The role of tumour-associated macrophages in bone metastasis

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A B S T R A C T

This overview addresses the recent research developments in the role of tumour-associated macrophages (TAM) in bone metastasis biology and management of breast and prostate cancer as well as in primary and lung metastatic osteosarcoma. Immunosuppressive M2-type TAMs have been shown to associate with poor prognosis. Throughout their life cycle, macrophages (Macs) can adapt to environmental cues and influence the surroundings by secreting different cytokines and enzymes crucial to matrix remodelling, infection fighting, immune regulation and/or inflammation. In general terms, there is a broad and complex spectrum of Mac polarization statuses from M1 (classically activated/inflammatory) to M2 (alternatively activated/wound healing/immune regulating) Macs. Often the activation status of TAMs resembles more the M2-type. Considering the physiological functions of M2 Macs, it is no surprise that TAMs appear to have a role in metastasis, participating in almost every step of the metastatic cascade, which we review and explore in selected bone tropic cancers.

1. Macrophages, osteomacs and osteoclasts

Macrophages (Macs) are immune cells derived both from embryonic precursors and circulating CD14+ monocytes which originate from the bone marrow [1]. Cell fate mapping studies in mice on adult microglia, bone marrow cells, alveolar macrophages and macrophages in mouse inflammation [2] have further demonstrated that tissue resident Macs can proliferate in situ, thereby bypassing the need of differentiation from newly recruited monocytes. Macs adopt different polarization/activation statuses as response to environmental stimuli and perform distinct physiologic functions from phagocytosis to antigen presenting, wound healing, immune regulation, tissue vascularization and inflammation [3]. Mac polarization spans a broad spectrum of intermediate statuses, with M1 or classically activated Macs at one extreme and M2 or alternatively activated Macs at the other extreme [4,5]. Human M2 Macs can be further classified as M2a, M2b and M2c (Fig. 1), the third being the most immunosuppressive Mac type. Recently, for in vitro differentiated macrophages, a nomenclature that clearly identifies the differentiation and activation stimuli used (e.g., M(IFN-γ), M(IL-4), M(IL-10), M(IFN-γ+LPS), etc.) has been proposed [1].

Bone marrow resident Macs (Osteomacs) are located in canopy-like structures in endosteal and periosteal surfaces, above osteoblasts [6]; osteoclasts result from the fusion of several myeloid osteoclast precursors [7]. Osteomacs constitute approximately 17% of the bone marrow cells and they differ from osteoclasts by the expression of F4/80 and CD68. In addition, osteomacs play an important role in bone repair and hematopoietic stem cell (HSC) niche maintenance [6].

2. Tumour-associated macrophages (TAMs) in the bone metastatic cascade

In primary breast tumours, 5–40% of the tumour mass consists of TAMs [9]. TAMs often resemble M2 Macs and the majority of the published studies report an association between poor disease outcome and the number of TAMs or low M1/M2 ratio [8]. In some studies, TAMs are associated with good prognosis (e.g., prostate, stomach, colon, cervix, lung and pancreas). However, the M1/M2 ratio or the location of the TAMs might - at least to some extent - explain these favourable outcomes [8].

In order to form bone metastases the cancer cells have to go through several steps, the so-called metastatic cascade. The metastatic cascade includes local invasion of surrounding healthy tissue, intravasation (formation of circulating tumour cells, CTCs), migration and survival in circulation, extravasation (formation of disseminated tumour cells, DTCs), angio- and lymphangiogenesis, matrix remodelling, premetastatic niche formation, survival at the new site either as dormant or proliferating DTCs, dormancy escape, proliferation and macrometastases formation [10]. We and others have recently reviewed the role of TAMs in each of the metastatic steps [11–13].

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3. TAMs’ role in bone metastasis and primary bone cancer: evidence from preclinical and clinical studies

The majority of preclinical and clinical studies assess TAMs in primary tumours and metastasis-associated macrophages (MAMs) in visceral metastases (e.g., lung, liver, kidney, spleen, brain). Some preclinical models require long progression times to form bone metastases which might limit their usefulness due to ethical reasons. Nevertheless, there is some indirect evidence of a role for TAMs in bone metastasis arising from studies in cancer models with systemic (Csf1op/op mice), conditional (MaFIA mouse model) or pharmacological macrophage ablation (e.g., the use of clodronate liposomes, CLO-LIP) and from retrospective clinical studies (see Table 1).

