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Research paper

The role of hematopoietic stem cell niche in prostate cancer bone metastasis

A.M. Decker [a], Y. Jung [a], F. Cackowski [a,b], R.S. Taichman [a,*]

[a] Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, 1011 North University, Ann Arbor, MI, USA
[b] Department of Internal Medicine, Division of Hematology and Oncology, University of Michigan School of Medicine, Ann Arbor, MI, USA

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ABSTRACT

Approximately 80% of prostate cancers exhibit some degree of bone metastasis. The role of the bone marrow and the hematopoietic stem cell (HSC) niche in attracting metastatic cells and maintaining dormancy of disseminated tumor cells (DTCs) is an increasingly important topic towards the development of novel prostate cancer therapies. This paper reviews aspects of the HSC niche that lead to prostate cancer cell homing and dormancy in the bone marrow. This review also discusses the role of DTCs in the niche environment and discusses the role of erythropoietin in targeting DTCs within the HSC niche.

1. Introduction

Cancer cells disseminate from a primary tumor and enter the circulation, of which less than 0.01% survive and produce metastases [1]. Hematogenous circulation and lymphatic routes appear to be major routes through which disseminating tumor cells (DTCs) navigate. There are many challenges that tumor cells must overcome during the metastatic process including dissociation from neighboring cells of the primary tumor, extravasation, survival, and establishment in distant sites. DTCs have a number of different fates including death, dormancy, or proliferation [2]. The role of the microenvironment in tumor cell fate regulation has been reported as early as Paget's "seed and soil hypothesis" [3]. This hypothesis was expanded upon by Fidler [2], who suggested that tumor cells (i.e. seed) extravagate into circulation, survive, and establish in a distant site (i.e. soil), and their fate (death, dormancy, or growth) is directly influenced by the microenvironment of the distant site. The "seed and soil" hypothesis has been used to describe many different tumor-related diseases, including prostate cancer, which has a particular predilection for metastasis to bone which also houses the hematopoietic stem cell (HSC). Toward this end, 80% of advanced prostate cancer cases exhibit distant site metastasis in bone accompanied by a median survival of approximately 40 months [4]. Here, we discuss insights into the role of the HSC niche in prostate cancer (PCa) bone metastasis.

2. Homing of DTCs to the HSC niche

The HSC niche is a complex microenvironment comprised of many cell types, including endothelial cells, adipocytes, osteoclasts, osteomacs, and cells of osteoblastic lineage [5]. A healthy HSC niche provides homing signals to healthy HSCs in order to promote their normal function [6]. Shiozawa et al. [7] demonstrated that increasing the number of HSC niches promoted increased number of bone marrow DTCs, which indicated these same homing signals can be exploited in PCa metastasis. Two important mediators of the HSC microenvironment are the chemo-attractant stromal derived factor-1 (SDF-1 or CXCL12) and the cell attachment factor (Annexin2 or ANXA2). CXCL12 regulates HSC homing to the bone marrow as well as mobilization into circulation, while ANXA2 is likely involved in HSC binding to the osteoblastic niche, and may act as an anchor of CXCL12 and aid in localization to the niche [8]. Recently, it was shown that bone marrow stromal cells expressing enhanced levels of CXCL12 and ANXA2 increases recruitment of PCa cells into the bone marrow, promotes proliferation of PCa cells, and protects PCa cells from chemotherapy induced apoptosis [9]. These data suggest that DTCs may home to the HSC niche using similar mechanisms to the HSCs themselves in the regulation of cell fate.

3. DTC dormancy in the HSC niche

The concept of a dormancy supportive/permissive microenvironment in the bone marrow is an increasingly important and complex area of investigation in clinical oncology due to the

* Corresponding author.
E-mail address: rtaich@umich.edu (R.S. Taichman).

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general mechanism by which chemotherapeutics act to target mitotic cells [10]. There are many mechanisms by which DTC mitotic cycling is affected including regulation of the immune system, angiogenesis/nutrients, tumor extracellular matrix, and hormones [11]. In the HSC niche, cancer cells are subject to HSC quiescent signaling mechanisms, and a number of possible contributing chemokines have been identified [7,12]. DTC dormancy can be achieved through lack of activating signals (e.g. Wnt, Notch) or directly due to inhibitory signals (BMPs) [13]. Aguirre-Ghiso et al. [14] demonstrated that the balance between p38 and ERK, both mitogen activated kinases (MAPKs), affect DTC mitotic state. When ERK is elevated compared to p38, proliferation is favored; conversely, elevation of p38 compared to ERK favors quiescence. The balance of ERK and p38 *in vivo* is further regulated by other ligands: down-regulation of urokinase receptor (uPAR) results in an $ERK^{Low}/p38^{High}$ signaling ratio, inducing proliferative behavior in squamous carcinoma cells (HEp3) [15]. BMP7 has also been demonstrated to induce dormancy in PCa cells through the p38 MAPK pathway [16].

Other HSC-mediated factors, such as low oxygen [17], angiogenic [18], or additional secreted factors can also control dormancy. Shiozawa et al. [19] reported that growth arrest specific-6 (GAS6), a ligand of TYRO3, AXL, and MER tyrosine kinase receptors produced by osteoblasts in the HSC niche, supported PCa cell dormancy with increased survival and additionally prevented proliferation. Additionally, angiogenic simulators c-myc, vascular endothelial factor (VEGF), and fibroblast growth factor 2(FGF2) may be involved in exit of DTCs from dormancy [20,21]. Understanding the mechanisms of DTC dormancy may lead to better targeted therapies for metastatic disease.

