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Mini-review

The bone marrow niche in support of breast cancer dormancy

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ABSTRACT

Despite the success in detecting breast cancer (BC) early and, with aggressive therapeutic intervention, BC remains a clinical problem. The bone marrow (BM) is a favorable metastatic site for breast cancer cells (BCCs). In BM, the survival of BCCs is partly achieved by the supporting microenvironment, including the presence of immune suppressive cells such as mesenchymal stem cells (MSCs). The heterogeneity of BCCs brings up the question of how each subset interacts with the BM microenvironment. The cancer stem cells (CSCs) survive in the BM as cycling quiescence cells and, forming gap junctional intercellular communication (GJIC) with the hematopoietic supporting stromal cells and MSCs. This type of communication has been identified close to the endosteum. Additionally, dormancy can occur by soluble mediators such as cytokines and also by the exchange of exosomes. These latter mechanisms are reviewed in the context of metastasis of BC to the BM for transition as dormant cells. The article also discusses how immune cells such as macrophages and regulatory T-cells facilitate BC dormancy. The challenges of studying BC dormancy in 2-dimensional (2-D) system are also incorporated by proposing 3-D system by engineering methods to recapitulate the BM microenvironment.

Introduction

The majority of cancer-associated deaths have been linked to cell invasion and metastasis. In fact, the majority of cancer cells in a patient were found at metastatic secondary sites as compared to the primary tumor [1]. Tumors are comprised of a heterogeneous population of cells such as the tumor initiating cells, also referred as cancer stem cells (CSCs), immune cells such as macrophages, fibroblasts and endothelial cells. Breast cancer (BC) cells (BCCs) show preference for the bone marrow (BM) [2]. Immediately after BCCs migration and invasion into the BM, they interact with mesenchymal stem cells (MSCs) that surround the abluminal region of BM's central sinus [3]. Their interaction promotes MSC protection of BCCs from immunosurveillance and clearance by increasing T-helper (Th) Th2 cytokines, the recruitment of regulatory T cell (T regs), and MSC mediated TGF-β1 secretion [4].

Invading BCCs can take advantage of the immune tolerant features and chemotactic properties of MSCs, to support tumor growth, metastasis and dormancy. First, by recruiting MSCs to the primary tumor site where they release cytokines that supports tumor survival [5]. Secondly, the BM can produce supporting growth factors and express adhesion molecules that make it easy for the BCCs to enter the cavity and survive [6].

As cancer cells adjust to its new microenvironment, the cells can communicate with the MSCs to attract other cells such as macrophages and immune suppressor cells [7]. Besides immune suppressive properties, MSCs can also aid in promoting cancer cell dormancy. Consequently, the multiple properties of MSCs would allow the BCCs to survive. These methods are expanded in this review article.

Currently two theories are postulated to explain tumor cell dormancy. One explanation is that slow proliferation is counterbalanced with apoptotic signals resulting in a stagnation of the tumor size [8]. The second explanation refers to cells that enter cycling quiescence in which they are maintained in G0/G1 phase of the cell cycle. The sum of these broad methods is the establishment of cycling quiescence of the BCCs, with the ability to recur and metastasis to tertiary sites at an undetermined time [8].

The ability of BCCs to show preference for metastasis to the BM can provide an additional level of protect for the BCCs. Specifically, the dormant BCCs, which are mostly located close to the endosteum, where they can also evade toxic anti-cancer agents. This type of protection is noted in patients with the primary tumor excised and then treated with adjuvant therapies. At 36 months after this treatment BCCs were identified in BM aspirates of patients [1].

To expand on the previous paragraph, there are reports showing that early removal of the primary BC could not prevent tertiary

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metastasis of the cancer initiating cells from the BM [9]. These findings indicated that the BCCs could exit the primary site early for integration within the BM. Indeed, the observations were clinically supported; showing a small number of BCCs exiting the primary tumor site and entering the BM during the early phase of tumor development [10]. Although the reports linked a small subset of CSCs as those that could establish and maintain dormancy, studies are needed to fully understand how tumor cells exit from the primary site and the method of integration into the secondary sites.

CSCs exhibit functions consistent with stem cells, including their ability to form more mature cells [11]. These latter properties are reminiscent of non-transformed cells that can differentiate along cell lineages, except that the developing cells are still transformed. These diverse properties of CSCs can also be noted during the later steps of metastasis when the CSCs begin to transition into invasive and metastatic cells [12].

The heterogeneity of tumors, combined with increased chemoresistance and cycling quiescence of cancer cells make it increasingly difficult to targeting the dormant cancer cells. As discussed above, this challenge is particularly relevant when trying to establish treatment protocols to targeting of dormant BCCs within the BM. The reasons for such challenges are multi-fold, and are discussed in the review. Also discussed, is an explanation of how the BCCs enter the BM to survive and adapt a dormant state in close proximity to the endosteum. The use of immune targeted therapy can eliminate the toxicity associated with current chemotherapeutic agents and is able to travel to the secondary metastatic sites to deliver therapy. Another topic for discussion is the utilization of 3-dimensional (3-D) biomaterials, as an experimental model to study BCCs interaction with the BM microenvironment.

