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REVIEW

The pre-metastatic niche: is metastasis random?

Thomas R Cox[1], Alison Gartland[2] and Janine T Erler[1]

[1]Biotech Research and Innovation Centre, University of Copenhagen, Copenhagen, Denmark. [2]The Mellanby Centre for Bone Research, Department Human Metabolism, The University of Sheffield, Sheffield, UK.

The metastasis of solid tumours is a vastly complex, dynamic and systemic process involving both primary tumour cells as well as a wide array of stromal and vascular cells. The recruitment and activation of host cells by tumours at both the primary and metastatic sites is crucial for successful metastatic dissemination highlighting the systemic nature of disease progression. The appropriation of distant metastatic sites by primary tumours and the generation of so-called pre-metastatic niches have gained much interest in the last decade complementing the century old 'seed and soil' hypothesis. The idea that tumours are capable of pre-defining future sites of metastasis is both exciting and terrifying as we try to understand the dynamic networks associated with solid tumour metastasis. Exactly how a tumour cell can alter the distant metastatic microenvironment is of great importance and will unlock novel strategies for successfully targeting these processes.

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Perspective

Metastasis as an obstacle. The dissemination of solid tumours from primary to secondary sites is a highly complex and dynamic process involving multiple stages and exhibiting a high degree of variability between both patients and tumours. Conceptually, the process of metastasis is often simplified into a classical, progressive and sequential series of irreversible changes, which allows the following obstacles to be overcome; invasion through the surrounding interstitial matrix, intravasation into the blood stream (either directly or indirectly via the lymphatic system), survival in circulation, extravasation at secondary sites and finally colonisation of often physiologically distinct and hostile environments. All of these steps are rate-limiting and make metastasis a highly inefficient process.[1,2] Indeed, metastasis has been described by some as a process of natural selection in which metastatic cells are challenged to acquire a specific and aggressive phenotype.[3] Despite this inherent inefficiency, patients most often succumb to the systematic complications of tumour metastasis, which currently accounts for over 90% of solid tumour patient deaths. While cancer research has traditionally focussed on the primary tumour, viewing adhesion, migration and metastasis as intrinsic properties of the tumour cells,[4] now, the microenvironmental regulation of the multiple stages of metastasis is under intense investigation and multiple genetic and epigenetic events are being attributed to these highly complex biological processes.[3,5] Solid tumour metastasis is an inherently reciprocal process with tumour cells and host cells modulating one another's behaviour creating a truly systemic disease. Dissecting and understanding the complex networks governing these reciprocal processes of metastasis will lead to the development of improved therapies to treat metastatic disease.

Metastatic organ specificity. It is currently unclear as to why some tumour cells preferentially metastasise to particular organs and others show a lesser degree of specificity, because metastasis as a process is inherently difficult to observe and study, since macro-metastases are the clinical endpoint of the process. Ewing[6] proposed that tumour metastasis was as a function of mechanical and anatomical features of the vascular system. The anatomy of the circulatory and lymphatic systems certainly helps to explain the delivery of tumour cells to distant organs, but does not appear to fully account for the site-specific bias of some metastatic disease. Importantly, it appears that the successful colonisation of macro-metastases is dependent on a receptive microenvironment and that the mere entry into and exit of tumour cells from the circulation is not enough to give rise to clinically detectable metastases. It has been estimated from experimental models that somewhere in the region of 1 million cells per gram of tumour tissue may escape from the tumour daily,[7] travelling around the body and lodging in various organs, yet only a miniscule percentage of these cells will propagate into overt metastases. Hence, a crucial step in tumour metastasis is the ability of tumour cells to successfully colonise either the same and perhaps more frighteningly, seemingly distinct organs with which they have previously had no connection. For example, prostate cancer will, in most cases, exhibit metastasis specifically to the bone,[8] whereas breast cancer will metasta-

