Hierarchy of Breast Cancer Cells: Key to Reverse Dormancy for Therapeutic Intervention

SARAH A. BLISS, STEVEN J. GRECO, PRANELA RAMESHWAR

Department of Medicine, Hematology/Oncology, New Jersey Medical School and Graduate School of Biomedical Sciences, Rutgers University, Newark, New Jersey, USA

SUMMARY

An understanding of how cancer cells adapt dormancy would allow for targeted treatment. The current literature suggests that the cancer stem cells might be the major cells with the ability to become quiescent and to resist current drug treatment. The properties of cancer stem cells and healthy stem cells are functionally similar, thereby posing a challenge to target the dormant cells. The bone marrow is particularly a challenge because the dormant breast cancer cells are close to the endosteum, which is also home to the endogenous hematopoietic stem cells. Here we discuss how research studies could bring an understanding of the cellular and molecular interactions between the cancer stem cells and cells within the bone marrow microenvironment. This will allow for intervention to reverse dormancy for targeted treatment. The treatment will require studies within the normal organ functions to ensure treatment without toxicity.

CANCER STEM CELL

Tumors are composed of a heterogeneous population of malignant cells. The heterogeneity is broadly categorized based on phenotype and function [1]. Although the details on the heterogeneity among tumor cells are not understood, the cancer stem cell (CSC) model does provide one explanation. This model is not new because it was proposed more than 150 years ago. However, recent advances in our understanding of stem cell biology, along with a demand for new strategies to target cancer, have renewed interest in the CSC subset [2].

Functionally, there are parallels between normal stem cells and CSCs. In adults, stem cells maintain the tissues and organs through self-renewal and differentiation into functional mature cells, as required by the specific organs or tissue. CSCs can also self-renew and differentiate within a hierarchy of tumor cells [3]. The hierarchy of cancer cells begins with the tumor-initiating cells followed by the more differentiated cancer cells referred to as the cancer progenitors [3, 4]. It is this heterogeneous mixture of cancer cells that creates the tumor; the bulk of the cells are the non-tumor-initiating CSC progeny. However, the most difficult to target with current therapies are the CSCs [5]. This is due to their ability to initiate tumors and yet resist cancer treatment. CSCs have low proliferative activity and are resistant to chemotherapy and radiation; such characteristics are often found in dormant cells, as discussed in the next section.

DORMANCY

Dormancy is defined as a state by which fully transformed cells retained a cycling quiescence phase with nontumorigenic properties that resists anticancer therapy. Clinical or metastatic dormancy has been defined as the time (5–25 years) between removing the primary tumor and relapse [6, 7]. This brings up the question of what is referred to as “cured” in oncology, because these cells exist by offsetting the proliferation of the cancer cells by apoptosis [7]. Of note, cell cycle quiescence, although a hallmark of dormancy, is not the only functional change during the “hidden” phase of the cancer. The cells with a dormant phenotype could be in areas of low angiogenesis and show increases in stress-related kinases (discussed in [7]).

In the case of breast cancer, the cells can reside in the bone marrow and other organs as dormant cells, long before clinical detection of the tumor [8–10]. Thus, the clinical goal for cancer treatment would be to eradicate the dormant cancer cells. However, this cannot be accomplished unless the method by which dormancy is achieved and maintained is understood. It is also necessary to determine whether dormancy can be adapted by specific cancer cell subsets.

