian excision can lessen the risk of breast cancer [14].

Some factors may pose a risk for breast cancer, but their influence is unclear. Research is being conducted to understand potential environmental effects on breast cancer risk. A special attention should be paid to environmental chemicals with estrogen-like characteristics. Examples of compounds that appear to have these qualities include those found in some plastics, specific cosmetics and personal care items, insecticides, and PCBs (polychlorinated biphenyls) [15]. These may have an impact on the risk of breast cancer. Although it is challenging to explore such effects in humans, research to date has not clearly linked exposure to these drugs to an increased risk of breast cancer. In this area, more research is required.

Breast Cancer Subtypes

Breast Cancer is molecularly classified according to the presence of certain biomarkers that are usually hormone receptors. Those hormonal Receptors include estrogen receptor (ER) and progesterone receptor (PR).

Luminal Subtypes

One of the first and most widely utilized BC biomarkers is ER. A pooled data set from numerous cohorts and cooperative studies indicate that roughly 80% of all BC are ER-positive (ER+). Compared to ER-negative (ER-) BC, ER-positive (ER+) BC is generally well-differentiated, less aggressive, and has a better prognosis [16].

The ER-positive type is further classified into subtypes A and B according to gene expression profiles of more than 500 genes: Luminal A and B subtypes. In contrast to the luminal B subtype, a higher expression of luminal genes and ER+ associated genes (such as PR) in the luminal-A subtype. Additionally, compared to the luminal-B subtype, the luminal A subtype shows a better

prognosis and overall survival. Poor response to luminal-B subtype endocrine treatments is frequently associated with low ER/PR expression, high Ki-67 expression, and atypical overexpression of HER2 according to numerous studies. As a result, the proliferative biomarker Ki-67 is also recommended as a second clinical biomarker to help distinguish between luminal-A and luminal-B subtypes [16].

HER2-Positive Breast Cancer

The Human Epidermal Receptors is a family of 4 members. The orphan tyrosine kinase receptor, also called human epidermal growth factor receptor 2 (HER2), is a member of the family. HER2 does not have a ligand, yet it either homo or hetero dimerizes with one of the 3 three other human epidermal factors. Thus, results in the activation of downstream tyrosine kinase signaling cascades, which leads to the promotion of cell growth, migration, invasion, and survival. In 15% of breast carcinomas, HER2 is amplified, resulting to HER2 overexpression [17]. The extracellular domain of HER2 is targeted by the monoclonal antibody trastuzumab along with chemotherapy, which alters the normal tyrosine kinase signaling. While on the one hand this mechanism confers a poor prognosis (due to the effect on cell proliferation, migration, invasion, and survival, all key aspects of cancer), on the other hand, it offers the unique possibility to use a targeted treatment approach [17].

Immunohistochemistry (IHC), which evaluates the levels of HER2 protein expression, and in situ hybridization (ISH), which determines the status of the *HER2* gene, are the only two methods with established clinical relevance. They are frequently used to determine the HER2 status in diagnostic pathology [17].

Triple Negative Breast Cancer

TNBC is a type of Breast Cancer where estrogen receptor ER, progesterone receptor PR, and human epidermal growth factor receptor 2 HER2 are not immunohistochemically expressed in triple-negative breast cancer (TNBC). It represents around 12–20% of all occurrences of breast cancer and is the most aggressive subtype. TNBC is linked to an earlier age of onset,

increased risk of metastasis, more relapses, and higher mortality rates [17].

TNBC usually appears in younger women and has a 40% mortality rate in advanced stages during the first 5 years following diagnosis compared to other types of Breast Cancer. Despite the development of novel biological and targeted medicines, cytotoxic chemotherapy remains the cornerstone of treatment for TNBC [17].

Breast Cancer Stem Cells

Breast Cancer Stem Cells and Relapse

Tumor recurrence and therapy failure are common outcomes in patients with breast cancer. Despite advances in diagnosis and treatment, therapeutic strategies have shown limited efficacy in patients with metastatic breast cancer. Therefore, it is essential to investigate the processes underlying this phenomenon to provide effective strategies for its treatment [18].

