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Research paper

The bone microenvironment – Multiple players involved in cancer progression

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The importance of the microenvironment for cancer development and progression is now widely recognised, but it is worth reminding ourselves that this is a relatively young area of research. Anyone searching PubMed today using the terms "*cancer, microenvironment*" will find over 22,000 scientific publications, with around 3000 new contributions in both 2014 and 2015. However, going back 10 years to 2004 there were only 364 papers published fitting these terms, hence keen researchers would have the capacity to read them all. Changing the search terms to "*cancer, microenvironment, bone*", results in 3400 papers with around 420 new contributions per year for 2014 and 2015, compared with 93 papers a decade earlier. Inclusion of the word *"therapy"* limits the number of hits further, but in all cases the trend is towards ever increasing numbers of annual scientific publications that include studies of the microenvironment, indicating an active research area in rapid growth.

The renewed interest in the microenvironment in cancer may in part come as a result of the realisation that the human genome project (completed 2003) did not deliver as much insight into cancer development as was hoped. Subsequent efforts to sequence cancer cell genomes and identify driver mutations were published from 2006 onwards, with the data on new cancer types regularly emerging, most recently in breast cancer [1]. However, it was clear that the translation of this wealth of new data into knowledge and increased understanding of cancer biology, ultimately identifying new therapeutic targets, was going to take time, and that new gene-based cancer therapies were not just round the corner [2]. The concerted effort of the Cancer Genome Atlas (TGCA) program (started in 2006) has yielded a wealth of information relating to cancer-subtypes and identified numerous mutations, but processing and interpreting its 20 petabytes of data, including 10 million mutations, is a mammoth task [3].

As the complexities of the cancer cell genome and its regulation became evident, scientists also started to consider how different cell types work together, and Hannahan and Weinberg predicted this to be of great importance in their original "*Hallmarks of Cancer*" paper published in 2000 [4]. In this they stated: *"Looking forward in time, we believe that important new inroads will come from regarding tumours as complex tissues in which mutant cancer cells have conscripted and subverted normal cell types to serve as active collaborators in their neoplastic agenda. The interactions between the genetically altered malignant cells and these supporting co-conspirators will prove critical to understanding cancer pathogenesis and to the development of novel, effective therapies."* This statement has turned out to be astonishingly accurate, reflecting the general view of the cancer biologists of today that understanding, and ultimately successfully treating, cancer, depends on unravelling how a multitude of cell types co-operate. How the components of the microenvironment contribute to tumour development was subsequently mapped on to the hallmarks of cancer in the 2012 paper from Hannahan and Coussens "*Accessories to the Crime: Functions of Cells Recruited to the Tumour Microenvironment*", which describes the contribution of a large number of both cellular and molecular components to tumour progression [5]. At the same time as huge efforts were made to characterise individual cancer cells, there was increasing realisation that tumours are heterogeneous, both in terms of the tumour cells [6] but also in their stromal component [7], and that the presence of different clones underpins the development of resistance to therapy. This heterogeneity introduces further layers of complexity in our quest to understand cancer, and highlights that analysis of individual components of a tumour and characterisation of single cells may not be fruitful.

For researchers with an interest in bone metastasis, the role of the microenvironment has always been an integral part of their thinking. As described by Rob Coleman in this issue (see page xx), bone-targeted therapies were shown to be effective in advanced disease in the early 1980s, initiating research into the connection between bone and tumour growth. This ultimately generated the hypothesis of the vicious cycle proposed by Mundy and colleagues, where tumour-bone interactions result in accelerated tumour growth and associated cancer-induced bone disease [8]. Initially,

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the focus of bone metastasis research was the osteoclast and its key role in cancer-induced bone disease. However, it soon became clear that even highly effective inhibition of bone resorption was insufficient to prevent tumour progression in bone, suggesting that other cell types and numerous molecular/cellular drivers were involved. As described by Le Pape and colleagues in this issue (see page xx), the role of the osteoclast in cancer-induced bone disease is now well characterised. However, the role of the osteoclast in the early stages of tumour cell colonization of bone is less clear, with recent studies using multi-photon microscopy to demonstrate that zoledronic acid treatment does not inhibit tumour cell homing to bone in mice [9]. The osteoclast may therefore have a role in the subsequent steps of stimulating the growth of disseminated tumour cells to form colonies.