4. The role of DTCs in niche formation

Following localization to the bone marrow, DTCs or their progeny can have osteoblastic or osteolytic effects, or both activities [22]. Osteoblastic lesions stimulate osteoblast formation and promote bone formation, albeit poorly woven bone [4]. Osteolytic lesions stimulate osteoclastic activity, which results in bone loss. In the instance of PCa, the impact of DTCs have been observed to be both osteoblastic and osteolytic in nature [23]. Joseph et al. [24] demonstrated that hematopoietic progenitor cells (HPCs) from mice inoculated with LNCaP-derived C4-2B induced osteoblastic differentiation of bone marrow stromal cells in co-culture through production of BMP-2. Conversely, these authors reported HSCs from mice inoculated with the PCa cell line, PC3, induced osteoclastic activity resulting in predominantly osteolytic lesions through an IL-6 mediated signaling pathway. Applications of this research include using HSC/HPC targeted therapy to limit the effects of bone metastasis. However, more research is needed to understand the precise mechanisms through which metastatic lesions may direct niche formation.

5. Erythropoietin and the HSC niche

Many molecules produced both locally and systemically, including erythropoietin (Epo) and adrenergic catecholamines, also appear to affect HSC niche and metastatic lesions in the bone marrow. EPO is a hematopoietic hormone produced predominantly in the kidneys in response to hypoxia. EPO functions through binding to a preformed homodimer transmembrane receptor (Epo-R). Interestingly, Epo-R is expressed in both hematopoietic and non-hematopoietic tissues, suggesting that the role of Epo may be more widespread beyond hematopoiesis [25]. The mRNA expression of Epo-R has been reported in tissues of the brain, testes, placenta, heart, lungs, bone marrow, spleen, and even tumor cells [26–28].

In cancer, Epo has both direct and indirect actions on DTCs in the HSC niche. First, Epo may directly act on tumor cells located within the bone marrow. Some studies provide evidence that Epo/Epo-R axis activation leads to tumor cell proliferation; while other studies demonstrate Epo/Epo-R activation did not support tumor cell growth specifically [29]. Interestingly, Shiozawa et al. [30] demonstrated that Epo did not stimulate PCa tumor cell (using PCa cell lines) proliferation *in vitro*, or enhance metastasis *in vivo*; however, these authors did demonstrate an increased tumor cell resistance to apoptosis resulting in PCa cell survival. Similarly, Todaro et al. [31] reported that Epo stimulation increased resistance of breast cancer stem-like cells (BCSC) isolated from patient tumors to chemotherapeutics, doxorubicin and 5-FU, *in vitro* and in a subcutaneous mouse model. Further, they reported stimulation with Epo upregulated known survival pathway mediators—Akt, Erk, and Bcl-xL—which may explain the mechanism by which BCSC cells achieved protection from chemotherapy [31]. These remain among the possible mechanisms that affect cancer therapy and may impact patient survival, through resistance of DTCs within the bone marrow to current chemotherapeutics [32].

There is also evidence that Epo increases niche formation, which may indirectly affect cancer cells or DTCs residing within the bone marrow niche [33,34]. It was previously reported that blood loss stimulates HSCs and may activate osteoprogenitor cells [35]. Further, Jung et al. [33] demonstrated that HSCs isolated from animals subjected to an acute bleed, showed increased capacity to induce osteoblastic differentiation, due to increased HSC-derived BMP-2 and BMP-6. Later, Shiozawa et al. [36] reported blood loss induced Epo production, causing BMP production in HSCs through a Jak/Stat signaling mechanism. In addition Epo regulates the bone microenvironment through direct action on mesenchymal cells, inducing osteoblast differentiation. Thus demonstrating that Epo can regulate bone metabolism through induction of osteoblast differentiation or production of BMPs by HSCs [34,36]. Further investigation showed increased angiogenesis as well as elevated numbers of red blood cells in the peripheral blood, and mesenchymal cells and hematopoietic stem cells in the bone marrow [34]. Taken together, understanding the role of Epo on the niche, within which DTCs reside may aid in the development of effective treatment modalities for cancer patients.

Together these data demonstrate interconnectivity of hematopoiesis and tumor metastasis. On one side the HSC niche is targeted by circulating tumor cells to facilitate the establishment of DTCs in marrow. Once there, DTCs are likely to undergo one of three fate decisions: apoptosis, dormancy or proliferation. Each of these fate choices are likely regulated by the niche by soluble and non-soluble factors. The niche, as an integrator of systemic demands for hematopoiesis, also is likely able to regulate DTC fate when occupied by these molecular parasites. Yet how this occurs, remains poorly understood. Similarly, progeny of DTCs are able to produce osteoblastic, osteolytic lesions or mixed. How this occurs is also not well understood and how the niche participates or regulates these activities is an active area of investigation (Fig. 1).

6. 10 outstanding questions in the field

1. What is dormancy – is it a lack of proliferation or does proliferation and cell death balance out for a zero gain of cell numbers?
2. Does signaling remain the same if metastatic tumor cells replace HSCs in the niche?
3. What mechanisms govern dormancy and disease recurrence in