An understanding of cancer dormancy would allow for targeted treatment to reverse the quiescence state of the cancer. Reverse dormancy refers to the process in which the resistance noncycling breast cancer cells are changed into cycling cells that have lost chemoresistance and can be targeted. It is expected that the cancer cells will interact with cells within the tissue microenvironment to acquire dormancy. Because the dormant cells will be among resident cells within an organ, the challenge will be to reverse dormancy without toxicity to the normal organ function. Breast cancer dormancy in the bone marrow is an excellent example of the complexity between the cancer cells and microenvironment, as discussed in the next section.
An example of how breast cancer cells can be targeted is extrapolated from our studies. Breast CSCs form gap junctional intercellular communication with resident bone marrow stroma [3]. MicroRNA (miRNA) can be shared between the contacting cells to form cycling quiescence [11]. The gap junction is formed by connexin 43, which is regulated by CXCL12 [3, 12]. These cellular and molecular findings can be intervened to break the intercellular connection. RNA therapeutics could be used to block the miRNA. At this point, another drug will be needed to induce the differentiation of the cancer stem cells to prevent them from dedifferentiating to CSCs; otherwise they will reform intercellular communication. In the future, biotechnology and pharmaceutical companies will have several targets as scientists focus on identifying genes that are involved in dormancy by CSCs [13].

**BREAST CANCER CELL DORMANCY**

In several cases of breast cancer resurgence, the bone marrow was identified as the source of initiating cells (CSCs) [14–16]. Breast cancer metastasis to the bone marrow results in a worse prognosis as compared with sentinel lymph nodes [17–19]. The clinical and experimental evidence indicates that cells within the bone marrow microenvironment close to the endostium can facilitate dormancy as well as resurgence of breast cancer [16, 20–24].

Stromal cells, which are part of the bone marrow or hematopoietic supporting niche, interact with breast cancer cells to regulate the production of cytokines. The changes in cytokines cause molecular changes in the cancer cells and the endogenous stroma to form gap junctional intercellular communication between the breast cancer cells and bone marrow stroma [11, 22, 25]. Through unclear mechanisms, after gap junctional communication the breast cancer cells adapt cycling quiescence [11, 22, 25]. Gap junctional intercellular communication with stroma can only be established by the most primitive subset of breast cancer cells (CSCs). This brings up questions about the identity of the cancer cell subset with a preference for dormancy and how the cancer cells interact with the endogenous cells within the bone marrow microenvironment. Indeed, the experimental evidence suggested that the most primitive breast cancer cells with tumor-initiating properties adapted dormancy and resisted chemotherapy [3]. The next section discusses the significance of research on CSCs as those with a preference for dormancy and, if this is the case, how the development of a hierarchy of breast cancer cells could be fundamental to reverse dormancy for targeted treatment.

**BONE MARROW NICHE IN BREAST CANCER DORMANCY**

A case for a hierarchy of breast cancer cells is underscored by the significance of poor clinical outcome when the cancer cells metastasize to the bone marrow [17, 26, 27]. Undoubtedly, breast cancer remains a clinical challenge despite education and aggressive intervention [28]. Mammograms with human compliance have led to early detection with some improvement in the overall survival [29]. Although breast cancer cells might adapt dormancy in several organs such as the brain, we discuss dormancy in the bone marrow because it is a major organ of metastasis [17, 26, 27]. Furthermore, the bone marrow has been identified as a source of initiating cells for tertiary metastasis in a significant number of cancer recurrences [15, 30, 31]. The fact that breast cancer resurgence from the bone marrow can occur more than 10 years after remission [14] supports a role for the bone marrow as the “home” for dormant breast cancer cells. At this time, it is unclear how the dormant breast cancer cells survive within the bone marrow niche for a long period without interrupting normal hematopoietic activity [25]. Thus, breast cancer dormancy will need to be studied in the context of hematopoiesis [25].

Cells within the bone marrow such as stroma and mesenchymal stem cells need to be investigated for their roles in breast cancer dormancy. Indeed, any tumor microenvironment is expected to be complex, with multiple intercellular interaction [32]. In bone marrow, stromal cells and mesenchymal stem cells are not the only components of the niche. However, because they are major components of the hematopoietic system, their role in dormancy is important because during clinical dormancy, there is no evidence of hematopoietic disruption [33–40]. Thus, it is important to understand how the major components of the hematopoietic niche can sustain dormancy and at the same time elicit normal hematopoietic function. The interplay among cells of the bone marrow niche and dormant breast cancer cells would lead to strategies to reverse dormancy for directed target of breast cancer cells within the bone marrow, without toxicity to the hematopoietic system [41–46].