Correspondence: Dr JT Erler, Biotech Research and Innovation Centre, University of Copenhagen, Ole Maaløes Vej 5, Copenhagen 2200, Denmark.
E-mail: jterler@gmail.com

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sise to bone, liver, lung and brain, and colorectal predominantly to liver. Although oncogenic transformation is considered the most important event in initiation of tumourigenesis, it is not sufficient for metastatic competence, as evidenced by many *in vivo* models of oncogene-driven tumourigenesis that fail to show establishment of distant metastases,[9] or indeed the fact that in some patients disseminated tumour cells are detectable, yet overt metastases fail to form.[10] Hence, the subtle difference between organ infiltration, the ability to extravasate from the circulation into a tissue, and organ colonisation, the ability to overtly proliferate within an organ, is a key driver in this apparent organ specificity of metastasis.

Over a century ago in 1889, the English surgeon Paget[11] first proposed the 'seed and soil' hypothesis to explain the seemingly predictable spread of solid tumours. By analysing autopsy records of 735 cases of advanced breast cancer, Paget discovered predictable patterns of bone and visceral metastasis. He introduced the concept of a receptive milieu and his hypothesis put forth that for a tumour cell (seed) to grow it requires the appropriate local microenvironment (soil). Seminal work by Fidler and colleagues[12,13] to support this showed that although circulating tumour cells are found in the tumour vasculature of multiple organs, they do not give rise to metastatic disease; however, other selective sites consistently develop metastatic tumour deposits and as such these sites must be more conducive to tumour cell colonisation. This work reignited an interest in the metastatic milieu of primary tumours and that of tumour cells at metastatic sites. A paper by Hiratsuka *et al.*[14] presented compelling evidence in support of Paget's original 'seed and soil' hypothesis showing that primary tumours are capable of appropriating secondary sites in advance of tumour cell arrival. This was closely followed by a paper in 2005 by Kaplan *et al.* in which they coined the term 'pre-metastatic niche'.[15,16] Since then, several groups have contributed to and refined the emerging and perhaps controversial theory of the pre-metastatic niche.

Creating a pro-metastatic milieu. The tissue microenvironment is a highly heterogeneous population of cells and the dynamic interactions between this diverse population can profoundly change the gene-expression patterns of residing cells and, in particular, cancer cells and hence their behaviour and growth ability (reviewed in Joyce and Pollard[17]; Bissell and Hines[18]). For example, the same tumour cells, grown experimentally in two different sites, show expression of different proteins, in particular proteolytic enzymes.[19,20] Cancer cells in different environments may also respond differently to chemotherapy,[21] which has an important role in therapeutic intervention. The tumour microenvironment is not only composed of cells, the surrounding extracellular matrix is also critically important in actively influencing cell behaviour, and remodelling of the extracellular matrix is an inherent aspect of tumour progression.[22] Perhaps the most striking concept of the pre-metastatic niche is the systemic signalling of tumour cells to activate normal host cells to begin remodelling of secondary site extracellular matrix, developing permissive niches in the absence of tumour cell presence, but which ultimately facilitate subsequent colonisation by these cells.

The pre-metastatic niche. The concept of the pre-metastatic niche stems from findings providing evidence that the distant secondary microenvironment can be primed and 'made ready' for future metastatic growth before the arrival of tumour cells. In elucidating the process of pre-metastatic niche formation, evidence has shown that it occurs as a temporal sequence of events predating the influx of tumour cells, effectually priming the target site of disease for the arrival, engraftment and colonisation of incoming metastasising tumour cells. Importantly, the existence of the pre-metastatic niche implies that metastasis to a particular organ is not a random occurrence, but rather a pre-determined event, with the tumour cells leaving the primary tumour with a target destination already defined. Whether the circulating tumour cells specifically home to these sites, or merely capitalise on the enhanced support provided by these pre-metastatic niches remains to be seen.