Breast cancer stem cells (BCSCs) are a minor cell population with plasticity and multipotent differentiation capabilities observed in breast tumors and are considered to be a factor contributing to the heterogeneity of primary tumors. BCSCs can originate from various sources, including normal stem cells that have acquired aberrations, or through the de-differentiation of malignant cells triggered by environmental elements in their surroundings. Although BCSCs represent a minor fraction of tumor cells, they are shown to have a critical role in the initiation, progression, and metastasis of breast cancer [20]. BCSCs can exist in various forms, each possessing resistance to severe environmental circumstances, anticancer therapies, and the immune system. BCSCs retain their plasticity and are potent shifters. The tumor microenvironment plays a significant role in shaping cancer stem cells. Importantly, it controls the fluctuation of cancer stem cells between a mesenchymal state with quiescent properties responsible for epithelial mesenchymal transition (EMT) and an epithelial state with proliferative traits required for mesenchymal epithelial transition (MET). EMT is a biological process involving the loss of strong cell-to-cell

adhesion and polarization in epithelial cells and the development of mesenchymal features including mobility and invasiveness [20]. MET is the term used to describe a reversal of EMT, where cells lose their mesenchymal characteristics and regain epithelial features. The conversion between EMT and MET is mediated by a series of transcriptional and epigenetic modifications. In fact, it has been postulated that EMT and MET are the two main processes indispensable for the metastatic spread of malignancy. Cells adopting the EMT phenotype will eventually reach the circulation and travel either as individual cancer circulating cells or in a cluster. Then, the MET program will be initiated to ensure the invasion of premetastatic sites. These cells can remain for up to two decades as disseminated tumor cells (DTCs) [20].

Characteristics of BCSCs

Tumorigenicity of BCSCs and Self-Renewal

Cancer stem cells (CSCs) are a heterogeneous population of cells that have been known to be particularly aggressive and tumorigenic. The ability of malignant stem cells to self-renew, similar to somatic cells, allows them to survive and multiply. A relatively small number of CSCs have been shown to initiate tumor growth in xenografts, demonstrating their tumorigenic nature. The great majority of these cells are dormant, which has been identified as critical for minimizing the incidence of replication errors and oxygen free radicals in CSCs. BCSCs also demonstrated the potential to withstand extreme environmental conditions, avoid anticancer therapies, and evade the immune system [4].

Surface Markers

CD44 is an important membrane glycoprotein that controls cell-extracellular matrix communication. It is abundant in BCSCs and is the most frequent recognition and classification marker for them. Remarkably, it is linked to the preservation of stem cell properties, epithelial-mesenchymal transition and tumor stem cell profiling [21,22] In addition, CD44 promotes tumor stem cell self-renewal and increases tumor stem cell invasion and dissemination by increasing growth factor and cytokine

signalling in the tumor microenvironment [21,22]. Downregulating CD44 overcomes BCSC treatment resistance and impairs self-renewal. In addition, CD44 is a receptor for both growth factors, the epidermal (EGF) and hepatocyte (HGF). Thus, it functions in cancer stem cell signaling. Furthermore, CD44 is a hyaluronic acid receptor that collaborates with HA to establish a matrix that governs cell adhesion and migration. The CD44- Hyaluronic acid interaction advocates malignant cell metastasis and infiltration into target tissues [21,22].

ALDH are a group of enzymes known as aldehyde dehydrogenases, which are primarily elevated in breast cancer [23]. ALDH is a marker used to detect and isolate BCSCs. It is closely linked to the survival of cancer stem cells and the maintenance of their stem-like properties. Furthermore, studies have demonstrated that specifically knocking out the Aldehyde Dehydrogenase 1 Family Member A1 (ALDH1A1) gene diminishes the production of stem biomarkers and the potential of cancer stem cells to develop into tumor [23]. Interestingly, ALDHs can stimulate cancer stem cell growth by neutralizing and removing toxic chemicals so as to escape oxidative stress. As a result, ALDH is critical in the proliferation and differentiation of tumor stem cells [23].

CD133 has been discovered to be a malignant marker for BCSCs. CD133 is a transmembrane glycoprotein expressed on the surface of hematopoietic stem cells and progenitor cells [21]. It is frequently associated with poor prognosis and limited metastasis survival in various types of breast tumors, such as Estrogen receptor positive (ER+) and human epidermal growth factor receptor 2 positive (HER2) [21].

The prevalence of cells expressing CD55 is associated with oncogenesis and tumor recurrence, indicating that CD55 is linked to a poor prognosis in breast cancer [24]. Yet, it isn’t validated whether CD55 can be used as a marker of BCSCs [24].

Ganglioside (GD2) represents a small fraction of BCSCs. It has been identified as a novel marker of BCSCs and is prevalent in cells actively undergoing EMT [20].