**HIERARCHY OF BREAST CANCER CELLS AND DORMANCY**

The above discussion makes a case for CSCs as the potential subset on breast cancer resurgence. However, the other breast cancer cell subsets cannot be eliminated as candidates that could adapt dormancy. We have developed a working hierarchy of breast cancer cells beginning with the most immature subtype that we identified as those with tumor initiating and self-renewal function [3]. The most immature breast cancer cells can form gap junctional intercellular communication with bone marrow stroma for miRNA exchange [3, 11]. Although several membrane proteins have been linked to the identity of breast CSCs, there are further studies to develop a hierarchy of breast cancer cells. This hierarchy should not be based only on phenotype but also function. As an example, we have used a stem cell gene, Octamer 4, to demarcate subsets of breast cancer cells. Through these studies, we have been able to initiate a working hierarchy of breast cancer cells [3]. Because we were interested in dormancy in the bone marrow, we compared the different subsets based on function. We studied intercellular communication with endogenous bone marrow stroma, tumor initiation, self-renewal, and dye efflux [3]. Intercellular communication, through gap junction, allowed the cells to share miRNA to regulate gene expression [11]. While these studies are at an early stage, one needs to keep in mind that there are other microenvironmental cells in the bone marrow, such as osteoblasts and mesenchymal stem cells. Also, because the subset of dormant cells in other tissues might be phenotypically similar to those in the bone marrow, functional studies will be required to show how the different subsets interact with the tissue microenvironment.

Despite the identification of cancer stem cells by several scientific groups, it is important to understand how the other subsets interact with cells of the tissue microenvironment because the cells lower within the hierarchy might be dedifferentiate to CSCs. If so, this information will be required to develop drugs. There are interests to target the CSCs and to allow the other subsets to...
undergo cell death because they would not be able to initiate further tumor development. One must be cautious that this could open the door for the cancer cells with early maturity to dedifferentiate back to CSCs. These are considerations for research, which will have to precede adequate drug development, going forward. Furthermore, the other subsets might interact differently with cells of the bone marrow microenvironment to transition into a dormant phase.

The development of a robust hierarchy of breast cancer cells and their interactions with cells of the bone marrow microenvironment is fundamental to the eradication of breast cancer cells. To explain the significance of how the bone marrow microenvironment supports breast cancer dormancy, we discuss the past failure of autologous bone marrow transplantation for breast cancer. This failure underscores the challenge to target dormant breast cancer cells in the bone marrow and also demonstrates the resistance of this population of breast cancer cells in the bone marrow.

The transplantation protocol entailed temporary removal of hematopoietic stem cells and their progenitors from the bone marrow of breast cancer patients for high doses of chemotherapy to eradicate the residing breast cancer cells. After this, the patients were reinfused with the autologous hematopoietic cells. The method resulted in poor outcome, indicating that the breast cancer cells survived the high-dose chemotherapy. We now know from the literature that the cancer stem cells and perhaps other subsets with an immature phenotype can survive close to the endosteum by forming gap junctional intercellular communication with the hematopoietic supporting bone marrow stroma [3, 22]. The breast cancer cell subsets located close to the endosteum have been shown to resist chemotherapy. The failed procedure indicated that it is not sufficient to study how cells within the bone marrow microenvironment support dormancy; one must also identify the particular subset of breast cancer cells and understand their interaction with the microenvironment. Progress in this area can only occur with the development of a robust hierarchy of breast cancer cells. At this time, perhaps progress in this field will not occur by scientists with one focused area of expertise, but by an interdisciplinary team. The basic biologists will need to acknowledge their limitations and invite bioengineers and material scientists.

