Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia

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Summary

When migrating away from a primary tumour, cancer cells interact with and remodel the extracellular matrix (ECM). Matrix metalloproteinases (MMPs), and in particular the transmembrane MT1-MMP (also known as MMP-14), are key enzymes in tumour-cell invasion. Results from recent in vitro studies highlight that MT1-MMP is implicated both in the breaching of basement membranes by tumour cells and in cell invasion through interstitial type-I collagen tissues. Remarkably, MT1-MMP accumulates at invadopodia, which are specialized ECM-degrading membrane protrusions of invasive cells. Here we review current knowledge about MT1-MMP trafficking and its importance for the regulation of protease activity at invadopodia. In invasive cells, endocytosis of MT1-MMP by clathrin- and caveolae-dependent pathways can be counteracted by several mechanisms, which leads to protease stabilization at the cell surface and increased pericellular degradation of the matrix. Furthermore, the recent identification of cellular components that control delivery of MT1-MMP to invadopodia brings new insight into mechanisms of cancer-cell invasion and reveals potential pharmacological targets.

This article is part of a Minifocus on invadopodia and podosomes. For further reading, please see related articles: 'Invadosomes at a glance' by Stefan Linder (*J. Cell Sci*. **122**, 3009-3013), 'Mechanisms for transcellular diapedesis: probing and pathfinding by 'invadosome-like protrusions'' by Christopher V. Carman (*J. Cell Sci*. **122**, 3025-3035) and 'Actin machinery and mechanosensitivity in invadopodia, podosomes and focal adhesions' by Corinne Albiges-Rizo et al. (*J. Cell Sci*. **122**, 3037-3049).

Key words: Extracellular matrix, Invadopodia, Matrix metalloproteinases, Metastasis

Introduction

Metastasis is a complex process that starts with the dissemination of cancer cells from a primary tumour to distant tissues. Along the metastatic cascade, tumour cells interact with and remodel the extracellular matrix (ECM). Among the many extracellular proteases expressed by human cells, matrix metalloproteinases (MMPs) have been identified as important enzymes engaged by tumour cells during metastasis (Egeblad and Werb, 2002).

Members of the MMP family (of which there are 25 in humans) are multifunctional zinc-dependent endopeptidases that can degrade a variety of ECM components. Almost all MMPs are secreted into the extracellular milieu, except six membrane-anchored (MT) MMPs (MT1-MMP to MT6-MMP, also called MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25, respectively). MMPs share a conserved structure that consists of a signal peptide, a propeptide and a catalytic domain. Several MMPs also contain a hemopexin (HPX) domain, which contributes to substrate recognition and degradation as well as to protein-protein interactions (Cao et al., 2004; Itoh et al., 2008; Li et al., 2008; Suenaga et al., 2005). MMPs are produced as zymogens (proenzymes) and require proteolytic cleavage of the propeptide for activation (Egeblad and Werb, 2002). In the case of MT1-MMP, proMT1-MMP (~64 kDa) is converted to a catalytically active enzyme (~55 kDa) by proteolytic cleavage by furin in the trans-Golgi network (TGN) prior to its arrival at the plasma membrane (PM) (Mazzone et al., 2004; Yana and Weiss, 2000); the process is similar for other MT-MMPs. By contrast, activation of the secreted MMPs proMMP-2 and proMMP-13 is mediated by a cell-surface complex that consists of a homodimer of MT1-MMP as well as a single molecule of tissue inhibitor of metalloproteinases-2 (TIMP-2; a natural inhibitor of MMPs) (Itoh et al., 2001; Strongin et al., 1995). TIMP-2 binds to the catalytic domain of one of the MT1-MMP molecules in the dimer and to the HPX domain of proMMP-2, thereby facilitating cleavage and activation of proMMP-2 by the second (TIMP-2-free) MT1-MMP molecule of the dimer (Butler et al., 1998; Kinoshita et al., 1998; Strongin et al., 1995). In addition to activation of pro-MMP-2 and pro-MMP-13, MT1-MMP also directly cleaves ECM components including type-I, -II and -III [and possibly type-IV (Hotary et al., 2006)] collagen, gelatin, laminins 1 and 5, fibronectin, vitronectin, aggrecan and fibrin, and cell-surface proteins such as CD44, αv integrins and syndecan 1 (Overall and Dean, 2006).

For almost three decades, interest in MMPs has been increasing in light of observations that several diseases, including cancer, are associated with high MMP expression levels (Deryugina and Quigley, 2006). Retrospective analyses of samples from individuals with cancer have revealed that expression of MMPs, such as MT1-MMP and MMP-1, -2, -3, -7, -9 and -13 is positively associated with tumour progression and metastasis, and MMP expression is therefore considered to be a valuable prognostic factor (Deryugina and Quigley, 2006; Hofmann et al., 2005). In addition, MT1-MMP promotes tumour growth and local invasion in a confined three-dimensional (3D) ECM environment (Hotary et al., 2003), and is a key factor in the high infiltration capacity of the brain tumours glioblastoma and medulloblastoma (Annabi et al., 2008; Belien et al., 1999). Most interestingly, MT1-MMP and MMP-2 were shown to accumulate at the invasive front of tumours (Hofmann et al., 2003; Ueno et al., 1997). Moreover, MT1-MMP (and secreted MMP-2 and MMP-9) are enriched at invadopodia,

which are specialized actin-based membrane protrusions that occur in invasive tumoural and transformed cells grown on ECM, and can degrade the matrix (Bowden et al., 1999; Buccione et al., 2004; Chen, 1989; Clark and Weaver, 2008; Gimona et al., 2008; Linder, 2007; Nakahara et al., 1997; Stylli et al., 2008). Invadopodia are small dot-shaped, F-actin-rich plasma-membrane extensions that are enriched in cell-matrix adhesion molecules, tyrosine kinases (including Src), tyrosine-phosphorylated proteins, actin-assembly regulators and matrix proteases (Buccione et al., 2004; Gimona et al., 2008; Linder, 2007; Stylli et al., 2008). Commonly, a limited number of invadopodia (≤20 per cell) form in the middle region of the adhesive surface of invasive tumour cells grown on ECM. Invadopodia have some similarities with podosomes, which are found in monocyte-derived cells and Src-transformed fibroblastic cells [see the Cell Science at a Glance article by S. Linder in this issue (Linder, 2009)].