Drug Resistance

Drug resistance is a protective feature of BCSCs that contributes to their malignant nature. Because of their extended dormant phase, BCSCs are a challenging target to chemotherapy [25,26]. Similarly, the ABC transporters are a superfamily of integral membrane proteins that are upregulated on the surface of cancer stem cells and are responsible for the efflux of wide range of substances along the membrane including anti-cancer drugs. ALDHs, on the other hand, are a class of enzymes that are abundant in BCSCs and are responsible for the oxidation of aldehydes. ALDHs can either inactivate anti-cancer treatments or aid in their excretion, keeping malignant stem cells free of oxidative stress [25,26]. BCSCs also display superior DNA repair activity upon radiotherapy and generate lower levels of reactive oxygen species than their non-stem cell counterparts due to their enhanced expression of free radical scavenging mechanisms [25,26].

Furthermore, several studies have also connected autophagy to therapeutic resistance in cancer cells and BCSCs. Autophagy was previously defined as a catabolic mechanism involved in the control of cell viability and senescence, preserving energy expenditure in the face of limited nutrition, stress, or oxygen deprivation [25,26]. In breast cancer, autophagy has been demonstrated to be critical for BCSCs tumorigenicity and a key element of BCSC survival, particularly in the presence of harsh microenvironments. Because dispersed breast cancer stem cells can survive for decades before resurfacing as aggressive secondary tumors, autophagy is especially important in the premetastatic contex [25,26].

Plasticity

Cancer stem cells are also very plastic, allowing them to switch between several developmental programs. They have been linked to cancer metastasis, which is a sequential process of (EMT) and MET [27-29]. EMT is the molecular process that converts adherent epithelial cells into individual mesenchymal cells with the ability to migrate and penetrate neighboring regions. The

stimulation of EMT causes an increase in the expression of genes linked with "stemness" and is also characterized by the loss of cell connections and the decrease in E cadherin expression which compromises cellular integrity [27-29]. Furthermore, the cytoskeleton shifts from keratin to vimentin and cellular polarity of the epithelial cells is disrupted, leading to a mesenchymal phenotype. During tumorigenesis, this process is reactivated in BCSCs, allowing them to traverse the basement membrane and extravasate into the vasculature [27-29]. The EMT-BCSCs are in quiescent- mesenchymal-like states defined by their capacity for migration and resistance to chemotherapy, seen near the tumor front and are characterized by having CD24− CD44+ surface markers [27-29].

When the cancerous cells reach a distal tissue, they adopt a MET phenotype, the inverse process of EMT. MET-BCSCs thus exhibit an increased E- cadherin expression and reduced vimentin expression. These cells are present near the core of the tumor, express aldehyde dehydrogenase (ALDH+), and feature characteristics of self-renewal and proliferation. The CD24+ BCSCs and ALDH+ BCSCs populations intertwine and are convertible to some extent [27-29].

Cytotoxic chemotherapy and radiation treatment are highly ineffective against EMT BCSCs. Furthermore, previous research has associated ALDH1 expression in BCSCs to a poor prognosis, suggesting that ALDH+ MET-BCSCs share features with EMT-BCSCs in terms of relapse and therapeutic resistance [27-29].

The growing evidence of BCSCs plasticity define this population as a flexible entity, prone to transition between EMT and MET developmental programs, and extremely influenced by the surrounding tumor microenvironment (Figure 1). BCSCs' plasticity is also demonstrated with the ability of non-breast cancer stem cells to convert into BCSCs, a process called de-differentiation [27-29].

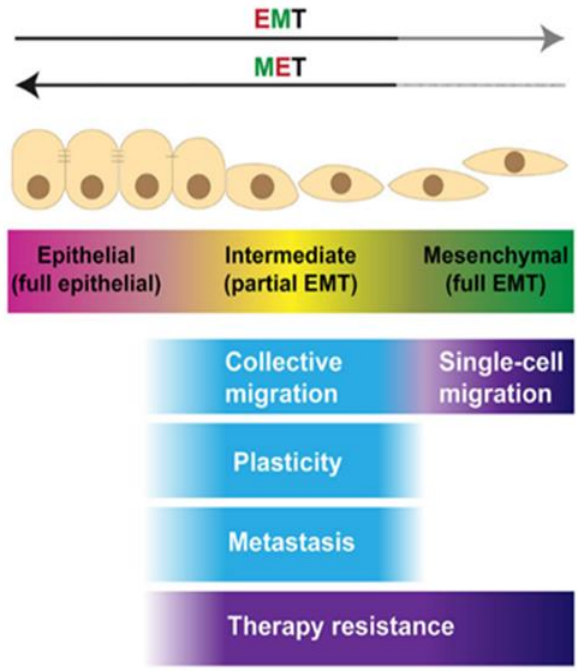

Figure 1: Characteristics of breast cancer stem cells. This figure illustrates the progression of Epithelial-Mesenchymal Transition (EMT) and Mesenchymal-Epithelial Transition (MET) stages. It captures the transformation of cells from a rounded form (representing full epithelial) to an elongated form (indicating full mesenchymal). Key characteristics associated with these transitions, such as migration, plasticity, metastasis, and therapy resistance, are also emphasized [30].

