MT1-MMP
A novel component of the macrophage cell fusion machinery

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Mice deficient in the matrix metalloproteinase MT1-MMP display defects in tissue development and angiogenesis, together with a complex bone phenotype characterized by several skeletal abnormalities and osteopenia. OCs and giant cells are multinucleated cells arising from the fusion of myeloid progenitors/macrophages that specialize respectively in bone resorption and engulfment of pathogens and foreign bodies. Our work identifies MT1-MMP as a novel component of the macrophage fusion machinery during OC and giant cell formation in vitro and in vivo. MT1-MMP is required for the proper lamellipodia formation and motility required to achieve proximity between fusion-competent myeloid cells; and roles of MT1-MMP in subsequent steps of the fusion process cannot be ruled out. For example, MT1-MMP might exert additional functions at fusion sites by forming molecular complexes with CD44 or tetraspanin proteins. Interestingly, the contribution of MT1-MMP to macrophage motility and fusion does not involve its catalytic activity. Instead, the MT1-MMP-cytosolic tail, in particular Tyr573, is required to bind the adaptor protein p130Cas and regulate localized Rac1 activity in myeloid progenitors. Modulation of this novel MT1-MMP-p130Cas-Rac1 signaling pathway in macrophages might have potential in the treatment of disorders involving increased OC activity or uncontrolled giant cell formation.

Matrix metalloproteinases (MMPs) are endopeptidases necessary for the processing of extracellular matrix components. However, deficient mouse models show that MMPs were mostly dispensable for mouse development and viability. MT1-MMP (also known as MMP14) was the first membrane-anchored MMP identified and was initially implicated in tumor invasion.2 Notably, MT1-MMP (Mmp14) null mice do display an obvious phenotype characterized by dwarfism; skeletal abnormalities; defects in the development of lung, submandibular gland and skeletal muscle; impaired adipose tissue growth, and altered angiogenesis.3-4 The bone phenotype, mostly in regard to bone formation, has been well characterized; the role of MT1-MMP in osteoclast (OC) development was, however, unclear. Previous data suggested a negative action of MT1-MMP on OC activity and development, since null mice have osteopenia and increased OC numbers at inflamed joints;3 defective shedding of RANKL in MT1-MMP-null osteoblasts (OB) also resulted in increased OC numbers in the absence of MT1-MMP.12 However, we could not detect differences in RANKL serum levels between WT and MT1-MMP-deficient mice (unpublished results), which suggests compensation by other RANKL sheddases such as ADAM10;13 nonetheless, we cannot rule out contributions of this pathway to the in vivo bone phenotype. In contrast, our data clearly establish a cell-autonomous and positive regulatory role of MT1-MMP in OC development via the modulation of Rac1-dependent motility, membrane protrusive activity and fusion in myeloid progenitors13 (Fig. 1). Consistent with these data, OC bone resorption is decreased...
in MT1-MMP null mice, as assessed by ICTP quantification; however, bone mass was not increased, likely due to an independent or coupled defect in OB differentiation, or to the perinatal stage analyzed.

The contribution of MT1-MMP to macrophage cell fusion is not restricted to the OC lineage; giant cell formation was also impaired in null mice, pointing to a role for MT1-MMP in chronic inflammatory disorders. Notably, MT1-MMP heterozygous mice, which show no obvious phenotype under steady-state conditions, have an impaired response to foreign body-induced giant cell formation and also to parathyroid hormone injection (and unpublished results). This would indicate that a minimal threshold amount of MT1-MMP is required for proper macrophage fusion in distinct pathophysiological contexts. This is in accordance with the defective vascular response to occlusion previously reported in heterozygous mice.14

