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Research Paper

The role of tumour-associated macrophages in bone metastasis

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ABSTRACT

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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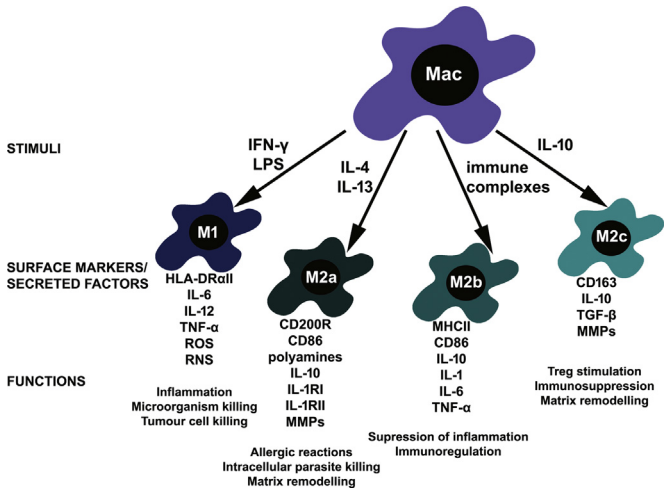

Fig. 1. Human macrophage (Mac) polarization. Polarizing cytokines, surface markers, secreted factors and physiologic functions.

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 ($Csf1^{op/op}$ 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 (premetastatic 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 premetastatic 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 premetastic 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 tumours [19]. Studies in murine breast adenocarcinoma models, where M-CSF blockade was applied, demonstrated a decrease in TAM infiltration and subsequent delay in angiogenesis [20]. Furthermore, M-CSFR blockade decreased lung metastasis in the

Table 1
Clinical studies of TAM infiltration and polarization status in cancer types known to have bone involvement.

Total TAMs	M2	M1	n	Main conclusions	Reference
Breast cancer					
CD68	–	–	1322	Association with other poor prognostic markers (> grade, ER-, PR- and > proliferation)	[9]
CD68	CD163		144	CD163 + TAMs in tumour stroma positively correlated with > grade, > tumour size, Ki67 +, ER-, PR-, and inversely correlated with ER + CD68 in tumour stroma was an independent prognostic factor for ↓breast cancer specific survival	[30]
CD68	CD163	HLA-DRα	562	$CD163^{+}$TAMs associated with other poor prognosis markers (> grade, ER-, node positivity, > proliferation and > tumour size) in the Cox multivariate model for RFS	[31]
Prostate cancer					
CD68	–	–	100	> TAMs density,↑Hexim1 expression,↑SMAD2 expression, and mild SMAD7 expression play important roles in the disease	[32]
Osteosarcoma					
CD14	CD163	HLA-DRα	145	Association of $CD14^{+}$TAMs with ↑OS, metastasis suppression in high-grade patients and ↑microvessel density. No associations of M1 or M2 TAMs with prognosis. Possible role for balanced M1/M2 TAMs response leading to ↑survival (Macs' subtype analysis was performed in a sub-cohort of n = 29)	[33]

Abbreviations: OS, overall survival; ER, oestrogen receptor; PR, progesterone receptor;TAM, tumour-associated macrophage; RFS, recurrence free survival; Macs, macrophages