

Mesenchymal Stem Cell-Breast Cancer Stem Cell: Relevance to Dormancy

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Abstract: Clinical advancement in cancer treatment has improved patient's outcome. Despite these advancements, cancer continues to remain a major clinical problem. However, treatment has been hindered by the ability of cancer to remain dormant for later resurgence. This review discusses dormant breast cancer (BC) with an emphasis on cancer stem cells (CSCs). BC cells (BCCs) have preference for the bone marrow (BM). We discuss how cells of the BM microenvironment such as mesenchymal stem cells (MSCs) and the hematopoietic supporting stroma interact with CSCs to support the latent or dormant phase of CSCs. The exchanges of small non-coding microRNAs between the CSCs and BM microenvironmental cells to induce cycling quiescence in CSCs are discussed. The article proposes the implications for treatments of BCCs and the challenges to target these cells in organs such as BM. Part of the challenges in this article include the similarity between the CSCs and the endogenous normal stem cells. The role of BM microenvironment with regards to oxygen levels and the ability to release cytokines as support of tumor growth is incorporated in the review.

Keywords: Breast cancer, cancer stem cells, mesenchymal stem cells, hematopoietic stem cells, stroma, bone marrow, exosomes, microenvironment, dormancy, cell cycle, immune modulator, immune suppressor, cytokine/chemokine.

ABBREVIATIONS

BCC: breast cancer cell

CSC: cancer stem cell

HSC: hematopoietic stem cell

MSC: mesenchymal stem cell

SC: stem cell

INTRODUCTION

Cancer metastasis is a leading cause of cancer-related mortality. Metastatic processes involve migration of cancer cells from its site of origin to secondary sites, where the cancer cells interact with the microenvironment/niche. The interaction between the cancer cells and respective niche can either lead to dormancy or cell growth into large tumors.

Despite clinical advancement in its diagnosis, death due to metastatic cancer cells remains a major issue. Drugs to target the proliferating cancer cells have improved with time, but the dormant cells seem to remain unaffected by this advancement [1, 2]. The drugs designed to target these cancer cells are unfortunately based on maximum cyto-

toxicity to proliferating cells leaving behind the dormant cells unaffected. Clinically, most cancer recurrence has been reported to originate from the dormant cells, in particular for breast cancer, the bone marrow [1, 2].

CSCs survive among the BM stromal cells that comprise non-hematopoietic cells such as fibroblasts and mesenchymal stem cells (MSCs) [2, 3]. Therefore understanding the interaction between cancer cells and the microenvironment will provide insights into how dormancy occur and thereby allow for the development of targeted treatment to reverse dormancy for effective treatments.

CANCER STEM CELLS (CSCs)

Cancer is a disease of mutation, where a tumor is composed of heterogeneous population of cells. The two prominent theories/models that explains the heterogeneity is stochastic and hierarchy/CSCs [4]. Stochastic model refers to every cell in a tumor as homogenous with the potential to initiate tumor and, heterogeneity varied function by populations of tumor cells. The CSC model proposes the existence of these cells within the tumor to sustain the growth by functioning as the initiating tumor cell [4, 5]. Like other stem cells (SCs), CSC can self-renew and give rise to a heterogeneous population of cancer cells within the tumor [6].

CSCs, with their long doubling times, can remain as dormant cells until the cancer cells review 'cues' from the

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microenvironment, resulting in tertiary metastasis, with proliferation and differentiation of the CSCs [7, 8]. Several studies including xenograft models have explained the tumor initiating potential of CSCs [8, 9]. The maintenance and reversal of dormancy by CSCs require several signaling pathways, transcription factors, cytokines/chemokines and other factors/proteins, most of which can also regulate normal stem cells [10–15]. Pathways involving Wnt, Notch, Sonic Hedgehog and NF κ B are the most common pathways that have been studied in both CSC and healthy stem cells [10–15].

Active regulation of the Wnt pathway has been reported in several sources of CSCs [16, 17]. Wnt signaling pathway can be β catenin-dependent or -independent [18, 19]. This pathway has a significant role in the self-renewal and maintenance of both stem cells, such as hematopoietic stem cells (HSCs), and those from the breast and colon [20]. The critical role of the Wnt pathways was demonstrated in studies that showed its inhibition affected the survival of CSCs [21].

The notch receptor is transmembrane, which is activated by direct contact with the ligand from another cell [22]. Notch signaling pathway has been shown to regulate healthy and malignant stem cell fate, partly through the initiation of transcription factors involved in cell differentiation [12, 13].