In an attempt to specify at which step(s) of the metastatic process matrix degradation is important, increasing efforts have focused on the effect of protease inhibition on cell migration in 3D cultures in vitro. Most studies describe the invasion of tumour cells through one of two models of the ECM: reconstituted basement membranes (BMs) and fibrillar type-I collagen networks. Thus, the available experimental data tend to provide a limited picture of the diversity of matrices, especially when compared with the variable composition and stiffness that cancer cells encounter in vivo (for details, see Even-Ram and Yamada, 2005; Rowe and Weiss, 2008; Sabeh et al., 2009).

In this Commentary, we briefly examine recent findings regarding tumour-cell invasion in these two models of the ECM, before discussing the mechanisms by which MT1-MMP activity is controlled. We focus particularly on the regulation of intracellular trafficking of MT1-MMP and on its polarized targeting to invadopodial structures in cancer cells.

Breaching of basement membranes by tumour cells

The BM is 50- to 100-μm thick, and is a highly cross-linked meshwork that separates epithelial cells from connective tissues and provides structural support. Type-IV collagen, laminin, entactin and heparan-sulphate proteoglycans are the most abundant BM components (Kalluri, 2003). These physically tough structures represent mechanical barriers to cancer-cell migration, because they do not contain pores that are large enough for passive invasion. During the metastatic process, however, cancer cells do breach BMs when leaving the primary tumour and entering or leaving blood vessels. BM degradation is one of the first detectable signs of metastatic development and it indicates a poor prognosis (Barsky et al., 1983).

Several models of the BM have been used for in vitro invasion assays. Matrigel, a laminin-, type-IV-collagen- and growth-factor-rich matrix derived from Engelbrecht-Holm-Swarm (EHS) mouse sarcoma cells, has been widely used as a BM mimic. When polymerized, it forms a dense gel with relatively small pores compared with the dimensions of a typical migrating cell (see Fig. 1), although it is less cross-linked and more susceptible to remodelling than native BMs (Even-Ram and Yamada, 2005; Kalluri, 2003). Tumour-cell migration and invasion through Matrigel has been shown to depend on MMP activity, and particularly MT1-MMP (Ueda et al., 2003; Zaman et al., 2006), although other studies reported that MMP inhibitors were inefficient in blocking cancer-cell invasion through 3D Matrigel (Hotary et al., 2006; Sahai and Marshall, 2003). Although the reason for these discrepancies is not precisely known, it should be emphasized that variation in Matrigel concentration influences the extent to which invasion is dependent on matrix proteolysis and the capacity of cancer cells to migrate and invade within the 3D matrix (Zaman et al., 2006).

Using native mesothelial BM and BM produced in vitro, it was demonstrated that activities of three MT-MMPs (MT1-MMP, MT2-MMP and MT3-MMP), but surprisingly not that of MMP-2 and MMP-9 (which are type-IV collagenases), are responsible for BM breaching by MDA-MB-231 human breast adenocarcinoma cells (Hotary et al., 2006). These findings point to a direct essential role of MT-MMPs in proteolytic remodelling and breaching of BMs, and argue against the idea that the proinvasive role of MT1-MMP depends on its activation of secreted MMPs (Hotary et al., 2006).

Tumour-cell invasion of interstitial collagen networks

Type-I collagen, which forms fibrillar networks, is commonly used as a model of interstitial matrix (Fig. 1). Several studies point to the necessity of matrix degradation for migration and invasion through interstitial collagen by normal and neoplastic cells (Chun et al., 2004; Filippov et al., 2005; Hotary et al., 2000; Li et al., 2008; Niggemann et al., 2004; Sabeh et al., 2004; Sodek et al., 2007). Strikingly, MT1-MMP is crucial for collagenolysis in these systems, whereas secreted MMPs are not (Hotary et al., 2006; Li et al., 2008; Sabeh et al., 2004). Furthermore, the need for matrix proteolysis during interstitial invasion is strengthened by consistent results that have been obtained using peritoneum-derived interstitial stroma or chicken chorioallantoic membrane (Li et al., 2008; Sabeh et al., 2004).

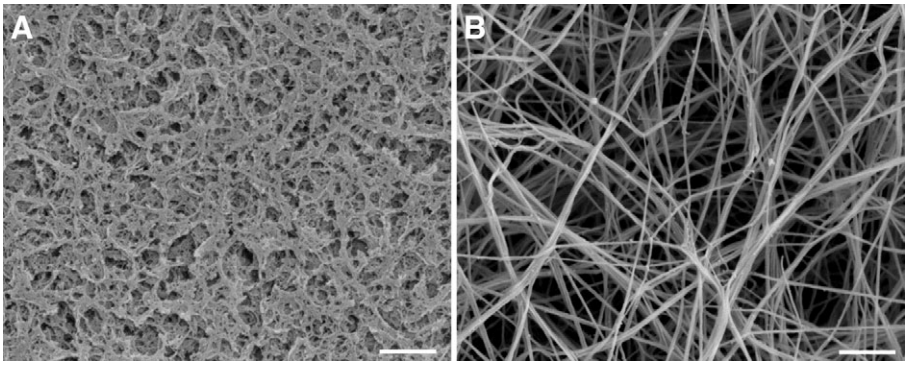

Fig. 1. Comparison of Matrigel and type-I-collagen matrix architecture. Matrigel (10 mg/ml, BD Biosciences) and acid-solubilized type-I collagen (3 mg/ml, Koken, Tokyo, Japan) matrices were prepared as described for ultrastructural analysis by scanning electron microscopy (Lizarraga et al., 2009; Steffen et al., 2008). (A) Matrigel, which is commonly used as a model of the BM, forms a dense gel with small pores compared with the dimensions of a cell. (B) By contrast, type-I collagen, which is often used as a model of the interstitial matrix, forms a typical fibrillar meshwork with large pores. Scale bars: 1 μm.