With improving models and technologies, including cell labeling and imaging, researchers are increasingly able to study the early stages of bone metastasis, prior to development of overt bone disease. This has generated new insights into the cellular and molecular composition of the putative "bone metastatic niche", introducing a host of new players with the potential to enhance tumour cell homing, survival and subsequent colonization of bone that in turn may represent novel therapeutic targets. For many of these we are only just starting to unravel their interactions with tumour cells. This special issue of JBO includes descriptions of a large number of different elements of the microenvironment affecting bone metastasis, from the early stages (preparing the niche) through tumour cell homing, survival and colonization to progression and advanced disease. At every step there is co-operation between cancer cells and their surroundings, highlighting that agents targeting these interactions may present novel therapeutic opportunities. The challenges are to hit the right cell types at the right locations at the right stage of the process, as the scope for off-target effects is considerable. For example, we want to eliminate tumour-associated vessels without damaging the normal vasculature. Similarly, it may be desirable to develop agents that will eliminate tumour promoting, but not tumour suppressing, macrophage subtypes.

1. Preparing the soil

If we consider microenvironmental influences at each stage of the metastatic process in order, the first is the role of systemic factors generated by the primary tumour in preparing the future site of secondary colonization, the pre-metastatic niche [10]. Ubellacker and McAllister (see page XXX) describe how primary tumour-derived factors interact with numerous cell populations in the bone marrow, mobilizing haematopoietic and mesenchymal cells into the circulation and in some cases recruitment into peripheral tumours. Despite intensive research, they point out that systemic instigation of bone metastases (in humans) remain to be demonstrated.

One such systemic factor, LOX, has recently been shown to increase bone metastasis in murine models. Gartland et al., (see page XXX) describe how the secretion of the enzyme LOX by tumour cells initiates alterations in the bone extracellular matrix and facilitates the development of pre-metastatic bone lesions. These lesions comprise areas of bone where the physical, biochemical and biomechanical properties of the extracellular matrix components are altered, creating an environment suitable for subsequent tumour cell colonization. Targeting of secreted proteins should therefore be considered as future therapeutic options in the context of bone metastasis.

Amongst the newest systemic factors to be described in relation to bone metastasis are miRNAs, and as described by Zoni and van der Pluijm (see page xx) a number of these have been suggested to be involved in different stages of the process, including in preparation of the pre-metastatic niche. With their capacity to modify multiple cell types (including osteoblasts and osteoclasts), these molecules may contribute to the cancer-induced alterations of the bone microenvironment that underpins subsequent metastatic progression. miRNAs are likely produced by all cell types and can be both pro- and anti-tumourigenic, hence their precise role in cancer progression and potential as therapeutic targets require further research.

2. Homing to bone

Megakaryocytes and platelets have also been shown to modify different steps of bone metastasis in model systems, covered by Leblanc and Peyruchaud in this issue (see page xx). Platelets are pro-metastatic, supporting survival of circulating tumour cells and their extravasation at secondary sites. The platelet-derived factor lysophosphatidic acid (LPA) is shown to enhance bone metastasis, and anti-platelet treatment combined with aspirin reduced bone metastasis in mice [11]. Platelet producing megakaryocytes also generate a number of factors that regulates bone turnover (like BMPs), and animals deficient in transcription factor required for megakaryocyte maturation have low platelet counts but highly increased bone mass due to elevated osteoblast numbers [12].

Recent technological advances have facilitated detailed charcterisation of the bone microvasculature, as described by Kusumbe (see page xx). As tumour cells reach bone through the circulation, the role of the vessels are clearly important in bone metastasis and the perivascular niche suggested to be a supportive environment for disseminated tumour cells. The identification of particular capillary types (H+ capillaries) that are surrounded by osteoprogenitor cells in the metaphysis of mouse long bones illustrate the close coupling between osteogenesis and angiogenesis, which also may have implications for disseminated tumour cells. Of particular interest is the report by Ghajar et al. [13] that dormant cancer cells are located close to stable vasculature, whereas vascular remodeling was associated with proliferating tumour cells.