The activation of hedgehog pathway in stem cells causes cell proliferation and self-renewal [14, 23]. This pathway was initially reported in drosophila as key during development [24, 25]. This pathway has also been shown to stimulate CSC maintenance at metastatic sites [26]. In mammals, there are three common hedgehog ligands – Sonic hedgehog, Desert hedgehog and Indian hedgehog, all of which bind to the Patch receptor to activate the transcription factor, Gli [27].

NF κ B activation is linked to varied functions such as regulating the immune system, oncogenesis and cell development. NF κ B is involved in the regulation of CSC proliferation as well as other functions linked to oncogenesis [28]. In colorectal cancer, NF κ B has been reported to activate the Wnt pathway [29]. Studies using the low invasive/metastatic cell line, MCF7, showed that inhibition of NF κ B prevented tumor growth by cells with multipotency property [15]. CSCs resist apoptosis by maintaining an imbalance between the pro and anti-apoptotic bodies with the former being the dominant function [30]. Cytokines can protect CSCs by activating signaling pathways to mediate DNA repair [31, 32].

Studies suggest that CSCs thrive in a hypoxic microenvironment, in part due to factors, which cause chemo-protection [7, 33]. The bone marrow (BM) endosteum, although vascularized, is not similar to the central vascular system, making this region the preferred metastatic site for CSCs survival [34, 35]. However, further studies are needed to determine how CSCs survive within the central vascular region. The interaction of CSCs and BM micro-

environment, which comprise stromal cells and MSCs, is important for acquiring cycling quiescence [36–38]. Quiescence can be achieved by the exchange of factors such as miRNAs between cells of the BM microenvironment and CSCs. Such interaction could be direct by gap junctional intracellular communication (GJIC)) or indirect by vesicular transport or cytokines [39, 40].

CSCs can be identified based on phenotype. Common markers found on different CSCs like CD133, aldehyde dehydrogenase isoform 1, Lgr5 have been reported [31, 41–47]. The reported markers for CSC need further stratification to fully understand how CSCs and the cells close to their maturation interact with the microenvironment. One of the hallmarks of CSCs is its ability to initiate a tumor, form tumorspheres, and can be serially transplanted [9]. Besides the heterogeneity, CSCs share several similarities with healthy stem cells (reviewed in [48, 49]). The similarities between normal and CSCs brought challenges to specifically target CSCs without untoward effects on the healthy stem cells. Thus, it is important to understand the interaction between the CSCs with cells of the microenvironment to identify drug targets for safe treatment.

MESENCHYMAL STEM CELLS (MSCs)

MSCs are a class of adult stem cells predominantly found in the BM and adipose tissues. MSCs are involved in regeneration of various connective tissues, supporting hematopoiesis within the BM, and serving as ‘gate-keepers’ of the BM to monitor afferent and efferent migration to the organ [50]. Beyond these roles, MSCs exhibit unique immunomodulatory effects, permitting them to act as immune enhancers and suppressors, depending on the microenvironment [51, 52]. These properties translate into MSCs exerting both pro- and anti-tumor behaviors, discussed below.

The aforementioned properties of MSCs, coupled with their ability to cross allogeneic barriers, have led to the idea that MSCs could be used as an ‘off the shelf’ therapy, using MSCs from any donor to treat any patient [53]. At the time of writing this article, over 1,400 clinical trials are registers with several of these using allogeneic MSCs. The indications for MSCs encompass disorders such as auto-immune and inflammatory diseases, tissue regeneration and drug delivery. MSCs are proving beneficial in these areas, in part, due to their pathotropic behavior: MSCs express a wide array of cytokine and chemokine receptors, permitting their rapid migration to target sites and subsequent action within these varied microenvironments [54]. Within an inflammatory microenvironment – as is present during tissue injury or insult, including tumorigenesis – the MSCs can be licensed into anti-inflammatory cells [55]. This could be beneficial to the cancer cells, in that the immune system is suppressed and thus cannot generate a robust attack against the tumor cells. The immunosuppressive functions of MSCs caused CD4⁺ (T_H) and CD8⁺

(T_C) T cells responses to be inhibited, while activating regulatory T cells (T_{reg}) (reviewed in [56]). Simultaneously, MSCs promote angiogenesis – a function that affects all cells, including tumor cells. Thus, the tumor supporting role of MSCs must be considered when using exogenous MSCs, lest these cells promote growth of an undiagnosed tumor, as well as when considering treatments for present tumors.