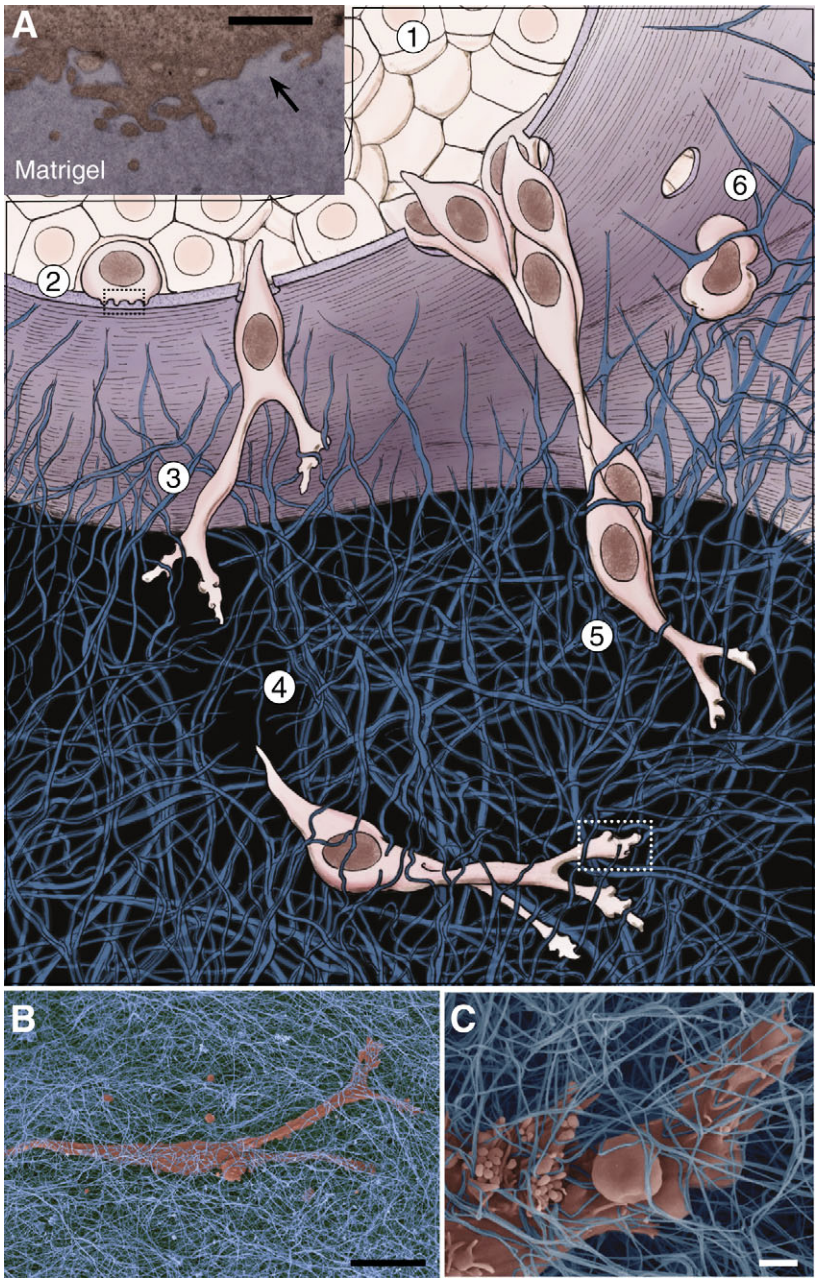

Fig. 2. Different modes of cancer-cell invasion. (A) Before beginning to migrate, cancer cells within the primary tumour acquire invasive capacities (1). The first detectable evidence of invasion is the degradation of the BM that separates the epithelium from the stroma (2). The region enclosed by the black dotted box is shown in the inset, which shows an electron micrograph of a vertical cross-section through an MDA-MB-231 (breast adenocarcinoma) cell (pseudocoloured in pink) invading a thick layer of Matrigel (indicated), the composition of which resembles BM [preparation for transmission electron microscopy was as described previously (Lizarraga et al., 2009)]. Invadopodium-like processes form at the ventral surface of the cell and invade within the matrix. The arrow points to a region of matrix degradation. After breaching the BM, tumour cells invade the underlying interstitial tissue, which consists mainly of type-I collagen. Inside the collagen meshwork, individual cells often use a mesenchymal mode of invasion, which is characterized by adhesion-based extension of long lamellipodial protrusions in the direction of migration (3). Pericellular proteolysis mediated by MT1-MMP allows tumour cells to remodel the matrix, supporting invasive migration through the 3D fibrillar collagen network. Proteolytic tracks that are left behind invasive cells might support the migration of other cells (4). The region enclosed by the white dotted box is shown in B and C, which show scanning electron micrographs of MDA-MB-231 cells invading through type-I collagen fibres. Cells were plated for 6 hours on 3 mg/ml acid-extracted type-I collagen and prepared for scanning electron microscopy as described (Lizarraga et al., 2009). Cells were pseudocoloured in red and matrices in blue using Adobe Photoshop. The anterior part of the long lamellipodial extension is covered by numerous small finger-like protrusions that might correspond to sites of pericellular proteolysis and matrix remodelling, as recently described (Wolf et al., 2007). Cancer cells can also migrate collectively as a multicellular sheet (5). Multicellular invasion requires the maintenance of cell-cell contacts and might involve MMP-mediated remodelling of the matrix. Tumour cells might also migrate individually through amoeboid motion, which is driven by RhoA- ROCK- and myosin-II-dependent contractility; this allows cells to squeeze between gaps in the 3D matrix independently of matrix proteolysis (6). Scale bars: 1 μm (inset in A; C); 10 μm (B).