Breast cancer dormancy

In general, clinical dormancy is considered as the phase between the removal of primary tumor and its resurgence [13]. Despite intense research studies on BC dormancy, the mechanisms underlying this phase of BC, in particular, in the BM remain poorly understood. Currently, there is a growing research interest to focus on how the different microenvironments affect dormancy and to determine if there are specific subtypes of BCCs that prefer to transition into dormant BCCs. One of the many challenges to target dormant BCCs is due to their ability to remain in cycling arrest for long periods. The importance of such studies is to develop new treatments to reverse and/or target the dormant BCCs [14–34].

A mathematical models of the development of cancer could reveal how dormant cancer cells could be established at different times, and provided insights into the challenges to eradicate these slow-dividing quiescence cancer cells [9]. The model, which was based on clinical and research data demonstrated how the tumor progressed from early tumor initiation upon epithelial to mesenchymal transition (EMT) unto metastatic cells. Based on the model, one would extrapolate that the fate of the metastatic cancer cells might be different depending on the host metastatic tissue. One would also assume that the type of targeting might be different, depending on the phase of the cancer [9]. As an example, the immune response would be different between initiation and the metastatic phase.

A systematic analysis of the method by which a tissue microenvironment facilitate dormancy would lead to targeted treatment to reverse the process, perhaps through combined therapies to prevent further proliferation and migration of the cancer cells to tertiary sites [35]. A single treatment with the current chemotherapy drugs might not be sufficient to target the dormant cancer cells because most of the anti-cancer drugs require that the cancer cells undergo rapid proliferation. Thus, there is a need to first induce the dormant cells to proliferation since the CSCs are generally slow dividing cells that evade chemotherapy for resurgence after decades of remission [36–41].

As discussed above, several subsets of cancer cells may be able to adapt dormancy within secondary microenvironment. However, it seems that the CSCs may be the preferred cells to transition into cycling quiescent phases as dormant cells [42]. Regardless, both the CSCs and the other dormant subsets are generally low-cycling cells that are mostly chemoresistant [43]. It should be noted that the mechanism of chemoresistance is a complex biological process that cannot be over-simplified. This article will discuss this topic in the subsequent sections.

As dormant cells, the CSCs are mostly in G_0 phase with increased level the cyclin dependent kinase inhibitor, p27 [44]. Reverse dormancy could occur by changes within the niche of the CSCs resulting in proliferation and migration to other tissues as metastatic sites. Upon receiving the signal to reverse dormancy, Kip1 ubiquitination complex degrades p27 to allow for the otherwise dormant CSCs to continue to the different cycling phases [45].

This paragraph discusses the behavior of CSCs within the BM. This organ is relevant to BC because the clinical data indicated that BCCs show preference for the BM [46–48]. In the BM, BCCs can remain in cycling quiescence close to the endosteum area [46]. The CSCs have been shown to interact with the BM microenvironment where the cells adapt a dormant phase [49]. These findings are consistent with the report that more than 50% of BC relapse are caused by the initiating cells from the BM [37,50].

The BM has a gradient of oxygen level, with the endosteum region being more hypoxic [51]. Thus, it is postulated that the supporting role of the BM for the survival of CSCs could be partly due to the low oxygen supply within the endosteum. Since CSCs share many of the properties of stem cells such as the HSCs, it is likely that the hypoxia area will be accommodating to the CSCs [52]. The hypoxic microenvironment may induce the CSCs to enter dormancy. Currently, the exact pathways by which hypoxia facilitates dormant state is unclear. This is an important issue to understand BC dormancy and should be a research area, open for investigation. There are some reports on the regulatory role of hypoxia on induced stress signals. An example is based on cycling arrest caused by stress signals to activate PI3K/AKT-1, resulting in inhibition of insulin like growth factor and decrease in phosphorylated AKT-1 [53,54].

Hypoxia can contribute to chemoresistance and malignancy. Inside the tumor microenvironment, macrophages (MΦ) are recruited and polarized into M2 phenotype to support tumor survival by promoting angiogenesis [55]. Oncostatin M and Eotaxin are cytokines with a key role in metastatic BC, due to the recruitment of MΦ into hypoxic regions of the tumor. Hypoxia induced factor (HIF1α) has a role in BC malignancy by activating of ten-eleven translocation (TET) enzymatic expression that causes DNA methylation to subsequently induce breast tumor initiating cells to undergo epigenetic changes, which is beneficial to tumor survival and perhaps tumor dormancy [56].

BM microenvironment – facilitator of cancer metastasis

In vitro co-culture of BCCs and BM stromal cells formed GJIC, resulting in cycling quiescence of the BCCs [47,57]. Connexin 43 (Cx43) has been shown to be responsible for the formation of GJIC with the non-hematopoietic cells of the BM [58]. Since Cx43 has been reported to exhibit a tumor suppressor role, it is not a surprise that the formation of GJIC resulted in cycling quiescence of the BCCs [59,60].

This paragraph briefly discusses the molecular events following contact between cells of the BM microenvironment and BCCs leading to BCC dormancy. GJIC between BCCs and stroma allows for the exchange of microRNAs (miRNA) between the two cell types to transition the BCCs into cycling quiescence [47,61]. Specifically,

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