Given that MSCs are capable of supporting tumor growth, it is tempting to eradicate the local MSCs at tumor sites. However, it is not currently possible to eliminate MSCs within a single target location – especially in the BM, where cancer dormancy occurs. Similarly, when targeting CSCs in cancer patients, the MSCs (and HSCs) must be protected lest the treatment kill the patient by eliminating stem cell reservoirs, including the pericytes, which surround blood vessels. Thus, further research requires identifying unique interactions between the CSCs and MSCs to target these cells.

MESENCHYMAL STEM CELL (MSCS) – BREAST CANCER CELL (BCC) INTERACTION

During the early stages of cancer, BCCs have the propensity to migrate to the BM where they achieve quiescence, evade immune surveillance and resist treatment. We have studied the mechanisms by which BCCs use genes and cells within the BM. The tachykinin gene and the receptor for the tachykinin peptides (*Tac1*) as well as MSCs and the hematopoietic stromal cells have been shown to facilitate the survival of BCCs [57].

Due to the immune suppressive functions of MSCs, their interactions with BCCs can impart immune protection to BCCs [57, 58]. The immunosuppressive abilities of MSCs to protect BCCs could occur early after the BCCs enter the BM. This possibility is due to MSCs being in contact with the linking central vascular sinus that allows cells to enter from the periphery into the BM cavity. MSCs and BCCs can interact by contact-independent communication. The mediators of such interaction could be due to extracellular vesicles and/or soluble factors such as cytokines and growth factors, known molecules involved in tumor development and progression, including metastasis [59].

Small membrane extracellular vesicles of endocytic origin called exosomes are believed to play a pivotal role in the crosstalk between BCCs and BM derived MSCs (BM-MSCs) [40]. Ranging between 40 and 150 nm in size, exosomes contain proteins and genetic materials, such as mRNA and miRNA, implying exosomes can influence the phenotype and function of the recipient cells by activating intracellular pathways [40].

Exosomes contain important signaling molecules such as mRNAs, miRNAs, lipids and proteins and function as a cell-to-cell communication vehicles, by delivering these important signaling molecules to other cells. Intercellular transfer of signaling molecules by exosomes have significant impact on physiological and pathological processes

such as development, cancer progression and immune response. Cancer cells can communicate with stroma by releasing exosomes into microenvironment and, in turn cancer cells can receive signals from stromal cells through exosomes secreted by stromal cells.

The experimental evidence indicated that exosomal mRNAs could be transferred to recipient cells for translation regulation [60]. This is very important because exosomes can alter recipient cell function by directly affecting their protein synthesis. However, the majority of investigated exosomal mRNAs are not full length mRNAs but rather, RNA fragments. Interestingly, one study reported that 3'UTR regions of mRNAs were significantly enriched in exosomal RNAs, implicating that exosomal RNA fragments can regulate gene expression and protein production in recipient cells [61]. MiRNAs also mediate communication between BCCs and MSCs. These small non-coding RNAs can be shuttled via exosomes and by GJ. The presence of GJ results in a bidirectional passaging of miRNA between MSCs and BCCs to promote dormancy [62, 63]. Once established in the BM, BCCs can form GJ with stromal cells and exchange miRNA resulting in phenotypic changes in the BCCs. Specifically, miR-127, -197, -222, and -223 decrease the production of CXCL12, resulting in decreased BCC proliferation and transition of the BCCs into cycling quiescence [64].

Makiko Ono, et al. reported when culturing MDA-MB-231-BM2 with conditioned media from BM-MSCs exosomes promoted dormancy in BM2 cells [65]. The authors confirmed the results by isolating BM-MSCs derived exosomes and characterizing the vesicles for known exosomes markers, CD9 and CD81 [65]. The interest in exosomes on different diseases, including their role in BC biology is rapidly increasing. By understanding the mechanism underlying crosstalk between BM-MSCs and tumor cells may be important to develop therapies to prevent the recurrence of BC, by avoiding dormancy and/or to reverse dormancy.

In addition to mRNAs/miRNAs, exosomal proteins have been shown to mediate intercellular communications in cancer. In particular Wnt proteins have been shown to be involved in BCC-stroma crosstalk [66]. Exosomes secreted by adipose-derived MSCs when added to MCF7 BCCs caused dose-dependent increase in migration through activation of Wnt signaling pathway [67]. Exosomes can also carry active Wnt proteins on their surface and deliver them to recipient cells and thereby induce Wnt signaling activation in recipient cells [68]. In another study fibroblast derived exosomes containing Wnt11 protein were shown to be taken up by BCCs, resulting in mobilization of autocrine Wnt-PCP signaling in BCCs and rendering BCCs to more invasive and metastatic in animal models [66]. In addition to receiving signals from the microenvironment, BCCs can also communicate with stroma by releasing exosomes into microenvironment. Adipose-

derived MSCs when treated by BCCs-derived exosomes converted to tumor-associated myofibroblasts through activation of SMAD-mediated signaling pathway [69]. Levels of angiogenic factors SDF-1 and VEGF1, prometastatic factor CCL5 and tumor growth promoting factor TGF β were also increased in exosome-treated adipose-derived MSCs [69].