Widely used cancer cell lines including HT-1080 fibrosarcoma cells, MDA-MB-231 mammary adenocarcinoma cells and MV3 melanoma cells display a predominantly mesenchymal mode of migration in 3D collagen matrices (Fig. 2). Mesenchymal migration requires matrix degradation mediated by MT1-MMP and depends on the formation of adherent β1-integrin- and Rac1-dependent lamellipodial protrusions that drive directional motility (Sanz-Moreno et al., 2008; Wolf et al., 2003; Wolf et al., 2007). MT1-MMP and β1 integrin co-cluster at sites of interaction with collagen fibres along the leading edge, where both cell traction and matrix degradation take place (Wolf et al., 2003; Wolf et al., 2007). Interestingly, it was recently argued that pericellular collagenolytic activity is restricted to a posterior region of the leading pseudopodium, segregating adhesion and matrix degradation and thereby enabling adhesive interactions with the ECM to drive efficient 3D migration (Wolf et al., 2007). However, another recent study, in which a novel collagen-cleavage-specific probe was used in live cells, reported that protease activity is localized to the leading edge of tumour cells (Packard et al., 2009). Furthermore, degradation and reorganization of collagen fibres during mesenchymal invasion leads to the formation of micro-tracks, which can be used and expanded by other cells (Fig. 2) (Friedl et al., 1997; Wolf et al., 2003; Wolf et al., 2007). Along these lines, it is known that cancer cells adopt different strategies to invade interstitial collagenous tissue, from single-cell to clustered-cell modes of migration (Fig. 2) (Friedl and Wolf, 2003).

Surprisingly, given the above findings, pharmacological inhibition of MMPs or of cysteine, serine and aspartic proteases does not affect the efficiency of migration of HT-1080 or MDA-MB-231 cells through reconstituted type-I collagen matrices.

Instead, cells switch from a mesenchymal to a degradation-independent amoeboid mode of motility (Wolf et al., 2003). Amoeboid migration (Fig. 2), which is named in reference to the particular motion of the slime mould *Dictyostelium discoideum*, is characterized by rounded cell morphology and enhanced RhoA-, ROCK- and myosin-II-dependent contractility, which allows cells to squeeze between gaps in the 3D matrix (Wilkinson et al., 2005; Wolf et al., 2003; Wyckoff et al., 2006). Interconversion between mesenchymal migration and RhoA- and ROCK-dependent migration that is similar to amoeboid movement has also been reported for tumour cells invading through 3D Matrigel (Sahai, 2005; Sahai and Marshall, 2003).

It has been proposed that conversion between migratory modes confers plasticity to cancer cells and the possibility of escape upon pharmacological inhibition of proteases, yet other parameters that influence the mode of migration should also be considered, including the degree of cross-linking of matrix components within native ECMs as opposed to reconstituted matrices (Rowe and Weiss, 2008; Sabeh et al., 2009). Indeed, migration through native, highly cross-linked fibrin or type-I-collagen matrices strictly depends on proteolysis of the matrix by MT1-MMP (Chun et al., 2004; Even-Ram and Yamada, 2005; Filippov et al., 2005; Hiraoka et al., 1998; Hotary et al., 2000; Li et al., 2008; Packard et al., 2009; Rowe and Weiss, 2008; Sabeh et al., 2004; Sabeh et al., 2009; Sodek et al., 2008). By contrast, poorly cross-linked reconstituted networks formed with pepsin-extracted (as opposed to acid-extracted) type-I collagen (or Matrigel) seem to be more permissive to amoeboid motion in the absence of protease activity (Wolf et al., 2003; Wolf et al., 2007). In addition, although it has been established that interstitial tissue invasion can occur under certain conditions by protease-independent mechanisms, it is also clear that degradation of BMs by pericellular MMP activities is a prerequisite for invasion and metastasis (see above).

Stromal cells, including fibroblasts and tumour-associated macrophages, are also emerging as essential matrix-remodelling cells during tumour-cell invasion. Interestingly, fibroblasts can support collective invasion by squamous cell carcinoma cells that are otherwise unable to invade (Gaggioli et al., 2007; Zhang et al., 2006). Fibroblast-expressed MT1-MMP is essential for this process (Zhang et al., 2006). Along the same lines, metastasis of MT1-MMP-negative mammary tumours in a genetically induced cancer model is reduced in a MT1-MMP-deficient genetic background in comparison with normal recipient mice, arguing that MT1-MMP-mediated ECM degradation by stromal cells promotes the metastatic development of cancer cells (Szabova et al., 2008).

Regulation of MT1-MMP intracellular trafficking

As discussed above, several converging studies establish that MT1-MMP comprises an essential arm of the tissue-invasive programme of tumour cells (Hotary et al., 2006; Li et al., 2008; Sabeh et al., 2004; Sodek et al., 2007; Wolf et al., 2007). Furthermore, MT1-MMP accumulates at invadopodia, where it is required for focal pericellular degradation of the ECM (Artym et al., 2006; Clark and Weaver, 2008; Nakahara et al., 1997; Sakurai-Yageta et al., 2008; Steffen et al., 2008).

As a multifunctional protease, MT1-MMP is subjected to various regulatory mechanisms at the levels of gene transcription, intracellular trafficking and proteolytic activation. Deregulation of these mechanisms is implicated in pathogenesis of several human diseases such as diabetes, vascular and connective-tissue diseases, and cancer (Deryugina and Quigley, 2006; Filippov et al., 2005; Holmbeck et al., 1999; Savinov and Strongin, 2007). In this section, we review control mechanisms of MT1-MMP activity, with a special focus on the regulation of intracellular trafficking of MT1-MMP and its polarized targeting to invadopodial structures of invasive cells.

Endocytosis of MT1-MMP

As a way of controlling the proteolytic activity of MT1-MMP on the cell surface, MT1-MMP is efficiently internalized by clathrin-mediated and caveolar endocytosis and reaches early- and late-endosomal and lysosomal compartments, where degradation can occur (Jiang et al., 2001; Li et al., 2008; Remacle et al., 2003; Remacle et al., 2005; Takino et al., 2003; Uekita et al., 2001). Recycling of MT1-MMP from endosomes has been proposed as a means of regenerating the active enzyme at the surface (Itoh and Seiki, 2006; Li et al., 2008; Remacle et al., 2003).