Interaction with Tumor Microenvironment (TME)

The microenvironment encompasses the extracellular matrix (ECM); soluble substances such as growth factors, hormones, and cytokines; and cellular components including cancer-associated fibroblasts, macrophages, endothelial cells, and immune cells. The collagen content of the ECM and its mechanical properties such as matrix stiffness have a substantial impact on cancer stemness [31]. In addition, the physiological properties of the TME, such as the oxygen level, acidity and nutrient status have been shown to influence the stemness and viciousness of breast cancers. The quiescence of CSCs in primary breast cancer and other malignancies is significantly

prevalent in regions of hypoxia, necrosis, and acidity [32]. Such unfavourable conditions have been indeed discovered to promote stemness, quiescence, and migration. Acidity in premetastatic locations provides a safe and nurturing environment for quiescent cancer cells, promoting dispersed malignant cell persistence and ultimately cancer progression. Several studies have revealed the impact of the tumor microenvironment on the modulation of EMT and MET states of BCSCs in response to a number of signalling molecules. For instance, it is evident that the EMT switchover stimulates various transcription factors in cancer stem cells under the influence of external stimuli [27].

Penetrating immune cells and activated fibroblasts, known as cancer associated fibroblasts (CAFs), induce inflammation in the tumor microenvironment, releasing cytokines, chemokines, and growth factors to which the tumor adjusts. It has been proposed that inflammation plays a critical role in tumorigenesis, angiogenesis, and dissemination. Tumor growth and metastasis are enhanced by IL-1, IL-6, IL-11, and transforming growth factor (TGF-β). TGF-β has two major roles in tumorigenesis. It acts as a tumor suppressor in the early stages of carcinogenesis; however, tumor cells in later stages evade this impact and advance in response to TGF-β signalling [33,34]. It also promotes EMT and the ability of BCSCs to initiate cancer. CAFs in breast cancer environment also produce IL-6, which promotes proliferation and tumorigenesis. IL-6 regulates self-renewal and initiates EMT in BCSCs. The activation of NF-κB by proinflammatory stimuli results in the conversion of normal cells to have a persistent malignant morphology with no alteration in Genomic DNA [33,34].

Cellular crosstalk in the TME is fundamental for cancer progression. As a matter of fact, BCSCs boost cancer progression by controlling immune cells and establishing an immunosuppressive environment thus minimizing cancer cell susceptibility and promoting resistance [4]. In particular, a range of chemokines released by BCSCs stimulates the recruitment of tumor-associated macrophages (TAMs) that help build the BCSC niche and promote immunological tolerance. They inhibit immune cells by secreting cytokines and growth factors, as well

as inducing the secretion of immunosuppressives found naturally in T cells [4]. In addition, macrophages secrete epidermal growth factor (EGF), which enhances the migration and invasion of malignant cells. Another molecule, the vascular endothelial growth factor (VEGF), induces vascularization and ECM remodeling. Macrophages also stimulate an increase in the expression of sex determining region Y-box 2 (SOX2), a transcription factor that correlates with stemness [35]. Cancer-associated fibroblasts (CAFs) are ubiquitous in connective tissues and one of the most frequent tumor microenvironment cells in all tumor types [36]. CAFs promote uncontrolled growth, vascularization, invasion, dissemination, and resistance to therapy in BCSCs. CAFs have also been shown to stimulate vascularization in breast cancer by attracting endothelial progenitor cells (EPCs) [37].

Similarly, cell-cell interactions of BCSCs with CD8 T lymphocytes in the microenvironment exhaust immune cells and induce immunological tolerance. This interaction occurs between PDL1, a T-cell suppressive molecule prevalent on the surface of BCSCs, and the PD1 receptor present on T-cells. Notably, PD-L1 also stimulates multidrug resistance and BCSC stemness by triggering the PI3K/Akt and ERK1/2 signalling pathways (Figure 2). In addition, ECM protects BCSCs from treatment pressure and promotes their metastatic development [38,39].

