BONE MICROENVIRONMENT AND ITS ROLE IN BONE METASTASIS

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Abstract

Bone is a common site for cancer metastasis. The development of bone metastases represents a serious progression in a patient with cancer, leading to treatment goals changing from curative to palliative. Cancers that have metastasised to the bone are resistant to therapies and lead to destructive and painful changes in bone structure. Here we describe the multiple steps in metastasis and the varying processes that drive tissue selectiveness. There is an emphasis on the basis of the fertile 'soil' for cancer growth that is provided by the bone microenvironment. The basic changes in host/cancer cell interactions as a metastatic cancer cell invades the bone, escapes dormancy to grow to a micro metastasis, induces angiogenesis and hijacks the normal physiological processes of bone remodelling to fuel its own growth are outlined. Steps in the process of metastasis that offer potential as therapeutic targets are briefly discussed.

Bone is a common site for the metastasis of solid cancers, particularly of breast cancer and prostate cancer, which are common cancers in women and men respectively. In advanced breast and prostate cancer, 70 to 80% of patients are found to have bone metastases. Once breast and prostate cancer invade bone, they have the ability to profoundly influence bone cells in their environment, resulting in predominantly destructive lytic lesions in breast cancer and painful osteosclerotic lesions in prostate cancer. In both diseases, the identification of bone metastases is usually associated with the change of clinical goals from curative to palliative, due to the resistance of disseminated skeletal metastases to current therapies.[1-3] The target tissue specificity of the metastatic process is indicative of the importance of the micro-environment the target tissues provide. This observation of cancer selectiveness for particular tissues has given rise to the seed (cancer cell) and soil (target tissue) analogy first suggested by James Paget in the 19th century.[4]

Steps in metastasis to bone

Intravasation

Metastasis of cancer cells is not a simple process and requires the successful completion of multiple steps. The first step of metastasis requires escape from the primary tumour. To escape from the primary tumour there are changes in cancer cell behaviour required. These include loss of cell – cell adhesion, loss of responsiveness to tumour chemo-attractive signals, and gain or maintenance of responsiveness to extra-tumoural chemo-attractive signals. Development of the capability to migrate through tissues is required to enable single cancer cells to escape from the primary tumour mass or local lymphatic tissues into blood vessels – a process called intravasation. These attributes then lay the foundation for escaping the blood vessel and establishment of these cells in a target tissue.[5-8] There is also evidence that prior to metastasis occurring, the primary tumour can act to condition or prime target tissues for metastasis to make them receptive to colonisation of cancer cells once they enter the circulation.[9] (See also articles in this issue from Moeller, Parker)

Extravasation

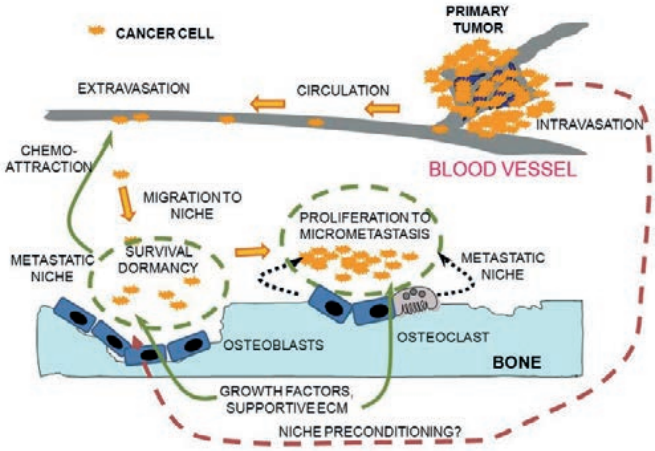
Once cancer cells have entered the circulation, their distribution throughout the body is initially a passive process dependent on the anatomic proximity to the primary cancer and the relative blood perfusion rate of the various tissues.[1] To establish in target tissues, the circulating cancer cells must escape the blood vessel that carries them by adhering to a blood vessel wall and migrating from the vessel into the surrounding tissues, a process called extravasation.[7] The local tissue microenvironment can influence extravasation via the nature of the vascular structure, with escape from the blood vessels in bone marrow likely to be enhanced by the thin-walled sinusoidal blood vessels present in bone. Additionally the presence of chemo-attractive agents within a tissue and diffusing into blood vessels may drive extravasation. Thus initial vascular deposition of cancer cells in a tissue may be random or may reflect active targeting (or both). Solid tumour cells tend to be large relative to haematopoietic cells. Intracardiac injection of breast and prostate cancer cells typically shows an initial rapid clearance of cancer cells from the blood and fairly broad distribution of cells in tissues, approximately consistent with the organ perfusion rate, supporting the concept of passive clearance of cancer cells from the blood and into tissues.[1] However, it is known that bone contains cytokines and growth factors that are chemo-attractive to cancer cells such as transforming growth factor beta (TGF),[8] and CXC12 (also known as SDF1),[10] for which the receptors are found on breast and cancer cells, and so there remains the possibility that there is also active homing of cancer cells to particular tissues including bone. This certainly occurs with haematopoietic sourced cancers such as multiple myeloma, however

