

Proactive for invasion: Reuse of matrix metalloproteinase for structural memory

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Migratory cells translocate membrane type-1 matrix metalloproteinase (MT1-MMP) to podosomes or invadosomes to break extracellular matrix barriers. In this issue, El Azzouzi et al. (2016. *J. Cell. Biol.* <http://dx.doi.org/10.1083/jcb.201510043>) describe an unexpected function for the MT1-MMP cytoplasmic domain in imprinting spatial memory for podosome reformation via assembly in membrane islets.

Invasion of most normal and cancer cells across basement membranes and collagen-rich interstitial tissues involves degradation of the ECM by membrane type-1 matrix metalloproteinase (MT1-MMP/MMP14; Willis et al., 2013). To fulfill this activity, MT1-MMP is transported to podosomes, the specialized ECM-degrading membrane protrusions found in highly migratory cells such as activated macrophages, osteoclasts, endothelial cells, and smooth muscle cells (Murphy and Courtneidge, 2011). In cancer cells, MT1-MMP is transported to ECM-degrading invasive structures called invadopodia (Poincloux et al., 2009). Both these membrane protrusions, collectively called invadosomes, are composed of an actin-rich core surrounded by scaffold and adhesion proteins, and numerous mechanisms of invadosome assembly, maturation, and dynamics have been identified (Poincloux et al., 2009; Murphy and Courtneidge, 2011). MT1-MMP activity is regulated at multiple levels to achieve targeted ECM degradation, cell surface protein processing, and protease activation (Sato et al., 1994; Osenkowski et al., 2004; Sugiyama et al., 2013; Willis et al., 2013; Itoh, 2015). Potential regulatory functions of MT1-MMP toward the cytoskeleton have, however, remained unclear. In this issue, El Azzouzi et al. describe an unexpected and novel function for MT1-MMP that goes beyond its traditional proteolytic activity: they show that MT1-MMP accumulates in membrane islets that provide macrophages with spatial information, or memory, in sites of podosome dissolution so as to enable efficient podosome reassembly.

El Azzouzi et al. (2016) first used total internal reflection fluorescence microscopy and a pH-sensitive version of MT1-MMP devised to fluoresce only when the MT1-MMP ectodomain is exposed to the extracellular environment's pH. With this approach, they show that, on the ventral surface of cultured human macrophages, MT1-MMP is localized at two different membrane compartments: underneath the podosome core, as

previously suggested based on matrix degradation and colocalization with podosome markers, and in distinct islets devoid of other podosome components, CD44, or integrin-mediated adhesion to the ECM (Fig. 1; Osiak et al., 2005). MT1-MMP islets were dependent on intact cortical actin, but became more apparent and persisted after podosome disruption by pharmacological perturbation of key components of podosome assembly and maturation, such as integrin adhesion, Src kinase activity, and the Arp2/3 complex essential for actin nucleation and branched actin cytoskeleton. Podosomes often reemerge at sites of previous podosome localization, and El Azzouzi et al. (2016) hypothesized that MT1-MMP islets might mark sites of podosome formation. They treated cells with an Arp2/3 inhibitor to disrupt podosomes and induce the appearance of MT1-MMP membrane clusters, and used time-lapse imaging to track what happens upon washout and podosome reformation. Interestingly, they show that these novel MT1-MMP structures serve as remarkably immobile cell membrane anchors capable of rerecruiting the podosomal actin cores/scaffolds to the same islets.

Further, by expressing mutant MT1-MMP proteins in cells silenced for the endogenous proteinase and using a podosome reformation assay (based on pharmacological dissolution of podosomes via Src inhibition, followed by podosome reformation after washout), El Azzouzi et al. (2016) pinpointed the region of MT1-MMP critical for islet formation, the LLY-sequence in its cytoplasmic domain. Moreover, when attached to the membrane by the MT1-MMP transmembrane domain, the 20-amino acid cytoplasmic tail appeared necessary and sufficient to form the islets. Considering the LLY sequence-dependent actin-binding ability of MT1-MMP (Yu et al., 2012) coupled with the observed necessity of cortical actin for islet appearance and podosome reformation, the direct interaction with unbranched cortical actin was suggested by the authors as a likely decisive mechanism for the remarkable MT1-MMP islet stabilization in podosome-free areas, although a possible indirect interaction was not ruled out. Actin binding through the MT1-MMP cytosolic tail was likewise suggested as a potential means for podosome rerecruitment by MT1-MMP memory islets.