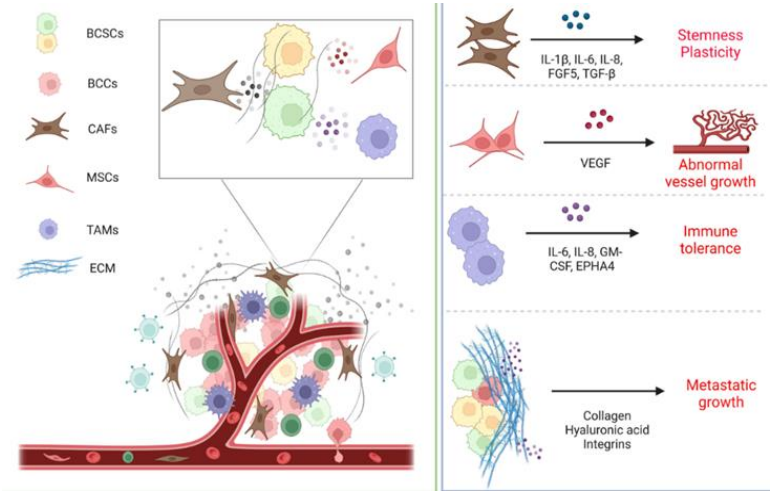

Figure 2: Interaction between tumor cell and the microenvironment. The biological activities of BCSCs are controlled by their microenvironment through direct interaction, the extracellular matrix (ECM), and signaling molecules. Cancer-associated fibroblasts (CAFs) release cytokines like IL-6, IL-8, and IL-1β, which enhance the stem-like properties and adaptability of BCSCs. Mesenchymal stem cells (MSCs) produce VEGF that nourishes BCSCs, resulting in irregular blood vessel formation. Similarly, macrophages emit a variety of cytokines that create a supportive environment for BCSCs and induce immune system tolerance. The ECM provides a shield for BCSCs against therapeutic stress and ensures their metastatic expansion [20].

Mechanisms of Relapse and Metastasis in BCSCs

Tumor metastasis is an intricate cascade involving a set of characteristics that should be adopted by metastatic tumor cells. These cells should be able to evacuate from the blood vessel walls by extravasation, withstand prolonged durations of mechanical stress in circulation, invade novel tissues, and multiply in target organs [40,41]. Despite the common misconception regarding metastasis as a latent event, malignant cells disseminate from primary tumors as an early event in oncogenesis. Recent findings indicate that 60- 70% of patients have already started the metastatic process by the time they are diagnosed. These data demonstrate that cancer growth and metastasis occur simultaneously. Gene expression studies in breast cancer demonstrate unique genetic modifications occurring in primary tumors and metastatic cells, providing

importance to the hypothesis of early metastatic spread [40,41]. Approximately half of the metastatic cells isolated from bone marrow of breast cancer patients contained fewer chromosomal abnormalities than the initial tumors, indicating that they disseminated prior to genomic instability events. These data imply that BCSCs have a metastatic potential [40,41].

A subset of breast cancer cells with characteristics such as self-renewal, quiescence, and strong metastasis and invasion capabilities reside in the circulation of patients with breast cancer who are undergoing or have completed treatment. These findings show that BCSCs have the capacity to migrate to distant organs and spread tumors to new locations [42] (Figure 3).

EMT promotes malignant cell dissemination, whereas MET promotes metastatic cell invasion. As previously stated, the tumor microenvironment influences cancer stem cells to upregulate transcription factors, such as Slug, Zebl, Zeb2, Snail, Twist, FOXC1, FOXC2, bHLH proteins, and TCF3 [43,44]. This provokes the synthesis of N-cadherin, vimentin, fibronectin, α-SMA, urging the shift to mesenchymal phenotype. EMT BCSCs in breast cancer promote tumor migration into the basal membrane, neighboring tissues, and even into the circulation where they survive because of their acquired quiescence and immunity to treatment [43,44].