3.1. TAMs in breast cancer bone metastasis

Primary breast cancer cells express a plethora of cytokines and growth factors into the local microenvironment and circulation. Amongst those factors, macrophage recruiting and differentiating factors such as VCAM-1 (vascular cell adhesion protein-1), M-CSF (macrophage colony-stimulating factor also known as CSF-1-colony stimulating factor-1) and MCP-1 (monocyte chemotactic protein-1) have been characterized. Additionally, breast cancer cells have been shown to set the scene for distant metastases (premetastic niche formation) long before actual CTC arrival to the potential metastatic site [14]. Among others, factors such as S100 proteins, MMPs (matrix metalloproteinases), VEGFs (vascular endothelial growth factors), fibronectin [14], and lysyl oxidase (LOX) [15] are crucial for the premetastastic niche formation. These factors elicit matrix remodelling at the new site, recruit bone marrow derived cells (e.g., Macs.) and provide “trails” (chemotaxis of CTCs by the secreted products) and “foot-holds” (premetastic niche expression of integrins and adhesion molecules) for colonization of the new site by DTCs.

The best described axes of crosstalk between breast cancer cell and TAM to date are the CSF-1 (cancer cell derived) CSF1R (TAM expressed) axis and the EGF (epidermal growth factor, TAM derived) and EGFR (EGF receptor, cancer cell expressed) axis. They are both known to have implications on early metastatic cascade steps of breast cancer cells such as cancer cell-TAM co-migration, invasion and intravasation [16]. A recent work has found that FLT1 expression (also known as VEGFR1) on MAMs is essential for CTC seeding of lungs and persistent metastatic growth, with no effect on primary tumour invasion and intravasation. FLT1+ macrophages were found to be substantially enriched in human breast cancer metastatic sites when compared with primary tumour sites. In mouse models of breast cancer lung metastasis, FLT1 was exclusively expressed by MAMs and not by monocytic precursor cells. These murine MAMs were shown to resemble tumour promoting TAMs. FLT1 inhibition decreased lung metastatic index without affecting MAM recruitment, but rather altering the inflammatory gene signature of MAMs. This included downregulation of CSF-1 expression through focal adhesion kinase 1 (FAK1) signalling [17]. The interaction between tumour cells, macrophages and endothelial cells (the so called tumour microenvironment of metastasis, TMEM) is essential to establish a spatially and temporally transient hyperpermeable tumour vasculature, which allows “streams” of tumour cells and TAMs to intravasate and disseminate. This study has shown that the macrophages at the TMEM are a subset of TAMs with high Tie2 and VEGFA expression [18].

Most of the early events described above translate into lung or liver metastases. However, recent studies [15] have shown bone premetastatic and metastatic results, with some indirect proof of Mac involvement. The latter study with intratibial and orthotopic MDA-MB-231 models showed that silencing the EGFR expression in the cancer cells decreased bone and mammary fat pad tumour growth, and reduced the production of M-CSF and MMP-9 in the cancer cells decreased lung metastatic index without affecting MAM recruitment, but rather altering the in...
PyMT murine breast cancer model [21]. Moreover, in pancreatic cancer M-CSF/M-CSFR blockade decreased TAM infiltration and reprogrammed the remaining TAMs to support antigen presentation and T-cell activation [22]. Although no TAM analysis was provided, it is reasonable to speculate that the reduced M-CSF and MMP-9 levels in the MDA-MB231 models reflected decreased TAM infiltration and decreased M2 Mac polarization. Thus, the observed decrease in bone and primary tumour growth of the MDA-MB231 model can be a combination of cancer cell, TAM and other microenvironmental effects [19]. Lysyl oxidase (LOX) secreted by hypoxic breast tumour cells accumulates in premetastatic niches where it crosslinks collagen IV at the basement membrane. This favours lung premetastatic niche formation in the 4T1 and MDA-MB231 models by enhancing metastatic tumour cell invasion and bone marrow derived cells’ recruitment to premetastatic lungs which further enhances matrix remodelling by Mac secreted MMPs [23]. A study on premetastatic bone lesions done with intracardiacally injected LOX silenced 4T1 cells demonstrated a role for LOX in osteoblast inhibition and osteoclastogenesis activation which favoured tumour cell colonization of bone [15]. It is again reasonable to think that LOX would also affect osteomacs and TAMs in the primary and bone metastasis sites, but further studies are required to elucidate that.