Lipid bilayer membrane of exosomes consists of lipid pool that includes cholesterol, diglycerides, sphingolipids, phospholipidson, phosphatidylserine and other raft-associated lipids. These bioactive lipids in addition to their crucial structural role play also important role in intercellular communications as they can be taken up by recipient cells and can affect cellular pathways. In the context of cancer, exosomes rich in bioactive lipids inhibited Notch signaling pathway, a critical cancer cell survival pathway, leading to apoptosis in pancreatic cancer cells [70].

Soluble factors such as tumor necrosis factor-alpha (TNF- α), interferon-gamma (INF- γ) and interleukin-6 (IL-6) can regulate CSCs *in vivo*, through intercellular communication with other cells [71, 72]. Interaction between MSCs and BCCs could occur directly by GJIC or Gap Junctions (GJs). GJs are intercellular channels that allow for the exchange of small molecules between adjacent cells. GJs are formed by integral membrane proteins known as connexins and may play a role in the survival of

cancer cells in different microenvironments [73]. Donahue *et al* described the role of gap junctions in establishing bone metastasis. In this model, BCCs establish GJIC with osteoblasts resulting in a cascade of events, which allow BCCs to migrate through the bone layers [39].

Although there are reports of BCCs and MSCs forming GJ, such interaction is not yet well understood [74]. Studies have shown BM-MSCs promote the growth and proliferation of BCCs in 3D cultures [75]. MSC extracts inhibited the growth of BCCs [62]. In contrast, there was no effect of BCC proliferation by supernatant from MSCs or conditioned media [62]. The effect of MSCs on the proliferation of BCCs could be suppressive or enhancement [62]. The reason for these differences is yet to be determined. However, GJIC seems to have an effect since its inhibition with a pharmacological agent resulted in significant decrease in BCC growth [62].

CANCER DORMANCY

Metastatic dissociation of cancer cells from primary site to secondary site worsens cancer prognosis, as they are difficult to target. Some of these metastatic cancer cells divide rapidly, but some, remain quiescent until suitable microenvironment signals them to reverse the dormancy [7, 8]. Clinically cycling cancer cells can be targeted but currently there are no drugs available that can target