Early work by Kaplan and colleagues in defining the pre-metastatic niche and its temporal and functional relationship to metastasis demonstrated that non-malignant bone marrow-derived haematopoietic progenitor cells (BMDCs) expressing the vascular endothelial growth factor receptor-1 (VEGFR-1) precede the arrival of even single metastatic tumour cells and macro-metastatic disease at distant sites.[16] They also demonstrated that tumour-specific factors upregulated VLA-4 ligand and fibronectin at distant pre-metastatic sites, recruiting PDGFR-expressing cells and resulting in the formation of permissive niches for incoming tumour cells. These observations provided the first evidence that non-neoplastic host cells can pre-define a future site for metastasis, however, it was later shown that although recruitment of VEGFR-1$^+$ BMDCs enhance metastasis in some cases, independent non-VEGFR-1-mediated mechanisms of spontaneous metastasis may also play a role (reviewed in Duda and Jain[23]). Concomitant to this, Hiraksuka *et al.*[14,24] showed that both MMP9 expression in endothelial cells and macrophages at the pulmonary pre-metastatic niche along with secretion of VEGF-A, TNF-α, TGFβ and the inflammatory chemo-attractants S100A8 and S100A9 drives recruitment of CD11b$^+$ (Mac1$^+$) myeloid cells to the pre-metastatic milieu. More recently they have shown that S100A8 and S100A9 drive expression of serum amyloid 3A in the pre-metastatic lung, which stimulates NF-kB via Toll-like receptor 4 leading to further chemo-attractant secretion and enhanced pulmonary metastasis. At the same time, we uncovered an important role for lysyl oxidase (LOX) in the formation of pulmonary, hepatic and cerebral pre-metastatic niches. Once secreted from the hypoxic tumour environment of primary breast tumours, LOX co-localised with fibronectin at these sites of future metastasis where it serves to facilitate matrix remodelling, enhancing CD11b$^+$ BMDC recruitment and increasing metastasis.[25] Indeed, it has been shown that inhibiting the recruitment of BMDCs to pre-metastatic sites either by antibody treatment or depletion is sufficient to block niche formation and metastatic progression.[16,25] That LOX has previously been shown to interact with fibronectin regulating its activity[26] suggests that it is likely that the initial deposition of fibronectin and LOX during pre-metastatic niche formation significantly contributes to the generation of suitable microenvironments that facilitates the recruitment of BMDCs and ultimately the successful colonisation by tumour cells. In studies of hepatic metastasis, factors secreted by melanoma cells appear to activate hepatic stellate cells to a myofibroblast-like phenotype associated with alpha smooth muscle actin expression and cytoskeletal changes creating growth-supportive milieu and colonisation of incoming tumour cells.[27,28]

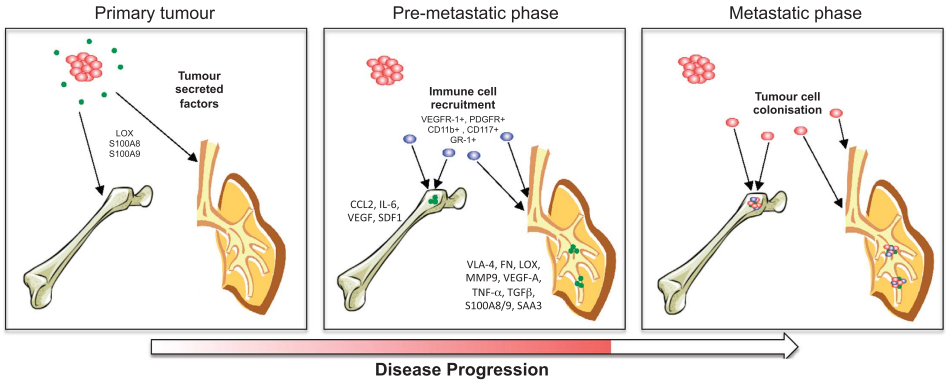

Figure 1 The pre-metastatic niche and disease progression. Tumour cells secrete factors during primary tumour progression, which act systemically at distant secondary sites, modifying the local environment and recruiting host immune cells further facilitating the appropriation of these sites for successful tumour cell colonisation.