Clathrin-mediated endocytosis of MT1-MMP

Internalization of MT1-MMP through the clathrin-mediated pathway requires the integrity of the 20-amino-acid MT1-MMP cytoplasmic domain, in particular a dileucine motif [Leu-Leu-Tyr573 (LLY573)] that functions as a high-affinity binding site for the AP-2–clathrin adaptor complex (Jiang et al., 2001; Lafleur et al., 2006; Li et al., 2008; Remacle et al., 2003; Uekita et al., 2001). Recent findings suggest that endocytosis of MT1-MMP can be linked to its activation or maturation status and is regulated in various ways, with consequences for pericellular collagenolysis. It has been reported that the internalization rate of proMT1-MMP by the clathrin-mediated pathway exceeds that of the mature enzyme, providing a mechanism for clearing inactive proMT1-MMP from the surface (Remacle et al., 2006). In addition, an inactive membrane-tethered 44-kD form of MT1-MMP that lacks the catalytic domain (through autocatalytic processing) interferes with endocytosis of the 55-kDa active enzyme (Cho et al., 2008; Toth et al., 2002). The mechanism behind this regulation is not known (but might involve competition between the two forms for the endocytic machinery), and might be important to preserve a basal level of functional protease at the cell surface. Several studies also show that TIMP-2, the major inhibitor of MT1-MMP, can be internalized in an MT1-MMP-dependent manner and that binding of TIMP-2 enhances uptake of MT1-MMP (Maquoi et al., 2000; Remacle et al., 2003; Wu et al., 2004; Zucker et al., 2004). It has been suggested that internalization of proteolytically inactive MT1-MMP–TIMP-2 complexes represents a mechanism to dissociate TIMP-2 from MT1-MMP within the endocytic pathway in order to regenerate an active protease (Li et al., 2008; Maquoi et al., 2000; Zucker et al., 2004).

It was recently reported that MT1-MMP can be phosphorylated on a tyrosine residue (Tyr573) of the cytoplasmic domain in a manner that is dependent on Src tyrosine kinase, and that this phosphorylation is required for tumour-cell proliferation and invasion of 3D collagen matrices and for tumour growth in nude mice (Nyalendo et al., 2008; Nyalendo et al., 2007). As noted above, the LLY573 sequence is required for clathrin-mediated uptake of MT1-MMP (Uekita et al., 2001), suggesting a possible regulation of endocytosis by phosphorylation downstream of Src. An additional regulatory role of Src in MT1-MMP function involves endophilin A2, a protein that generates membrane curvature during the formation of endocytic vesicles. It was found that phosphorylation of endophilin A2 by a focal adhesion kinase (FAK)-Src complex leads to reduced endocytosis of MT1-MMP

and increased matrix-degradation activity (Wu et al., 2005). Compared with its non-phosphorylated form, phosphorylated endophilin A2 demonstrates reduced association with the large GTPase dynamin 2 (Dyn2), which controls fission of endocytic membranes and caveolae and is involved in endocytosis of MT1-MMP (Jiang et al., 2001; Wu et al., 2005). Notably, Dyn2 localizes at invadopodia and is required for their formation and activity, although its role at invadopodia seems to be related to the regulation of actin and cortactin dynamics (see below) and membrane-protrusion formation, rather than the regulation of endocytosis (Baldassarre et al., 2003; Kruchten and McNiven, 2006). These findings led to the hypothesis that FAK, in complex with Src, inhibits endocytosis of MT1-MMP by negatively regulating the association of endophilin A2 and Dyn2 in tumour cells. Overexpression of FAK is found in many types of tumours, and correlates with invasiveness and enhanced metastasis (Cance et al., 2000). Thus, the finding that FAK can counteract MT1-MMP uptake might provide a molecular framework for its proinvasive activity. However, it should be emphasized that the localization of FAK at invadopodia remains controversial [the presence of FAK at invadopodia of invasive tumour cells and related podosome structures of Src-transformed fibroblasts has been reported (Alexander et al., 2008; Hauck et al., 2002; Wu et al., 2005), as has the absence of FAK at invadopodia (Bowden et al., 2006; Chan et al., 2009)].

The recent observation that type-I collagen can interfere with clathrin-mediated uptake of MT1-MMP is interesting, as this might represent a mechanism to increase the level of active protease at cell-matrix interaction sites (Lafleur et al., 2006). In addition, inhibition of MT1-MMP endocytosis was shown to be partially responsible for induction of activity of MMP-2 (which is activated by the MT1-MMP–TIMP2 complex; see above) by type-I collagen in tumour-derived cell lines (Lafleur et al., 2006). Similarly, activation of proMMP-2 by concanavalin A in HT-1080 cells might also involve inhibition of MT1-MMP endocytosis (Jiang et al., 2001). Inhibition of MT1-MMP endocytosis by type-I collagen requires the HPX domain of the protease (Lafleur et al., 2006); however, the underlying mechanism is not precisely known. Interestingly, the engagement and clustering of β1 integrins on endothelial cells by type-I collagen induces a physical interaction between MT1-MMP and β1 integrin, which correlates with an inhibition of MT1-MMP internalization (Galvez et al., 2002). However, in contrast to the situation in tumour cells, MT1-MMP proteolytic activity at the surface of endothelial cells growing on type-I collagen is reduced despite its increased surface expression; this points to some intrinsic difference between normal and tumour cells in the regulation of adhesion and migration by MT1-MMP (Galvez et al., 2002).

Caveolar endocytosis

Several reports document the association of MT1-MMP with caveolae and suggest that a caveolae- and dynamin-dependent internalization route is important for the control of MT1-MMP localization and surface expression (Annabi et al., 2001; Galvez et al., 2004; Remacle et al., 2003). Studies in endothelial cells and in tumour cell lines have shown that MT1-MMP and secreted MMPs localize to caveolar detergent-resistant membranes, and that caveolae are required for proper MT1-MMP localization and function in cell migration (Annabi et al., 2001; Galvez et al., 2004; Puyraimond et al., 2001). Overexpression of caveolin-1, the major component of caveolae, was found to reduce metastasis and invasion of a highly invasive mammary-derived carcinoma cell line. This effect was correlated with reduced expression of the gelatinases MMP-2 and MMP-9 in the external milieu, possibly linking the tumour-suppressor role of caveolin-1 with the regulation of MMP availability (Williams et al., 2004). The cytoplasmic domain of MT1-MMP has been shown to interact with tyrosine (Tyr14)-phosphorylated (Tyr14-*P*) caveolin-1, suggesting a possible mechanism for the caveolar association of MT1-MMP (Labrecque et al., 2004). Interestingly, Tyr14-*P* caveolin-1 is required for integrin-dependent internalization of lipid-raft signalling domains in a mechanism regulating cell adhesion and migration (del Pozo et al., 2005). Whether this (or a similar) mechanism operates during the regulation of MT1-MMP internalization by caveolae is unclear, but it is an attractive possibility.