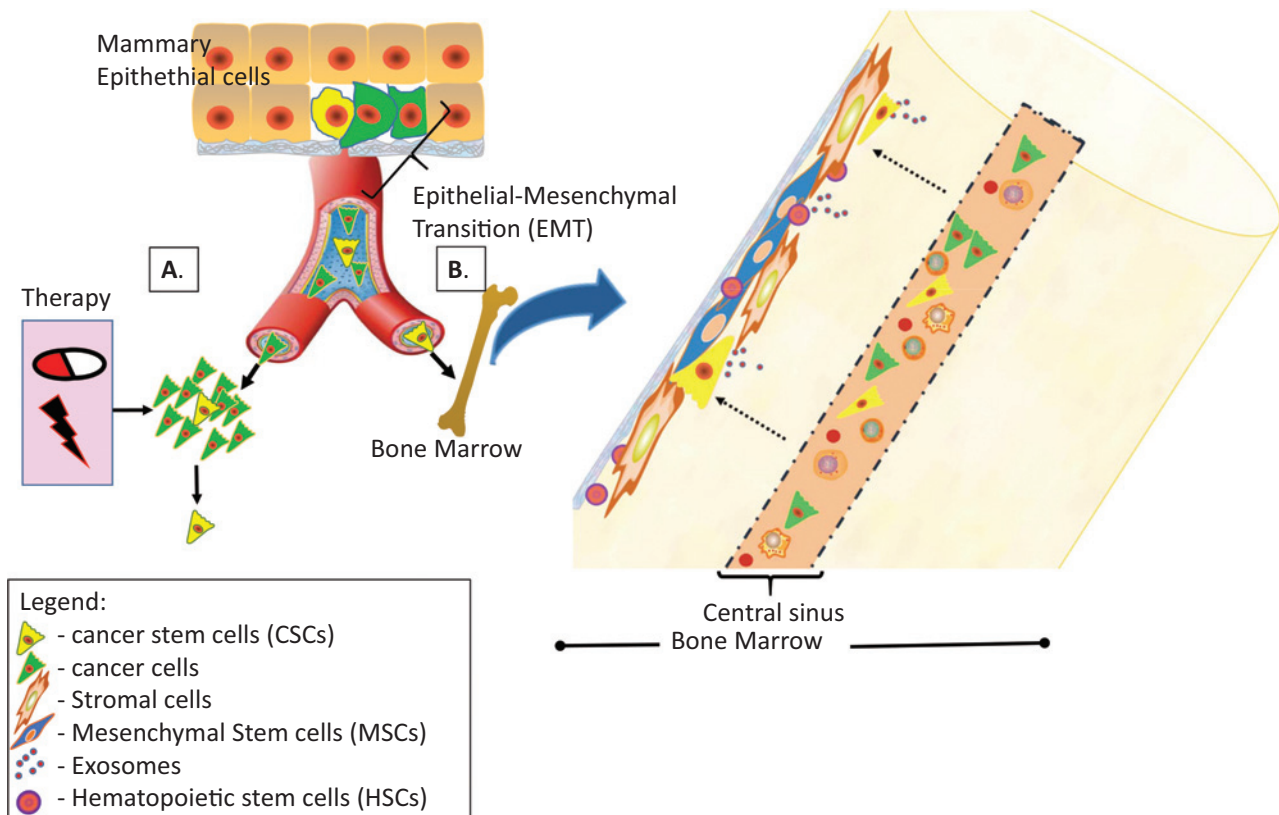


Figure 1. Shown are transformed mammary epithelial cells undergoing EMT. **A.** The CSCs survive treatment whereas the proliferating cancer cells are targeted. **B.** CSCs preferentially migrate to the endosteum where interaction (either directly or via microvesicles) with the endogenous MSCs and stromal cells, resulting in the cancer cells adapting a dormant phase. Shown are HSCs within the same region of the dormant CSCs.

dormant CSCs, leading to subsequent metastatic recurrence. These dormant cancer cells undergo cell cycle arrest i.e. remain in G₀-G₁ phase and predominantly migrate to BM [35]. Microenvironment plays an important role in acquiring, maintaining and reversing cancer dormancy, where microenvironment usually includes stromal cells and MSCs [76]. These growth-restricted cells remain undetected by altering several signaling pathway for a long period until the microenvironment supports its proliferation.

As discussed before, CSCs prefer hypoxic condition for survival and maintenance, it releases stress related signals, which promotes dormancy. Signaling pathway PI3K/AKT-1 regulates cell cycle and has been widely studied in cancer dormancy. In an *in vitro* model, when cancer cells were grown in serum free media, i.e. nutritional stress, clusterin (stress protein) bound to insulin like growth factor -1 and inhibited its binding to PI3K, thus negatively regulating PI3K-Akt pathway by reducing Akt phosphorylation [77] leading to quiescence on CSCs.

BM can significantly maintain HSCs and MSCs; hence, it is very likely that the signaling cascade involved in maintaining its quiescence will play a crucial role in maintaining dormancy of CSCs. Three most significant proteins that have shown its involvement in HSCs and CSCs are - growth arrest specific protein 6 (GAS6)- a ligand derived from stromal cells regulates its survival [78, 79], bone morphogenetic protein 4 (BMP-4)- blockage of BMP-4 suppresses differentiation of stem cell in BM [80, 81] and transforming growth factor β (TGF- β) - BMP-7, which belongs to TGF- β important in maintaining homeostasis by downregulating ERK and upregulating p38, when blocked reduced cancer dormancy [82, 83]. Phosphatase and tensin homolog (PTEN), a tumor suppressor gene downregulates PI3K-AKT signaling, and its absence increase unregulated proliferation of hematopoietic system leading to leukemia [84, 85]. Hence, an understanding of BM microenvironment is essential for targeting CSC.

CONCLUSIONS

Current treatments have limitation with regards to targeting the CSCs within the BM microenvironment. Several drugs require proliferating cells. More importantly, the CSCs within the BM are in the same area as the endogenous HSCs (Figure 1). Since the CSCs share properties with HSCs, it is a challenge to target the CSCs without harm to the HSCs, which can result in hematopoietic failure (reviewed in [86]). Another limitation is that the markers reported for CSCs identify a heterogeneous population and therefore cannot be used for targeted therapy. Thus understanding the interactions between the CSCs and the BM stromal cells to achieve dormancy will lead to an improved treatment of cancer patient.

CONFLICT OF INTEREST

The authors have no conflict to declare.

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