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Experimental models of bone metastasis: Opportunities for the study of cancer dormancy☆

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ABSTRACT

Skeletal metastasis is prevalent in many cancers, and has been the subject of intense research, yielding innovative models to study the multiple stages of metastasis. It is now evident that, in the early stages of metastatic spread, disseminated tumour cells in the bone undergo an extended period of growth arrest in response to the microenvironment, a phenomenon known as "dormancy". Dormancy has been implicated with drug resistance, while enforced dormancy has also been seen as a radical method to control cancer, and engineering of dormant states has emerged as a novel clinical strategy. Understanding of the subject, however, is limited by the availability of models to describe early stages of metastatic spread. This mini-review provides a summary of experimental models currently being used in the study of bone metastasis and the applications of these models in the study of dormancy. Current research in developing improved models is described, leading to a discussion of challenges involved in future developments.

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1. Motivation

Cancer dormancy refers to a protracted symptom-free period following the successful removal of a primary tumour until recurrence of the disease. Such clinical behaviour is commonly observed in cancers of the breast [1], skin [2] and prostate [3], with time to relapse ranging from years to decades. Recurrence is often manifested as metastatic lesions in distant tissue, particularly bone [4,5]. Currently, the evidence suggests that metastasis-initiating cells are tumour cells that escaped from the primary site at an early stage of disease, disseminating to distant tissue, where they are initially clinically undetectable due to their small volumes. There, they remain undetectable due to microenvironmental cues that limit the proliferation of the "occult" metastatic tumour [6,7], and the metastasis can only progress when the tumour is able to overcome these imposed metastatic blocks. Much research in the past decade has focussed on elucidating these mechanisms, in order to identify therapeutic targets that may limit the progression of the disease, as well as to understand the implications of dormancy on chemoresistance. Indeed, experiments on mouse models of breast cancer dormancy have confirmed quiescent cells to be refractory to conventional chemotherapy [8], and it was further suggested that

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chemotherapy was only able to eradicate tumour cells that had exited dormancy during treatment.

Despite the importance of understanding dormancy, advances in the field have thus far been severely limited by the scarcity of adequate cancer metastasis models, particularly those which are amenable to induction and monitoring of early post-metastatic events [9]. Indeed, the range of in vitro and in vivo experimental models available neither possess sufficient sophistication and finesse in recapitulating physiological processes during the early stages of metastasis, nor are they amenable to long-term serial, single-cell monitoring, required for the study of cancer cell dormancy. Thus, while classical models of cancer metastasis have proved integral in the dissecting of the processes of metastatic invasion and dissemination [10,11], a significant gap exists in recapitulating the events between the steps of initial seeding and eventual outgrowth that encompass dormancy. Consequently, hypotheses on the governing mechanisms or even existence of metastatic dormancy have hitherto been largely built on mathematical models based on clinical data [12]. Serendipitously, technological advances have led to increasingly sophisticated models of metastatic disease being developed in recent years. In particular, much attention has been paid to replicating tissue-specific cues that influence cancer cell behaviour, in accordance with Paget's seed–soil theory, where metastatic "seeds" will only thrive in tissues with appropriate microenvironmental "soil" [13]. Bone, a common metastatic focus for many cancers [14], is one such tissue under intense scrutiny. Of note, the bone marrow is an established stem cell niche, with possible overlapping mechanisms governing dormancy of cancer and haemopoietic stem cells [15]. It follows that novel models of bone metastasis may provide an avenue to study metastatic dormancy, and will be the subject of this review. This mini-review serves to provide a summary of in vitro and in vivo models commonly used in cancer metastasis studies, and to highlight the current research efforts being undertaken to tailor these models for the study of dormancy. Finally, the challenges that remain to be addressed are summarised, leading to future research directions. To facilitate the discussion, an overview of cancer dormancy and the bone marrow niche will first be described.

2. Dormancy and the role of the bone marrow

Solid tumours often undergo an extended period of very slow growth, in which time the patient presents little or no symptoms of the disease [5]. This is manifested in some patients who have undergone apparently successful treatment for cancer, only to develop overt metastasis, sometimes more than a decade later [16]. In other patients, minimal residual disease may persist in the form of circulating tumour cells (CTC) in circulation even over twenty years after removal of the primary tumour [17]. Mathematical models describing the kinetics of the disease demonstrate that disease progression is inconsistent with a continuous-growth model [18], instead showing that the natural history of the disease likely involves dormancy and metastatic reactivation. In the light of these findings, it has been suggested that cancer cells are disseminated to distant tissue early, where they remain dormant until activated, triggering metastatic relapse [19]. It has been proposed that two categories of dormancy exist: (1) cellular dormancy, in which single cells enter into a non-proliferative state and (2) tumour-mass dormancy, where the growth of the tumour mass is limited by a state of matched turnover between proliferative and apoptotic cells. By this definition, cellular dormancy refers to a state in which the disseminated tumour cells (DTC) enter G0/G1 arrest, whereas tumour mass dormancy occurs where growth of micrometastases are limited by factors inducing cell death, including vascular insufficiencies and immune surveillance. The similarities with primary tumour dormancy are evident; the scope of this article will thus be limited to cellular dormancy as a critical step in the progression of metastatic disease.