Although cortical actin is instrumental for the emergence of the spatially and temporally stable MT1-MMP islets upon podosome dissolution in macrophages and direct actin-MT1-MMP interaction has been proven in vitro and suggested as a

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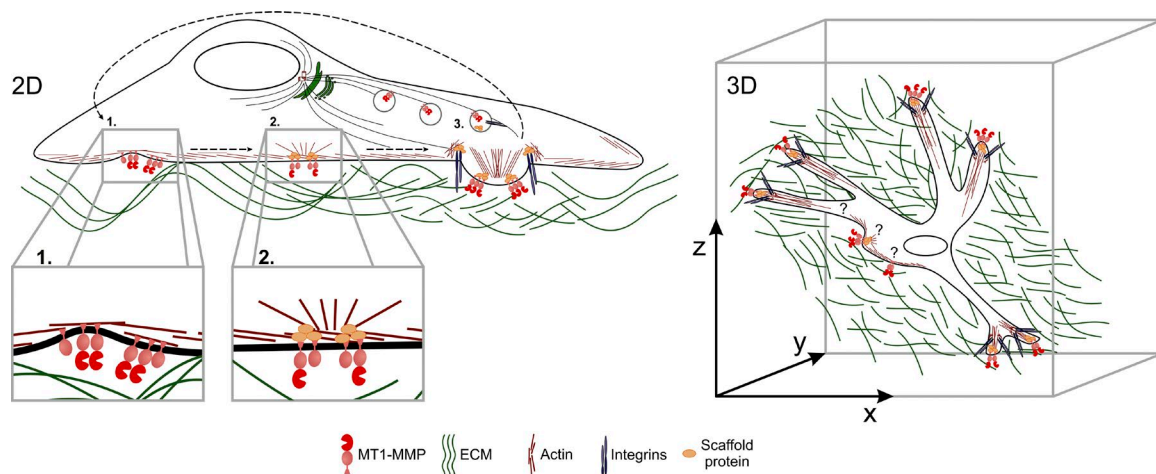


Figure 1. **MT1-MMP islets as memory sites for podosome reformation.** Migratory cells translocate MT1-MMP (red) to podosomes or invadosomes to degrade the ECM (green fibers). These membrane structures are composed of an actin-rich core (brown) surrounded by adhesion and scaffold proteins (beige) such as integrins (blue). El Azzouzi et al. (2016) show a function for MT1-MMP accumulation in membrane “islets” (1), where they imprint spatial memory for podosome reemergence after podosome disassembly (2). Unlike dynamic mature podosomes (3), MT1-MMP assembles in stable islets via anchorage to cortical actin. Future work in the fields of inflammation, cancer, and angiogenesis will need to address the nature of the cytoskeletal dynamics mediating islet formation, the involvement of microtubules in islet formation, the exact islet protein composition, and the relevance of these memory sites to 2D or 3D environments and to other cell types beyond macrophages, including endothelial cells and invasive cancer cells.

means for retaining MT1-MMP in invadopodia, a Src-regulated interaction between MT1-MMP’s cytoplasmic domain and the actin-binding scaffold protein palladin has also been shown to regulate MT1-MMP targeting into invadopodia (Yu et al., 2012; von Nandelstadh et al., 2014). Moreover, the LLY sequence in MT1-MMP’s cytoplasmic tail harbors a Src substrate sequence and mediates an interaction between MT1-MMP and AP-2 that is important for MT1-MMP internalization and dynamics in cell migration and invasion (Uekita et al., 2001; Nyalendo et al., 2007). Intriguingly, El Azzouzi et al. (2016) did not find evidence of involvement of dynamin-dependent membrane trafficking events in the ability of MT1-MMP islets to function as memory sites. However, their results after treatment with the microtubule inhibitor nocodazole indicated that although the islets themselves remained intact, podosome reappearance was mislocalized, suggesting that microtubules contribute by as yet undefined mechanisms to the ability of MT1-MMP islets to provide spatial memory and to facilitate podosome reassembly. Therefore, further identification of drivers and specific regulatory mechanisms of the actin–MT1-MMP interaction dynamics in podosomes, of the stable actin–MT1-MMP interaction and structures in podosome-free areas, and of microtubule-dependent podosome reassembly will be of interest.