the cells in these cancers are much smaller and arise from known cell types that naturally home to the bone marrow.[11] The presence of cancer cells in tissues non-receptive to metastasis rapidly reduces after intracardiac systemic injection, indicating that failure to survive and clearance from the body is the most common destiny for most cancer cells entering the vascular system.[12]

Targeting to the metastatic niche in bone and dormancy

Once cancer cells have been immobilised in blood vessels within the bone, there is the potential that chemo-attractive signals and tissue adhesion molecules specific to a target tissue, such as bone, can drive extravasation, enabling metastatic cancer cells to enter a microenvironment conducive to their survival. It is apparent that very few cancer cells escaping from a tumour are responsible for giving rise to a secondary tumour. Many cancer cells entering the circulation do not survive and disappear completely. Others escape from the vasculature but remain as single cells, identifiable in tissues, but remaining as single cells even years after a primary tumour has been removed, surviving in a state of apparently permanent dormancy.[7] The initial establishment of a cancer metastasis in bone depends on the presence of a microenvironment which induces the cancer cells to extravasate, survive and escape dormancy. These requirements probably are dependent on the nature of the environment in which the cancer cells find themselves, with bone providing a particularly fertile 'soil'. The rarity of all these events occurring is indicated by the initial small number of metastases observed in patients and after intracardiac of breast and prostate cancer cell injection of mice, which has given rise to the concept of the presence of a metastastic niche within bone.[13,14] It is known that there are particular niches within bone for both haematopoietic and mesenchymal stem cells. These appear to be closely dependent on the presence of a bone surface and osteoblasts, the bone lining cells which are able to synthesise bone (figure 1).

Figure 1: Initial steps in cancer metastasis to bone. Prior to metastasis, the primary cancer may condition the bone tissues to receive cancer cells (dashed line). Cells escape the primary tumour by extravasation into a blood vessel, which involves adherence to a blood vessel wall, invasion into the surrounding tissues and migration to a receptive niche. These cells may be initially dormant, but can be triggered by signals (dotted line) to proliferate and form micro metastases.



It is thought that important components of the niche are the expression of chemo-attractive signals that retain cells in the niche, the expression of cell surface adhesion proteins such as integrins on both the cancer and niche cells, and the presence of extracellular matrix proteins with ability to signal to cells through the presence of surface signals such as RGD domains (arginine-gycine-asprtate). Another important factor is likely to be the expression in the niche of various growth factors and chemokines.

How the metastatic niche maintains the survival of cancer cells and at some point allows their escape for dormancy is not known, but may be dependent on the varying expression of growth factors and cytokines which cycle during the normal periodic remodelling of bone, with migration of bone remodelling units across the surface of bone participating in a process that removes and rebuilds the skeleton in a seven to ten year cycle.[15] Bone tissue itself contains significant amounts of a wide range of growth factors that are released during the bone resorptive phase of this process, including many that are potentially able to act as growth factors for cancer cells able to drive their proliferation and migration. These include TGF beta, IGF1, fibroblast growth factors and bone morphogenetic proteins.[16] It has been demonstrated that increasing background rates of bone remodelling through calcium deficiency, vitamin D deficiency or by ovariectomy could each increase the growth rate of metastatic tumours in bone,[17-20] while reduction of bone remodelling inhibits the ability of tumours to grow in bone.[21]