On the basis of the data described here, the emerging picture is that basal surface expression of MT1-MMP is low in most cell types, because MT1-MMP is efficiently removed from the cell surface by the clathrin-mediated and caveolar endocytic routes (see Fig. 3A), and that several mechanisms exist and cooperate in invasive tumour cells to counteract clearance of the active protease from the cell surface. Moreover, accumulation of proteolytically active MT1-MMP can be visualized at invadopodia and invasive membrane protrusions of tumour cells both in two-dimensional (2D) and 3D matrices (Artym et al., 2006; Clark and Weaver, 2008; Nakahara et al., 1997; Sakurai-Yageta et al., 2008; Steffen et al., 2008; Wolf et al., 2003; Wolf et al., 2007). According to this view, invadopodia and any structures involved in pericellular degradation of the matrix can be considered as plasma-membrane domains that have a reduced endocytosis and increased exocytic rate for MT1-MMP (see below).

An exocytic machinery for focal delivery of MT1-MMP to invadopodia

The current view of localized matrix degradation at invadopodia is that activation of integrins or stimulation of cells by growth factors [e.g. epidermal growth factor (EGF)] leads to the assembly of a core invadopodial structure of F-actin and cytoskeletal proteins, including the N-WASP–Arp2/3 complex, Diaphanous-related formins and cortactin (Ayala et al., 2008; Bowden et al., 1999; Bowden et al., 2006; Clark et al., 2007; Lizarraga et al., 2009; Yamaguchi et al., 2005). Aggregation of F-actin and cortactin initiates the accumulation of MT1-MMP at nascent invadopodia, which can further mature and stabilize into proteolytically active structures (Artym et al., 2006). Recent observations also suggest that MT1-MMP itself contributes to the maturation and/or stabilization of invadopodia, because depletion of the protease or inhibition of its activity impairs F-actin and cortactin accumulation at the ventral surface, as well as reducing matrix degradation (Clark et al., 2007; Sakurai-Yageta et al., 2008; Steffen et al., 2008). Several questions arise from this model. First, what is the origin of MT1-MMP that accumulates at invadopodia? Next, what is the mechanism behind its targeted delivery? Finally, how do the cellular machineries involved in actin-driven membrane protrusion and exocytosis cooperate to deliver and concentrate MT1-MMP, integrins and other components at invadopodia? Below, we describe how the answers to these questions are currently conceptualized.

Origins of invadopodial MT1-MMP

A significant fraction of internalized MT1-MMP recycles back to the surface, suggesting that the active protease can be returned from