There is also growing evidence that other immature BMDC populations may be important in the development of the pre-metastatic niche, such as the undifferentiated myeloid CD11b$^+$ Gr-1$^+$ cells, so called myeloid-derived suppressor cells (MDSCs), which act to suppress antigen-specific T-cell responses consequently favouring metastatic behaviour.[29] Work by Yan *et al.*[30] has revealed a novel pro-tumour mechanism for these CD11b$^+$ Gr-1$^+$ cells whereby they drive changes in the pre-metastatic lung creating an inflammatory and proliferative environment, diminishing immune protection and promoting metastasis through aberrant vasculature formation. Of particular note is that S100A8 and S100A9 upregulation at distant pulmonary pre-metastatic sites likely serves as the main mechanism of MDSC recruitment to these sites[24] as well as impairing dendritic cell maturation, further increasing MDSC accumulation and enhancing inflammation.[31]

Critically, the changes that occur at the pre-metastatic niche are crucial in generating a milieu to which the tumour cells can metastasise. Despite that, the exact role of the individual cell types involved in pre-metastatic niche formation and metastasis is not yet clear. Similarly, the pre-metastatic niche has been predominantly studied in pulmonary metastasis and the precise mechanism by which osteolytic cancer cells (breast, prostate) colonise the bone is as yet unclear. Bone and the bone marrow environment is an intricate vascular network consisting of a dense mesenchymal-derived stroma harbouring numerous essential growth factors, cytokines, chemokines and extracellular matrix components. The majority of evidence in support of a pre-metastatic niche in bone comes in the context of endocrine-like actions, often referred to as the 'vicious cycle' model. In this model, cancer cells secrete pro-inflammatory factors that lead to both osteoblast-mediated and direct osteoclast activation. This results in increased bone resorption with the subsequent release of bone-stored factors that facilitate rapid metastatic colonisation and tumour cell growth.

As in the lungs, the importance of haematopoietic cells in facilitating tumour cell colonisation of secondary organs also pertains to bone metastasis of prostate cancer. Bone, the preferred soil for metastasising prostate cancer cells, is a complex microenvironment rich in multiple cell types and highly capable of supporting the growth of skeletal metastases. In particular, CD11b$^+$ cells in the bone marrow have been shown to support the metastatic growth of prostate cancer cells likely through elevated levels of CCL2, IL-6 and VEGF.[32] Indeed, there is a significant overlap in the molecular machinery between metastasising tumour cells and haematopoietic cells, and so it is not surprising that many cancers show a lethal proclivity to establish within the bone and bone marrow. It has been shown that this commonality in homing can occur via similar mechanisms that osteoblasts and bone marrow stromal cells use, namely the CXCL12/SDF1-CXCR4 axis to attract haematopoietic stem cells (HSCs).[33] Indeed, many osteotrophic cancers such as breast, ovarian, prostate and neuroblastoma express high levels of the CXCR4 receptor and it is thought that they metastasise to bone in a SDF-1-dependent manner.[34–37] It has recently been shown that prostate tumour cells may compete directly with HSCs for occupancy of the bone marrow niche, 'hi-jacking' and significantly altering the microenvironment to facilitate the formation of bone metastases while driving HSCs into the peripheral blood pools.[38] It is unclear at present, however, primarily due to experimental limitations precluding such investigations, whether appropriation of the bone marrow niche occurs in advance of tumour cell arrival in the same manner as observed in pulmonary pre-metastatic niche formation. An overview of the pre-metastatic during cancer progression is summarised in **Figure 1**.