A striking observation of this study is that MT1-MMP islets do not display degradative activity in matrix degradation assays. In addition, inhibition of the proteolytic activity of MT1-MMP through pharmacological agents or via an inactivating mutation did not impact islet appearance or podosome reemergence at sites of MT1-MMP clustering. Overall, on the extracellular side of the plasma membrane, the apparent lack of contact and degradation of the ECM as well as the relatively minor impact of the N-terminal MT1-MMP ectodomain on islet formation and podosome reemergence are peculiar features of the MT1-MMP islets. However, El Azzouzi et al. (2016) show evidence for somewhat impaired islet formation in cells expressing an MT1-MMP mutant lacking the entire ectodomain, and they demonstrate that endogenous MT1-MMP must be silenced for the LLY MT1-MMP mutant to disrupt islet localization. Based

on these results, the authors suggest the possible influence of MT1-MMP oligomerization and of MT1-MMP–ECM binding on islet recruitment and stabilization. Nevertheless, these observations altogether indicate that the adhesive and degradative activities of MT1-MMP memory islets toward the ECM are minor and, intriguingly, do not influence the structure or function of these islets as currently characterized in 2D cultures.

Furthermore, the aforementioned results raise questions about the possible contribution of the different molecular forms of MT1-MMP (e.g., cleaved or uncleaved and inhibitor bound or not) to the stabilization and podosome reassembly function of MT1-MMP islets. In cells and conditions in which MT1-MMP activity is high, MT1-MMP turnover is typically fast via autocatalytic cleavage or shedding of the N-terminal catalytic domain (Lehti et al., 1998; Yana and Weiss, 2000; Itoh et al., 2001; Osenkowski et al., 2004). After interaction with inhibitors such as tissue inhibitors of metalloproteinases, active endocytosed MT1-MMP may dissociate from the bound inhibitor to be recycled to the plasma membrane (Jiang et al., 2001; Remacle et al., 2003). However, in the absence of interaction with a protease inhibitor or collagen/matrix substrate, MT1-MMP oligomerization facilitates MT1-MMP turnover via autocatalytic inactivating cleavage (Itoh et al., 2001; Lehti et al., 2002; Osenkowski et al., 2004). In the current study, El Azzouzi et al. (2016) used MT1-MMP proteins with a pH sensor inserted N-terminally to the transmembrane domain, so that the probe is located extracellularly on the surface-exposed protease. The fluorescence signal from these constructs is not expected to be affected by proteolytic processing or shedding of the catalytic domain, so it is unclear whether the MT1-MMP proteins clustered in islets are cleaved or not. However, FRAP results showed that the turnover of MT1-MMP molecules within the islets is relatively slow. It thus remains to be clarified if and how the proteolytically active or possibly processed or protease inhibitor–bound inactive forms of MT1-MMP are stabilized in these MT1-MMP islets.

As podosomes are highly dynamic protrusions, their rapid turnover implicates a constant disassembly at the rear and

formation at the front of migrating macrophages. Assembly and disassembly are known to depend on Arp2/3-mediated actin nucleation and fission of preexisting podosomes, respectively (Linder et al., 2000). Both of these mechanisms may contribute to podosome reassembly at MT1-MMP memory sites. Considering that these islets are laterally immobile and overall stable in at least unpolarized cells, it is unclear how migrating cells coordinate their actin and microtubule cytoskeletons for podosome reassembly at the front using islets formed upon podosome dissolution at the rear of the cell (Fig. 1). Moreover, the structural and functional features of MT1-MMP islets in the scenario of 3D cell–ECM microenvironments is intriguing and will need to be investigated at high resolution, as cytoskeletal dynamics, cell polarity, and matrix stiffness greatly differ in 3D tissues and matrices from the simple 2D setting of cultured cells, and all are known to influence cell behavior. Although the transient nature of these MT1-MMP islets in bridging podosome disassembly and reassembly exemplifies and reflects the efficiency of podosome reuse, probing the protein composition of these islets as well as the dynamics of podosome reassembly will likely be challenging. Future studies comparing MT1-MMP state, dynamics, reuse, and turnover in different types of invadosomes, islets, and other subcellular compartments will be instrumental to better understand how cells integrate the different types of MT1-MMP membrane structures and cell–ECM communication with other cellular signals and with drivers of cytoskeletal dynamics.

The identification of the molecular mechanisms of structural and functional podosome memory are not only relevant to the fields of macrophage biology and inflammation but also more broadly to those of tissue invasion and matrix remodeling. For instance, endothelial cells, smooth muscle cells, and cancer cells are also known to target MT1-MMP to podosomes or related invadosomes. Examining MT1-MMP memory in such specialized subcellular compartments will be interesting beyond the podosome field, as the podosome counterparts in cancer cells may display and use MT1-MMP or other metalloproteinases in a similar manner. By shedding light on the mechanisms of dynamic protrusion formation and function, this paper not only opens new avenues of investigation into the cellular structures marking protrusion sites as “memory devices” but also brings about a new concept to the fields of cell invasion, angiogenesis, and cancer.

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