MT1-MMP plays a critical role in pre-fusion stages by affecting myeloid cell morphology, membrane protrusive activity and motility (Fig. 1). Although a direct fusogenic role for MT1-MMP is unlikely, it is possible that MT1-MMP might participate in later events such as membrane recognition, apposition or fusion between myeloid progenitors. One possible way in which MT1-MMP might participate in later fusion events is through interaction with CD44, which contributes to macrophage cell fusion through a variety of mechanisms including its shedding.15-17 Since MT1-MMP associates with CD44 and mediates its shedding, it is possible that CD44-MT1-MMP complexes are relevant to cell fusion.18 However, the fact that we could not rescue the fusion phenotype of MT1-MMP null myeloid progenitors by overexpressing various forms of CD44 suggests a predominant role of MT1-MMP at pre-fusion stages. An alternative interpretation is that CD44 might facilitate MT1-MMP localization at fusion sites, as it does at the leading edge of tumor cells and at the poles of epithelial
MT1-MMP can also associate with tetraspanin (Tspan) family members that are relevant to membrane fusion during fertilization and giant cell formation. MT1-MMP-Tspan complexes are found at endothelial and cancer cell-cell contacts, and it is possible that inclusion of MT1-MMP into Tspan microdomains also contributes to MT1-MMP localization and function at the fusion sites (Fig. 1).

A surprising finding of our study was that the contribution of MT1-MMP to myeloid cell fusion was independent of its catalytic activity, similarly to the developmental defects in lung and submandibular gland reported in MT1-MMP null mice. Instead, MT1-MMP regulates Rac-1-dependent myeloid cell morphology and migration via interaction through its cytosolic tail with p130Cas. It will be interesting to investigate whether this MT1-MMP-dependent signalling pathway is also relevant to the achievement of optimal Rac1 activity in other contexts in which both MT1-MMP and Rac1 are critical players, such as endothelial cells and angiogenesis. The requirement for MT1-MMP, and in particular its catalytic activity, for 2D and 3D cell migration has remained controversial.

Our current findings, together with previous reports, suggest a model in which MT1-MMP contributes to 2D-migration through catalytic-independent and cytosolic domain-dependent mechanisms by regulating Rac1 GTPase activity or some other processes; however, when cells are involved in an invasive program within a 3D-scenario, MT1-MMP catalytic activity is mandatory for cell invasiveness. In contexts in which low Rac1 activity is required (3D migration) or in which Rac1 activity is deregulated (tumor cells) the requirement of MT1-MMP for migration might be circumvented.

The function of the MT1-MMP cytosolic tail in migration and invasion is similarly unclear and complex. A potential problem is that many studies have used MT1-MMP mutants with the whole cytosolic tail deleted, thus removing putative positive and negative regulatory elements. This led to seemingly contradictory findings, with the cytosolic tail apparently dispensable for the catalytic activity of MT1-MMP in 3D contexts, while partial deletion mutants (Δ567 and Δ573) pointed to these sequences as important for MT1-MMP localization at the leading edge and Matrigel invasion. Our findings clearly show the mechanism by which the MT1-MMP catalytic tail contributes to myeloid cell fusion. In particular, our findings show that Tyr573 is important for the association of MT1-MMP with p130Cas, an issue not addressed previously. Interestingly, MT1-Tyr573 can be phosphorylated by Src kinase, which is essential for normal OC development. We also show that MT1-MMP-p130Cas association is important for optimal membrane targeting and activity of Rac1 (and probably also cdc42) in myeloid progenitors (Fig. 2). It seems that MT1-MMP, like integrins, is important for the proper regulation of GTPases in distinct contexts: MT1-MMP cooperates with cdc42 in the formation of tunnels for invasion within 3D matrices, the MT1-MMP catalytic tail can bind p27RF-Rho, a regulator of RhoA activity, and MT1-MMP deficiency decreases the amounts of Rac1 and cdc42 and increases RhoA activity at the lamellipodia of myeloid progenitors (reviewed in ref. 13 and unpublished results). Through this latter mechanism, MT1-MMP might also impact the assembly/disassembly of focal adhesions (FA), thus explaining the larger and thicker FA observed in MT1-MMP null myeloid progenitors. The possibility that MT1-MMP plays additional roles in FA turnover through ECM processing or through the regulation of integrin recycling deserves further investigation.

The newly-identified contribution of MT1-MMP to myeloid cell fusion suggests new therapeutic possibilities. Specific modulation of the MT1-MMP-p130Cas-Rac1 pathway in macrophages might be a valuable approach in diseases associated with enhanced OC activity, such as osteoporosis or bone metastasis, and in chronic inflammatory disorders involving uncontrolled giant cell formation, such as foreign body reactions and granulomatous disease.

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