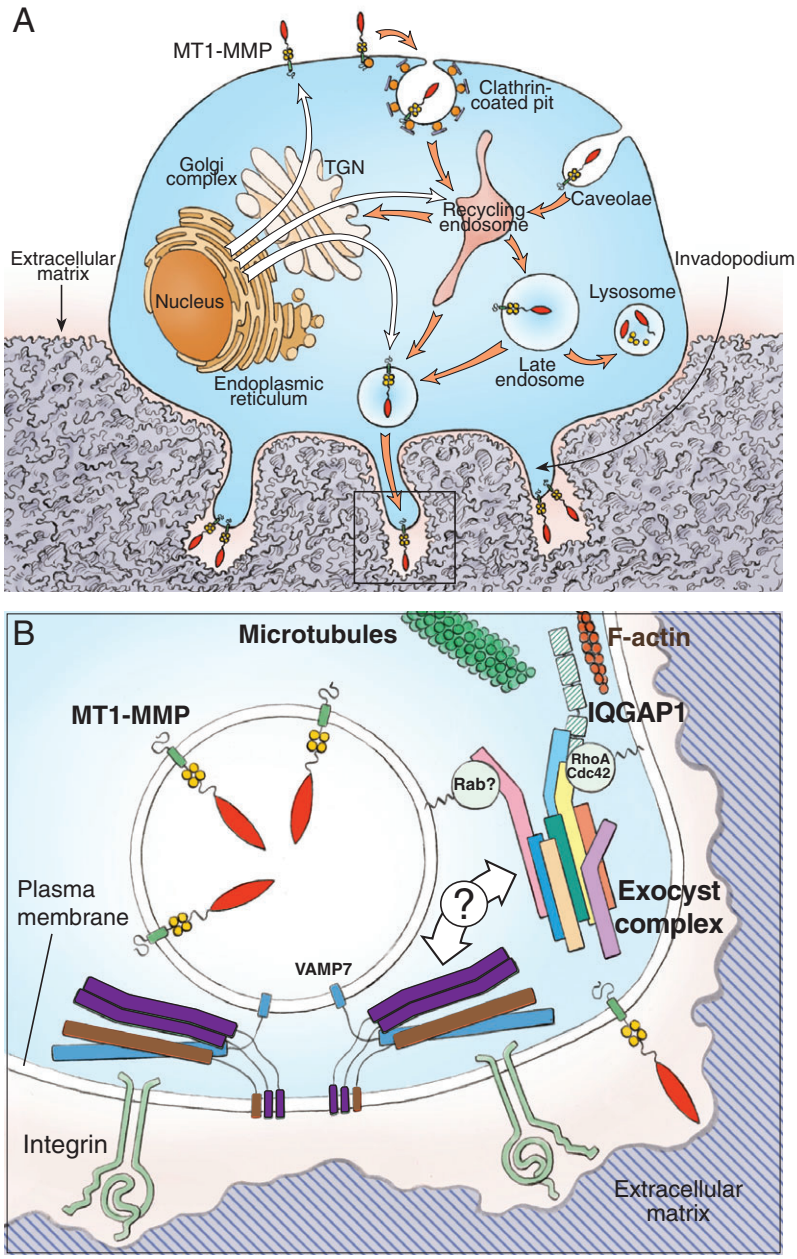

Fig. 3. A road map of MT1-MMP intracellular transport. (A) MT1-MMP is synthesized as a latent proenzyme and is transported through the biosynthetic pathway to the plasma membrane. Activation of MT1-MMP occurs in a post-Golgi compartment through proteolytic cleavage of the propeptide by furin (not shown). MT1-MMP is efficiently internalized from the plasma membrane by the clathrin-dependent and caveolar endocytic routes, and reaches early- and late-endosomal compartments. A fraction of the protease is degraded in late endosomes and lysosomes. Activation of integrin-mediated adhesion events at cell-matrix contact sites triggers the assembly of invadopodia, which are specialized actin-based membrane protrusions of invasive cells that have the capacity to degrade the matrix. Polarized targeting of MT1-MMP to invadopodia occurs through mobilization from different intracellular storage compartments in the biosynthetic and endocytic recycling pathways (see text for details). (B) The boxed area in A is shown. A specialized exocytic machinery controls polarized delivery of MT1-MMP at invadopodia. The cytoskeletal scaffolding protein IQGAP1, downstream of the small GTP-binding proteins Cdc42 and RhoA, has a central role in invadopodium function: it coordinates actin assembly and the exocytic machinery via the vesicle-docking exocyst complex, and might also act through microtubule plus-end anchoring. The proteins that mediate the recruitment of the eight-subunit exocyst complex to MT1-MMP transport vesicles are unknown (but might include a Rab GTPase). The v-SNARE protein VAMP7 has recently been found to regulate delivery of MT1-MMP to invadopodia, indicating that the SNARE machinery regulates the fusion of MT1-MMP-containing vesicles with the plasma membrane. Invadopodial plasma-membrane SNAREs and syntaxins have not been identified (represented in brown and dark purple). The degree of coordination between exocyst-dependent docking events and SNARE-mediated fusion is not known (see text for details).

endosomes to cell-matrix contact sites in order to make new invadopodia (Itoh and Seiki, 2006; Li et al., 2008; Remacle et al., 2003; Wang et al., 2004b). This mechanism would be similar to the ‘front-to-back’ model of endocytic recycling of integrins (in which integrins are endocytosed at the rear of the cell and returned to the leading edge), which has long been proposed as a mechanism to regenerate new attachments at the leading edge of migrating cells (Bretscher, 2008). Alternatively, or additionally, MT1-MMP might be mobilized from the secretory pathway, as was recently suggested by the discovery of a Rab8-dependent pathway for delivery of MT1-MMP from a storage compartment to collagen contact sites (Bravo-Cordero et al., 2007). Recent studies of polarized epithelial cells have revealed that recycling endosomes can operate as a way-station in biosynthetic protein transport from the Golgi to the PM (Ang et al., 2004; Lock and Stow, 2005). Interestingly, endocytosed MT-MMPs have also been found to recycle through the TGN, and this is dependent on the presence of a conserved C-terminal tripeptide motif (Asp-Lys-Val582 in MT1-MMP) in the cytoplasmic domain, which is similar to PDZ (postsynaptic density-95/Discs large/zona occludens-1)-domain-binding motifs (Wang et al., 2004a; Wang et al., 2004b). In the case of MT5-MMP, this tripeptide signal serves as a binding site for the PDZ domains of Mint-3 (Wang et al., 2004a), a protein that interacts with clathrin and small ADP-ribosylation factor (ARF)-family GTPases at the TGN and endosomal compartments, and functions as an ARF-dependent adaptor in protein trafficking (Hill et al., 2003). Altogether, these findings suggest that newly synthesized MT1-MMP that travels through the biosynthetic pathway and pre-existing MT1-MMP molecules that have been internalized from the cell surface intersect in a post-TGN or endosomal recycling compartment, from which delivery to invadopodia might occur (Fig. 3A).

Mechanism of MT1-MMP delivery to invadopodia: coordination between cytoskeletal reorganization and exocytosis?

Progress has recently been made in the identification of cellular components involved in the delivery of MT1-MMP to invadopodia. Key components include cortactin, IQGAP1, the exocyst complex and VAMP7, and these are discussed below.

Cortactin, which is a substrate of Src that has been implicated in cancer progression and metastasis (Buday and Downward, 2007), has an early and essential role during invadopodium formation (Artym et al., 2006; Ayala et al., 2008; Bowden et al., 1999; Bowden et al., 2006; Clark et al., 2007). Although it is not completely understood, the function of cortactin at invadopodia might involve its capacity to interact with actin filaments and activate Arp2/3-complex-mediated branching (Ayala et al., 2008; Weaver et al., 2001). Cortactin has an important role in membrane traffic, being a regulator of Arp2/3-mediated actin branching, a process that is required for the generation and/or fission of vesicles (Hehnly and Stamnes, 2007). A recent study has brought novel insights to the function of cortactin in invasion by showing that cortactin might regulate secretion of MT1-MMP and MMP-2 and/or MMP-9 at invadopodia (Clark and Weaver, 2008). Together, these results indicate that cortactin might coordinate MT1-MMP exocytosis and actin reorganization at invadopodia.

A further indication that cytoskeletal assembly and exocytosis are coordinated at invadopodia is provided by the observation that IQGAP1 [a key cell-polarity regulator that links microtubule and actin cytoskeleton networks (Brown and Sacks, 2006; Noritake et al., 2005)] and the exocyst vesicle-docking complex are required for invadopodium formation and activity (Sakurai-Yageta et al., 2008). The exocyst complex, which consists of eight subunits (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84), mediates the tethering of post-Golgi and endocytic recycling vesicles at the PM for exocytosis (Hsu et al., 2004; Prigent et al., 2003; Yeaman et al., 2001). We recently showed that, in MDA-MB-231 cells, IQGAP1 and the exocyst component Sec8 colocalize at invadopodia, and that a complex of IQGAP1 with the exocyst assembles upon activation of the Rho GTPases Cdc42 and RhoA, both of which are essential for invadopodium formation. RNA-interference (RNAi)-mediated knockdown of exocyst-complex subunits or IQGAP1 prevents focal delivery of MT1-MMP to invadopodia (Sakurai-Yageta et al., 2008). These findings suggest that the exocyst complex controls docking of MT1-MMP transport vesicles to the invadopodial PM, in a mechanism that requires association of the exocyst with invadopodium-enriched IQGAP1 (see Fig. 3B) (Sakurai-Yageta et al., 2008).