Targeting the pre-metastatic niche. Bone is the most common metastatic site for prostate and often breast cancer and prognosis is extremely poor for patients with metastatic bone disease. As such, therapies that target metastasis are critically needed. Two important questions must be addressed when targeting metastasis; first, is the target clinically relevant, and second, is it biologically a good target. For example, by the time a primary tumour is clinically detectable it is likely to have seeded tumour cells to secondary sites. Hence, targeting early stages of metastasis, that is, cells leaving the primary tumour may not be feasible. Similarly, from a biological approach, it would be better to target the most inefficient stage of metastasis, namely secondary organ colonisation, thus complementing the natural selection of tumour cells during the metastatic process. Thus, the successful inhibition of metastasis requires targeting of both the metastasising cell and the supportive microenvironment, and will ultimately require the simultaneous administration of anti-metastatic agents with established primary therapies. Therefore, targeting the pro-metastatic milieu created in secondary organs to prevent tumour cell colonisation is an attractive option. Similarly, targeting the mechanisms by which circulating tumour cells may home to pre-metastatic niches may also be feasible. Indeed, some drugs already appear

to fulfil some of these criteria, such as bisphosphonates, which have been shown to prevent the growth of bone metastases. Bisphosphonates work by preventing osteoclast bone resorption, which prevents the release of bone-stored growth factors that promote tumour cell survival and growth, thus disrupting the so-called vicious cycle.[39] Hence, they are considered as targeting the 'soil' rather than the 'seed'.[40,41] However, there is increasing evidence for a direct effect of the nitrogen-containing bisphosphonates on tumour cells outside of bone.[42–44] At the same time there is tantalising evidence from some randomised clinical trials that supports the use of adjuvant bone-targeted treatments to prevent tumour metastasis and increase patient survival, however, emerging evidence from some trials and interim reports from the AZURE trial suggest that this benefit is only observed in sub-populations of patients (reviewed in Gnant[45]). Notably, zoledronic acid has been shown in studies to improve disease-free survival in a sub-set of women with primary breast cancer, most notably in breast cancer patients who are post-menopausal or treated with aromatase inhibitors and exhibit high bone turnover.[46] In men with prostate cancer and a rapidly rising prostate serum antigen, denosumab (a fully human monoclonal antibody against RANKL, which will also reduce osteoclastic bone resorption) has been shown to delay the development of bone metastases. It is tempting to speculate in these cases that preventing bone turnover abrogates the formation of pre-metastatic niches (albeit indirectly) thereby decreasing metastatic bone lesions. However, although these results prove promising, the treatment benefits do not appear to extend to all patients and it is still too early to know whether such treatments offer long-term therapeutic benefit. Other major targets for the inhibition of bone metastasis through directly or indirectly modulating bone turnover include: Src Kinase inhibitors, Cathepsin K inhibitors, parathyroid hormone-related peptide inhibitors, TGFβ inhibitors and chemokine receptor inhibitors, all of which are showing promise and have been systematically reviewed in Onishi[47].

The future. As we progress towards more personalised medicine, there is an ever-increasing need to understand the complex underlying networks of metastasis. As a result of the recent explosion of interest in this field, we are rapidly advancing our understanding of how metastasis develops, especially from the point of view of tumour cell-intrinsic mechanisms. However, it is the authors' belief that a greater emphasis needs to be placed on the study of systemic and microenvironmental factors associated with metastasis, and more importantly, how the different steps of this dynamic process may be amenable to therapy. Moving forward, there is the need for greater integration of clinical data with basic research in terms of the metastatic cascade, organ sites and the temporal aspects of these processes in order to fully understand the site-specific nature of tumour metastasis. At present it is still unclear whether future metastatic organs are intrinsically permissive to tumour growth or whether 'conditioning' of these sites mediated by the primary tumour is necessary for metastatic colonisation. However, the appropriation and metastatic colonisation of secondary sites represents a temporally broad target, which is both biologically and clinically appropriate. Given the current scarcity of effective therapies that specifically target metastasis, the concept of the 'seed and soil' leading to organ-specific metastatic growth is in itself a highly relevant therapeutic target, and further understanding of the factors that influence this process is essential to the development of new therapeutics.

Conflict of Interest

The authors declare no conflict of interest.

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