The region of bone marrow close to the endosteum is relatively hypoxic [35, 36]. Tumor dormancy was proposed as a failure of angiogenesis to delay the recurrence of tumors [47, 48]. Thus, dormancy close to the endosteum is consistent with the hypoxic condition of the region. In addition to cancer stem cells interacting with cells of the bone marrow microenvironment, the reduced vasculature close to the endosteum might partly account for the ability of the cancer stem cells to remain dormant. In the absence of substantial vasculature, it would be difficult to deliver drugs to the endosteal region, as well as in other tissues of dormancy. This limitation would bring forth other methods to deliver drugs. In the case of the bone marrow, mesenchymal stem cells could be used to deliver drugs because these cells would home to the bone marrow.

**THE CHALLENGE**

Ideally, the goal of any cancer treatment is to target the dormant cancer cells, especially those responsible for initiating the tumor. The CSCs appear to be the subset responsible for tumor initiation. There are several challenges to achieve success in targeting dormant cancer cells. First, the cancer stem cells have common properties with the normal stem cells, which are present in all organs. It will be a challenge to target the cancer stem cells without toxic effects on the endogenous normal stem cells. Second, there is
a major challenge to target dormant and CSCs within the bone marrow. The literature indicates that dormant cancer cells, in particular breast cancer cells, are present close to the endostium (Fig. 1). This region is also home to hematopoietic stem cells and hematopoiesis (Fig. 1). It will be difficult to target the cancer cells without toxicity to hematopoiesis because the drug that targets the CSCs is likely to also target the healthy hematopoietic stem cells (Fig. 1). Also, this area has reduced vascularity as compared with the central sinus, making it difficult to get drugs to this area. We propose cell therapy as a method to deliver drugs and perhaps RNA to dormant cancer cells within the endosteal region. This would result in hematopoietic ablation, underscoring the need for in-depth research studies on the molecular and cellular interactions between the dormant cancer cells and cells within the endogenous organ microenvironment. It is expected that the dormant cells will need to be reversed to proliferating cells for effective targeting.

Another challenge is the reports of cancer stem cells or similar cells within the circulation of patients [3, 49]. Research studies are required to determine whether the circulating cancer stem cells differ from those in other organs. Live imaging in animals can track cancer cells [50]. However, because the frequency of cancer stem cells is low, it will be technically challenging to use live imaging to study the movement of this particular subset.

The role of cytokines in cancer dormancy has been studied [20]. However, cytokines generally act in paracrine manner. Thus, it is likely that through intercellular interaction between the CSCs and cells of the tissue microenvironment, the cells could communicate with each other, either through soluble factors or through small vesicles such as exosomes, which would dictate the function of each cells, such as the production of cytokines. The cytokines are redundant with regards to their functions and would be difficult for direct targeting.

It could be noted that dormant tumor cells could never become a clinical problem. Similarly, CSCs could also remain dormant and never become a clinical problem. However, when there is a clinical diagnosis of cancer, one has to keep in mind that these cells exist, and additional intervention would be needed to eliminate them, even as dormant cells.

CONCLUSION

This brief perspective summarizes the complexity of targeting dormant breast cancer cells in the bone marrow. Regardless of the methods used to target the cancer cells, the targets will need to be studied in the context of the hematopoietic system. These studies will require a team with a multidisciplinary background in stem cell research, cancer biology, immunology, and hematopoiesis. Although not discussed in this perspective, the inclusion of bioengineers and investigators with mathematical modeling experience would be an advantage, because this could supplement the biological findings to investigate how small changes within the tissue microenvironment might affect the outcome of the dormant cancer cells. Mathematical modeling will be able to insert the different parameters to account for the heterogeneity of cancer and to include the underlying clinical "picture" of the patient to accomplish the following: to determine how the patient might respond with other drugs, to determine any untoward effects of the other pathological conditions, and to predict what combination of drugs could be effective for the cancer.

Finally, the development of a hierarchy of cancer cells is still in the early stage. This perspective discusses the need to invest in this approach because this might be key to the development of effective and safe targets to eradicate the majority of cancer cells and prevent or prolong the time before recurrences of cancer.

AUTHOR CONTRIBUTIONS

S.A.B., S.J.G., and P.R.: manuscript writing, final approval of the manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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