The finding that IQGAP1 is enriched at invadopodia and is required for invadopodial function in matrix degradation suggests a mechanism for the demonstrated role of IQGAP1 in the dissemination of invasive carcinoma cells in human tumours (Clark et al., 2000; Jadeski et al., 2008; Mataraza et al., 2003; Nabeshima et al., 2002). Another interesting aspect of the function of IQGAP1 is that it can capture microtubule plus-ends, via its association with plus-end-associated proteins such as CLIP-170 and APC (Fukata et al., 2002; Watanabe et al., 2004). Microtubules and microtubule motors are important for the formation and dynamics of podosomes in human macrophages (Kopp et al., 2006; Linder, 2009). Whether invadopodium formation and activity also involve microtubules (for instance, for directional trafficking of MMPs) is poorly understood (Kikuchi and Takahashi, 2008; Remacle et al., 2005; Schnaeker et al., 2004).

SNARES in MT1-MMP exocytosis

Exocytosis is dependent upon soluble N-ethylmaleimide-sensitive factor (NSF)-attachment protein receptors (SNAREs), which drive membrane fusion between transport vesicles and the PM (Jahn and Scheller, 2006). Syntaxin-4, a PM SNARE, is involved in the trafficking of MT1-MMP to the PM and in the invasiveness of human gastric cancer cells (Miyata et al., 2004). We recently showed that the vesicular SNARE Ti-VAMP (VAMP-7) colocalizes with MT1-MMP in late endosomes and in lysosomal structures, and at the PM at sites of matrix degradation. Upon RNAi-mediated knockdown of Ti-VAMP in MDA-MB-231 cells, significantly less MT1-MMP accumulated at the PM, the number of invadopodia decreased and invasion was impaired, suggesting a specific role of Ti-VAMP in targeting MT1-MMP to invadopodia (Steffen et al., 2008).

So far, it is unknown to what extent trafficking of MT1-MMP to late endosomes and lysosomes represents a degradative pathway for the protease (Remacle et al., 2003; Takino et al., 2003). Ti-VAMP, which is present on late-endosomal and lysosomal structures and on the TGN, contributes to various exocytic events (Proux-Gillardeaux et al., 2005), and thus might regulate delivery and exocytosis of MT1-MMP from these compartments (Fig. 3B). It remains to be determined which additional PM-target (t)-SNARE(s) are involved in Ti-VAMP-mediated exocytosis of MT1-MMP. In addition, there is genetic and biochemical evidence in yeast and mammals that exocyst-dependent vesicle docking and SNARE-mediated fusion machineries are linked (Aalto et al., 1993; Bao et al., 2008; Knop et al., 2005; Sivaram et al., 2005; Wiederkehr et al., 2004). Future studies should determine whether targeting of MT1-MMP to invadopodia also requires cooperation between exocyst and SNARE components (Fig. 3B).

Conclusions and perspectives

It is becoming clear that MT1-MMP, along with secreted MMPs and possibly other MT-MMPs, has an essential role in both the remodelling and breaching of BMs and in collagenolysis during tumour-cell invasion through interstitial tissues. However, our understanding of the mechanisms leading to degradation-dependent cell invasion remains limited. The observations that tumour cells assemble specialized degradative structures (invadopodia) when cultured on a 2D matrix and that MT1-MMP mediates focal degradation at invadopodia have provided a powerful and straightforward model for the understanding of pericellular matrix degradation. The picture that is emerging is that formation of invadopodia and subsequent matrix degradation in invasive tumour cells rely on the local coordination of cytoskeletal assembly and exocytosis events.

Several cellular components involved in cytoskeleton organization and in the regulation of MT1-MMP endocytosis and polarized targeting to invadopodia have been identified recently. More will become available in the near future, which should provide novel and potentially meaningful therapeutic targets. Among the possible candidates are members of the ARF-GTPase family, in particular ARF6 and some of its signalling partners such as the ARF6 guanine-nucleotide exchange factor GEP100, because they are overexpressed in highly invasive breast-cancer cells and contribute to the invasive phenotype of melanoma, glioma and breast-cancer cell lines and to metastasis in an in vivo mouse model (Hashimoto et al., 2004; Li et al., 2006; Morishige et al., 2008; Muralidharan-Chari et al., 2009; Sabe et al., 2006; Tague et al., 2004). ARF6 has been implicated in the regulation of invadopodial

dynamics and activity (Tague et al., 2004), and it might regulate MT1-MMP and protease trafficking through its effects on endocytic recycling and actin remodelling, because both of these processes impinge on the acquisition of migratory and invasive potential (D'Souza-Schorey and Chavrier, 2006). Other players are the related adaptor proteins Tks5 (Fish) and Tks4, which are linked to Src signalling and are required for podosome and invadopodium formation, ECM degradation and invasion in a variety of tumour cell lines (Buschman et al., 2009; Oikawa et al., 2008; Seals et al., 2005). Although their mechanism of action is not precisely known, these proteins [which comprise an N-terminal Phox homology (PX) domain and several (four or five) Src-homology 3 (SH3) domains] have clear roles during invadopodial actin assembly and in the recruitment of matrix proteases at invadopodia. Proteins involved in directional transport along microtubules, as well as those that regulate dynamics and anchoring of microtubules at the leading edge, might also turn out to be instrumental for polarized delivery of invadopodial components (Kopp et al., 2006; Remacle et al., 2005; Schnaeker et al., 2004).

Beyond completion of the molecular and cellular description of invadopodia, a challenging issue will be to monitor matrix remodelling and invadopodial dynamics in invasive cells in the tumour microenvironment. High spatial- and temporal-resolution imaging techniques are available that allow visualization of invadopodial components in action in living cells in 3D reconstituted matrices (Lizarraga et al., 2009; Wolf et al., 2007), and recent developments in intravital imaging methods make it possible to visualize invasive cells in situ (Kedrin et al., 2008; Sahai et al., 2005). Improvement of the spatial and temporal resolution of intravital microscopy will be necessary to visualize the dynamics of cellular components and invasive structures of tumour cells in their native environment.

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