Recent investigations on circulating tumor cells (CTCs) and disseminated tumor cells, both of which demonstrate EMT and sternness, support the involvement of EMT in tumor cell dissemination. In fact, tumors release thousands of cells into the circulation daily; however, only a minority of them cause secondary tumors to emerge. Metastasis-initiating cells (MICs) are a subpopulation of CTCs capable of generating clonal metastatic growth in distant organs [45] Clinically, the presence of five or more CTCs in 7.5 ml of blood plasma is a sign of tumor progression. The number of CTCs in patients with metastatic breast cancer is a stronger predictor of cancer outcome than other diagnostic methods. Recent research on CTCs from breast cancer patients has found a link between mesenchymal CTCs and tumor progression [45]. CTCs can exist as single cells

or multicellular clusters and they have been shown to display recognized mesenchymal markers and EMT markers such as the transforming growth factor beta (TGFB) signalling pathway and the transcription factor FOXC1. As a result, CTCs can withstand mechanical stress accompanied during their migration to distal organs through blood [45].

Upon extravasation from the vasculature, these EMT BCSCs produce micro metastasis in target organs. Although BCSCs derived from primary cancer tissues and CTCs have been demonstrated to exhibit typical EMT characteristics, the majority of metastatic cancers exhibit an epithelial architecture indicating that the reversal of the EMT program is required for dissemination [46]. (MET) is induced at pre-metastatic locations, resulting in the establishment of substantial macro-metastatic populations at remote sites. Indeed, the interaction between EMT and MET transition is the main driving mechanism of metastasis [46].

Recent studies have also revealed that stimulating MET with miRNA regulatory networks, particularly the miR-200 group, can facilitate breast cancer metastatic proliferation. In another study, selective activation of the Id1 gene in EMT-induced breast cancer cells induced MET via Twist1 inhibition, and this shift was essential for metastatic proliferation in the lung [46].

Given that primary cancerous masses discharge a significant proportion of malignant cells to circulate the bloodstream, only a small proportion of these cells (2%) are capable of initiating another tumor as a micro metastasis, and only 0.02% of CTCs are expected to become substantial macro metastases at distant sites [45].

Some secondary cancers could include CSCs with EMT and MET features. The presence of invasive and proliferative CSCs in a single tumor mass may contribute to a particularly aggressive subtype of breast cancer [45].

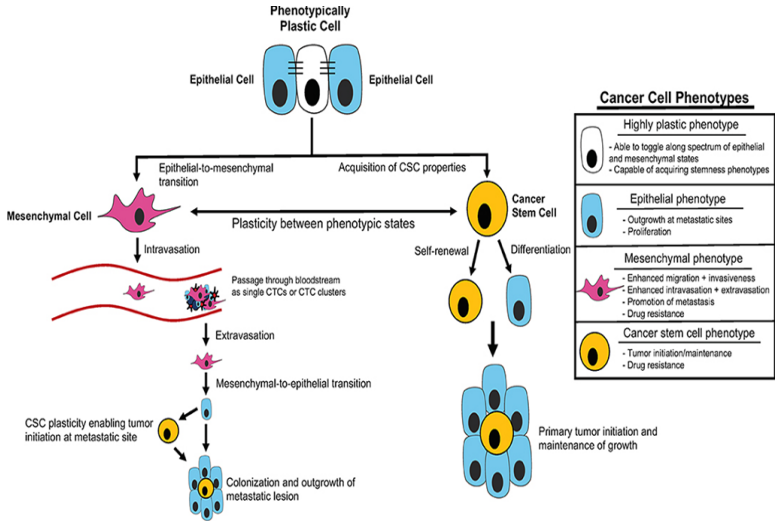

Figure 3: Tumor metastasis and cancer cell phenotypes. The versatility of cancer cells during the onset of a tumor and the metastatic process is discussed. This includes the role of epithelial-mesenchymal plasticity (EMP) and the development of cancer stem cell (CSC) traits in starting a tumor and elements of the metastatic process, such as entering and exiting blood vessels, and the colonization and expansion of metastases. Each stage is associated with unique cell phenotypes and features [47].

Conclusion

In conclusion, this review highlights the critical role of BCSCs in breast cancer's persistence, recurrence, and metastasis. BCSCs' distinct characteristics, drug resistance, and interaction with the tumor environment have been explored. Their metastatic potential challenges the traditional view of metastasis as a late-stage event, emphasizing the need for early targeting. The tumorigenicity, self-renewal, and surface markers of BCSCs, along with their plasticity and ability to transition between states, add complexity to our understanding. The significance of BCSCs in early tumor dissemination and the role of EMT and MET processes in malignant cell dissemination and invasion are discussed. Recent studies on circulating tumor cells (CTCs) and metastasis-initiating cells (MICs) support the involvement of EMT in tumor cell dissemination. The heterogeneity within breast cancer subtypes is underscored by the potential role of CSCs with EMT and MET features in secondary cancers.

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