3.2. TAMs in prostate cancer bone metastasis

Prostate cancer bone metastases are often osteoblastic or mixed osteoblastic/osteolytic lesions. Rodent models of prostate cancer have revealed similar TAM associations with early steps of the metastatic cascade as seen for breast cancer with perhaps a more predominant proangiogenic component [11]. Additionally, a study using the intratibial PC-3 mouse model of tumour growth in bone has shown that cancer cell derived IL-6 recruited Macs to the tumour site and promoted tumour aggressiveness, whereas Mac depletion or IL-6 silencing decreased the size of bone lesions, the degree of bone lysis and the incidence of lymph node metastases [24]. This is in line with the findings by Bonapace and colleagues [25] in breast cancer lung metastatic models, where IL-6 production by pulmonary recruited inflammatory Macs of tumour bearing mice increased VEGF-A signalling which subsequently unleashed lung metastasis after anti-CCL2 treatment cessation [25].

More recently, the RM-1 and PC-3 prostate cancer models were established in macrophage deficient mouse models (the inducible macrophage deficient Csf1op/op model, the macrophage ablation model MaFIA and the CLO-LIP Mac depleted model) [26]. In these models, primary tumour growth was impaired in Mac depleted mice compared to controls with normal Mac numbers. Tumour-bearing bones of control mice had larger numbers of Macs than tumour-free bones. However, the Mac and bone tumour growth association held true in the Mac depleted models suggesting that osteomacs and TAMs may have a role in supporting also the prostate cancer bone lesion formation [26]. Studies performed in TRAMP and TRAMP-PSA models indicate a more complex scenario, showing a mixed M1/M2 Mac polarization of TAMs. In vivo Mac depletion increased tumour growth, suggesting an anti-tumour role for TAMs in the early stages of these prostate cancer models [27]. This perhaps reflects a predominant M1 polarization of TAMs. Based on the protein expression data, mostly only M1 markers (IL-1β and TNF-α) were significantly elevated in TAMs compared with peritoneal Macs with the exception of IL-10 an M2 marker [27]. Although the authors claim that TAM polarization is mixed M1/M2 and base it on mRNA relative expression data of M1 and M2 markers [27], this is questionable as it may not fully reflect TAMs polarization and function which can only be assessed by surface markers and protein expression/secretion analysis.

3.3. TAMs in osteosarcoma

The role of osteoclasts and TAMs in osteosarcoma and osteosarcoma metastasis is often confounded, probably due to the relevance and closeness of both cell types in this cancer type [28]. A recent study recurring to ex vivo and in vivo techniques has demonstrated that IL-34 and M-CSF are expressed by osteosarcoma cells, and IL-34 overexpression contributes to tumour growth and lung metastasis by recruiting M2 Macs and by increasing neoangiogenesis [29]. Clinical data supporting these preclinical results on the role of TAMs in osteosarcoma is provided in Table 1.

4. TAM targeting therapeutic opportunities

Considering every TAM/bone metastasis aspect discussed so far, it is clear that TAMs like many other cells of the tumour microenvironment are almost ideal therapeutic targets, as they are genetically stable, seem to adopt a different polarization in cancer compared to the physiological polarization status in a given tissue, and are recruited and educated by cancer cell secreted factors. Thus, agents targeting recruitment and polarization (e.g., anti-M-CSF antibodies and small molecule inhibitors of M-CSFR and bisphosphonates), M1 activating agents (e.g. mifamurtide, IL-2, zoledronate), agents interfering with the cancer cell/TAM crosstalk (e.g., VCAM-1/α4 integrin inhibition) and Mac depleting agents (e.g., CLO-LIP) are all strategies being pursued, mostly still in the preclinical setting [11]. However, with the ever evolving understanding of the roles of TAMs, it is reasonable to think that in the future TAM modulating therapies might be at the disposal of clinicians and patients.

5. Outstanding questions

1. What is the origin of TAMs? Are TAMs derived from resident Macs educated by the growing mass of dormancy escaped DTCs? Are TAMs educated by the premetastatic niche factors elicited by primary tumour cells remote signalling? Or are TAMs newly recruited monocytes locally differentiated by similar players?

2. Do TAMs co-migrate with CTCs?

3. Are osteomacs prone to similar re-education by DTCs, premetastatic niche and primary tumour factors, potentially becoming metastases-associated macrophages (MAMs)?

4. Are TAMs involved in the CTC to DTC transition, by means other than facilitating extravasation of CTCs?

5. Are TAMs involved in the dormancy escape of DTCs? Which are the triggers for this phenomenon?

6. Does the immunological status of each particular breast, prostate and other bone seeking cancer patient predispose him/her to develop bone metastases?

7. What are the other immune and microenvironmental players affected by TAMs further contributing for a worse prognosis?

8. Is TAM targeting/re-education a real therapeutic option to bone metastatic patients?

Conflict of interest

The authors have no conflict of interests to declare.
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