Angiogenesis

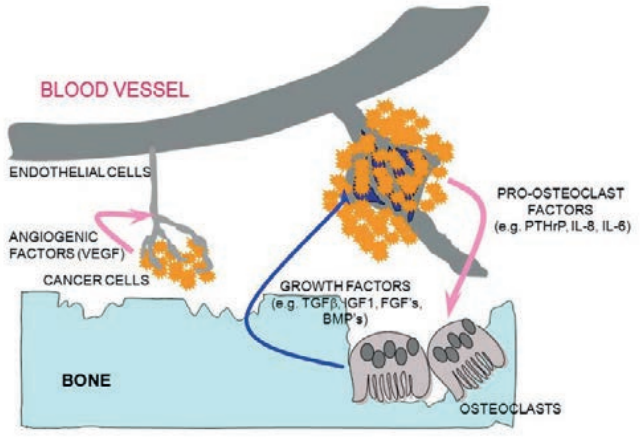
It is likely that the initial factors driving bone metastasis establishment are reliant on the pre-existing bone microenvironment into which the invading cancer cell migrates. However, as the cancer cells proliferate and form micro-metastases, they develop more and more ability to modulate the microenvironment in which they find themselves. In some patients, small cancer foci or micro metastases can be observed, in which initial proliferation of cancer cells occurred but progression has been inhibited. The development of a capability to induce neo-angiogenesis becomes essential for progression when a tumour reaches about 1mm in diameter, as its further growth is then impaired unless blood vessel invasion of the tumour can occur to provide the necessary nutrient supplies and waste removal. At this point, further growth becomes dependent on development of a vascular supply for the tumour, which can be achieved if the cancer cells are able to produce angiogenic signals that drive the vascularisation of the growing tumour mass.[22] The elevated expression of VEGF by breast cancer cells is associated with poor prognosis (see figure 2).[23]

Hijacking host regulator systems

As tumours grow further, their ability to modulate the signalling in host tissues to support their own further growth increases. The metastatic tumours now demonstrate the ability to mimic the regulation of normal bone tissue processes and so to hijack normal signalling processes in bone to induce increased bone resorption by host tissue osteoclasts. This has the potential to initiate self-amplifying cycles through the osteoclast mediated release from bone

of growth factors able to further expand tumour growth. The initial cycle described by Mundy and colleagues,[6] was termed a 'vicious' cycle in bone metastasis. In this cycle, they identified the ability of breast cancer cells in bone to secrete parathyroid hormone-related protein (PTHrP), to induce the formation of osteoclasts via increased local production of the osteoclast inducing cytokine, receptor activator of NF kappa B ligand (RANKL), by cells of the osteoblast lineage.[7,16,24] They were also able to identify the release of TGF from the bone matrix and its activation by the acid conditions produced by osteoclasts within the resorption sealed space between the osteoclast and the bone surface. TGF beta could then be demonstrated to increase cancer cell proliferation. Thus a vicious cycle was developed, in which cancer cells were able to cause osteoclastic bone resorption of the surrounding bone, both removing the physical limits on tumour growth and providing a source of growth factors to drive further cancer cell proliferation and PTHrP production, and thus more bone resorption and so on (see figure 2).

Figure 2: Progression of micro metastases into larger vascularised tumours regulating their own environment. To grow beyond micro metastases, cancer cells must induce neo-angiogenesis. As tumours grow further, they can produce pro-resorptive factors to drive bone resorption, thus releasing growth factors in a cyclic process driving more resorption and thus more growth.