As depicted in Fig. 1, tumour cells are disseminated from the primary site, and are transported to distal tissue, where they get trapped singly. During these initial stages, endogenous pro-metastatic cues are balanced by exogenous microenvironmental cues limiting disease progression, which must be overcome in order for overt metastasis to develop. This microenvironment is largely the result of cells present in the bone marrow, including osteogenic cells and endothelial cells, as well as associated extracellular matrix, which is thought to be initially unsuitable for cancer proliferation. Correspondingly, it has been reported that although occult lesions can be found in almost all healthy adults, only a fraction of these acquire malignancy [20]. For example, occult carcinomas have a prevalence rate of 99.9% in the thyroid, yet the incidence of thyroid cancer stands at only 0.1% [21]. It is thus evident that cancer cell response is largely dependent on interactions with the dissemination site and, in line with the cancer stem cell hypothesis, the concept of shared niche interactions with normal stem cells was developed [22]. For example, it has been established that osteoblasts and endothelial cells within the bone marrow perform a common role in the recruitment of haematopoietic stem cell (HSC) and prostate cancer cells via Annexin II [23]. Corollary to this, metastatic quiescence may be governed by similar cues dictating HSC dormancy in the stromal microenvironment. This was demonstrated by Shiozawa et al., where binding to Annexin II was succeeded by expression of growth arrest-specific 6 (GAS6) receptors on prostate cancer cell, inducing dormancy of the cancer cells [24] and effectively mirroring the HSC–niche interactions [25]. Similar observations were also made by the same team in their studies on acute lymphoblastic leukemias [26].

Within the bone marrow, two distinct stem cell niches may be identified: the endosteal niche and the perivascular niche [27]. The endosteal niche is composed primarily of mesenchymal stem cells (MSC) and osteoblasts, which perform central roles in regulating haematopoietic stem cells dormancy through stem/progenitor pathways [28]. It is of note that osteocytes derived from normal bones and those associated with metastatic disease present distinct transcriptional signatures, although it remains unclear if these changes were the cause or result of metastastic colonisation, nor if these changes impact stem cell behaviour [29]. Depletion of osteolineage cells is known to result in loss of HSC from the bone marrow and obtunds extramedullary haematopoiesis, further highlighting the links between the osteogenic and haematopoietic systems [30]. In a study by Greenbaum et al., a similar effect could be achieved by CXCL-12 deletion in the bone marrow [31]; CXCL-12 is also implicated in HSC [32] and breast cancer cell quiescence [33]. Interestingly, when CXCL-12 deletion was performed selectively on specific cell populations in the bone marrow, it was found that modifying mineralising (mature) osteoblasts had no effect, whereas targeting the early mesenchymal progenitors resulted in constitutive mobilisation, leading to a debate on the contribution of mature osteoblasts to HSC maintenance [31]. Other recent studies involving MSC demonstrate an exchange of microRNAs between MSC and cancer cells via gap junctions [33] and exosome signalling [34] promote breast cancer cellular dormancy. BMP-7 is another secretory factor from bone stromal cells known to induce cancer dormancy [35]. Incubation of prostate cancer cells with BMP-7 was shown to activate p38, NDRG1, p21 and p21, while suppressing the ERK–MAPK pathway. P38 activation, in particular was found to drive NDRG1, a metastasis suppressor gene. BMP-7 injected into a mouse model of skeletal metastasis effectively suppressed tumour growth; this effect was lost upon withdrawal of the BMP.

In contrast to the endosteal niche, the perivascular niche has conventionally been associated with activated HSC, although recent evidence suggests that almost all HSCs occupy a perivascular location, even within the endosteal region [36]. Similarly in cancers, metastatic tumour cells require a perivascular location in order to survive [37]. This niche is populated by endothelial cells (EC), MSC and other stromal cells characterised by a high CXCL-12 expression. Recently, EC have been shown to play a key role in HSC regulation, where EC-specific Scf deletion was found to deplete HSC from the bone marrow [38]. Crucially, it was shown that EC in stable microvasculature express