Hematopoietic stem cell in tumour cell homing to bone has been established in models of prostate cancer, and as described by Taichman and colleagues (see page xx), chemo-attractants and attachment factors generated in the HSC niche may also act to facilitate the homing and survival of prostate cancer cells to these niches. The concept that cancer cells reside in the HSC niche and are able to respond to the same signals as govern the dormancy/proliferation status of HSC opens up new therapeutic avenues. Whether different cancer types (most notably breast cancer) also locate to HSC niches remain to be established.

As covered by Morris and Edwards (see page xx), an adipocyte-rich bone marrow microenvironment may contribute to creating a permissive niche for cancer cells, as well as provide the energy that help fuel their progression into overt tumours. Adipocytes also produce a range of pro-tumourigenic chemokines, and targeting of these adipokines may contribute to reduced tumour growth.

Whether cells of the osteoblast lineage are key components of the metastatic niche has been the subject of some debate, as described by Ottewell (see page xx). Their contribution to tumour cell homing, survival, maintenance of dormancy remains to be established although a number of reports suggest that tumour cells locate to the endosteal niche in models of breast and prostate cancer. How alterations in bone turnover rates, decreasing or increasing osteoblast activity, affects disseminated tumour cells is unclear.

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3. Established colonies and tumour progression

Once tumour cells have successfully colonized bone, they start to recruit other cell types to support their expansion. Prajapati and Lambert highlight that compared to the role of cancer-associated fibroblasts (CAFs) in primary tumours, their involvement in bone metastasis remains poorly understood (see page XXX). This is in part due to their heterogeneity and lack of specific markers to identify subtypes with different organ preference. As CAFs are abundant in tumours and a source of numerous pro-tumourigenic growth factors and cytokines, it is highly likely that they are also a part of the bone metastatic microenvironment. Their relative genetic stability (compared to tumour cells) suggests that they are unlikely to develop resistance to therapy and may therefore be suitable targets for intervention.

Bone is highly innervated and bone homeostasis is regulated through the release of norepinephrine by sympathetic nerves. Although the involvement of the nervous system has been recognised in the context of cancer-induced bone pain in advanced disease, Elefteriou (see page XXX) describes how there could also be involvement at earlier stages of metastatic spread. Chronic immobilization stress has been shown to increase the number of bone metastasis in an *in vivo* model of bone metastasis, as well as increase the severity of the associated lytic lesions. Whether this is relevant for human disease remains an intriguing possibility, opening novel avenues for therapeutic intervention.

Although established as key players in the development of metastases in general, it has been more difficult to pin down the precise role of macrophages in bone metastasis, as described by Sousa and Maatta (see page XXX). In part this is due to the heterogeneity of and plasticity of these cells, but also the lack of macrophage-specific agents that can be used to elucidate their functions *in vivo*. The ideal scenario is to develop therapeutic strategies to prevent the formation of pro-tumorigenic macrophages and at the same time stimulate the tumour-suppressing populations, suppressing secondary tumour formation.

Many of the components described above contribute to tumour response to anti-cancer agents, including the vessels, osteoclasts *etc.* Less is known about how the endocrine system, in particular hormones, modify bone metastasis and therapeutic response. The article by Wilson (see page xx), describes the potential role of female reproductive hormones in breast cancer bone metastasis. As recent clinical trials have shown a difference between pre- and post-menopausal women in terms of survival benefit from adjuvant bisphosphonates [14], understanding how the hormonal status of the microenvironment affects disseminated tumour cells is the subject of intensive research efforts.

In addition to the cellular and molecular components described above, physical/chemical properties of the bone microenvironment are also likely to influence development and progression of metastasis. Alterations in the level of extracellular calcium (see article by Brenner, page xxx) can affect tumour cells through calcium sensing receptors (CaSR) or calcium binding proteins. A number of studies have manipulated the expression of CaSR in tumour cells and found that this reduces Ca-stimulated migration/invasion. Specific CaSRs have been shown to be associated with increased ability to form bone metastasis *in vivo*. These and other data suggest that a reduction in extracellular calcium levels caused by anti-resorptive therapy may contribute to reduced tumour growth through reduced signaling by CaSRs.

As highlighted by many of the authors contributing to this issue, the complexity of the bone microenvironment, and the dynamic nature of its interaction with cancer cells, presents both challenges and opportunities in the context of therapeutic targeting. However, the overall consensus is that continued research efforts in this area will result in new agents and treatment strategies that will improve the outcome for patients with skeletal metastases.

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