Since this initial description of the vicious cycle, it has become apparent that additional amplification loops and intermediates are active in this cycle. In addition to TGF, other growth factors such as IGF1, endothelin-1 and fibroblast growth factor 2 also may contribute to cancer cell proliferation. Similarly, other cytokines secreted by cancer cells, such as IL-8,[25] and MIP -1alpha,[26] can drive increased bone resorption. Recently, a parallel amplification loop was identified in which tumour secretion of interleukin-6 (IL-6) was found to be induced by RANKL secreted by cells of the osteoblast lineage. In turn, IL-6 is known to be able to increase RANKL production by bone cells to further increase bone resorptive activity. Interestingly, IL-6 secreted by the tumour was also able to increase tumour RANK expression, further sensitising the tumour to the actions of RANKL. Knockdown of RANK or IL-6 in the cancer cells was able to reduce tumour growth in the bone, but not in the mammary gland, again emphasising the importance of cancer cell/bone cell interactions in driving bone metastasis.[27] This parallel loop supplements the actions of the vicious cycle to further increase bone resorption. It is apparent that resorption is a primary process driving tumour growth and that there are multiple pathways by which the tumour cells are able to modulate bone resorption to fuel their own growth.

In the final stages of metastatic cancer, the seriousness of the disease increases and the tumour, through its local effects, begins to impact the whole skeletal element in which it resides, frequently inducing bone pain, pathologic fracture and nerve compression.[2]

Therapeutic opportunities

The prevention or control of metastatic disease remains an area of significant unmet medical need. There are many potential steps, as outlined in this review, which provide potential targets for the prevention of metastasis or of the adverse effects. Ideally, the prevention of the development of actively growing metastases would be the most effective therapeutic approach. Therapies directed against intravasation, extravasation and tissue invasion represent a possible strategy. However, by the time primary tumours have been identified and removed as a source of metastasis, many cancer cells are likely to be already resident in the patient's tissues.

The metastatic niche also represents a valid target whose disruption could impair the survival of cancer cells in the metastatic target tissue, or prolong cancer cell dormancy. Arresting the transformation of dormant cancer cells to rapidly proliferating cells represents a compelling target for developing new therapies, as often patients show no evidence of tumours after primary tumour removal, but relapse with metastatic disease sometimes years later.

The lack of knowledge of the requirements for achieving cancer survival through dormancy, and of the nature of signals that initiate escape from dormancy, has limited progress in this area.[28] Another approach would be to change the bone environment to make it less supportive of bone metastasis. There is considerable mouse model evidence that increased bone remodelling makes the bone a more supportive place for cancer metastasis, while reducing bone remodelling has the opposite effect.[17-21] Initial treatment to reduce bone remodelling would be to correct common causes of high bone remodelling, such as calcium and vitamin deficiency,[29] with the latter particularly common in women at the time of breast cancer diagnosis.[30] These can each be readily diagnosed and addressed by providing oral supplements. As described in more detail below, the bone remodelling rate can also be reduced pharmacologically with bisphosphonate or anti-RANKL (denosumab) therapies.[31]

Inhibiting tumour angiogenesis is a highly promising treatment paradigm for metastatic disease, and while some initial approaches have proved somewhat disappointing, especially in terms of overall survival, much research activity is directed to this strategy.[32]

Inhibiting the development of vicious cycles within bone has proved an effective palliative strategy for prostate and breast cancer. The most developed and effective approach has been to target osteoclast activity, either through inhibition of osteoclast function with bisphosphonate treatment, or by preventing osteoclast formation with denosumab treatments.[32,33] Both of these strategies significantly reduce the incidence of skeletally related events in clinical trials. Therapies targeting other components of vicious cycles, such as PTHrP, are also showing some promise.[34] However, it appears that osteoclastic bone resorption is a fundamental mediator of the cycles so far identified, and may prove to be the most effective point of intervention. Multiple potential mediators have been implicated for pro-resorptive and cancer cell proliferative effects, and thus targeting single candidates may have only limited effects. Interestingly, there is some limited evidence that blocking bone resorption can delay the development of bone metastatic disease in prostate cancer patients,[35] and can increase patient survival in breast cancer patients.[36, 37]

In summary, the development of bone metastases is common in both breast and prostate cancer due to the fertile soil that the bone microenvironment provides for these cancer cell types. Once in bone, the tumours can lie dormant or be activated to proliferate and eventually produce destructive and painful metastatic lesions. This is a multi-step process with many potential points of therapeutic intervention, but therapies remain limited and primarily palliative in nature.

Acknowledgements

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