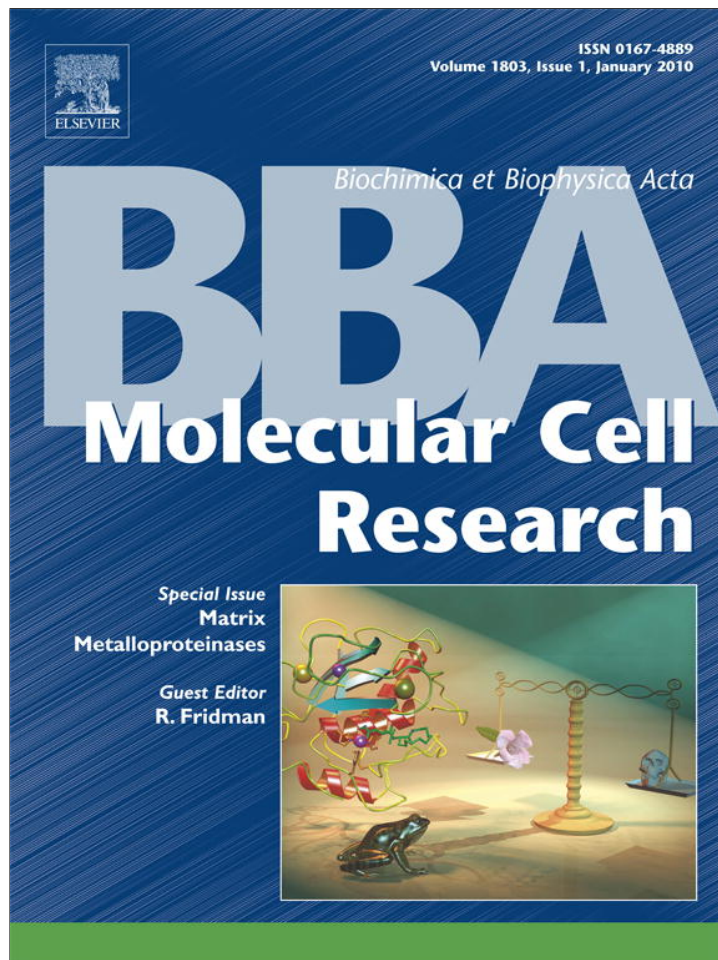


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Review

Emerging concepts in the regulation of membrane-type 1 matrix metalloproteinase activity[☆]Denis Gingras, Richard Béliveau^{*}

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ABSTRACT

Pericellular proteolysis mediated by membrane-type 1 matrix metalloproteinase (MT1-MMP) represents an essential component of the cellular machinery involved in the dissolution and penetration of ECM barriers by tumor cells. Although most studies on the proinvasive properties of MT1-MMP have focused on its unusually broad proteolytic activity towards several ECM components and cell surface receptors, recent evidence indicate that the cytoplasmic domain of the enzyme also actively participates in tumor cell invasion by regulating the cell surface localization of MT1-MMP as well as the activation of signal transduction cascades. The identification of the molecular events by which the intracellular domain of MT1-MMP links proteolysis of the surrounding matrix by the enzyme to modification of cell function may thus provide important new information on the mechanisms by which this enzyme controls the invasive behavior of neoplastic cells *in vivo*.

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1. Introduction

Matrix metalloproteinases (MMPs, EC 3.4.24) comprise a large family of proteolytic enzymes that play an essential function in cellular processes that require coordinate dissolution of the extracellular matrix (ECM), such as those involved in tumor invasion and angiogenesis [1,2]. Among the twenty-five members of this family that have been described to date, there is now considerable evidence that MMPs that are intrinsically associated with the plasma membrane, the so-called membrane-type MMPs (MT-MMP), represent key components involved in pericellular proteolysis and subsequent cell locomotion [3,4]. The prototypical member of this unique subclass of MMPs, MT1-MMP, possesses an overall domain structure similar to secreted MMPs i.e. with a zinc-containing catalytic domain, a flexible linker peptide and a hemopexin-like domain but, unlike its soluble counterparts, the enzyme is tethered to the cell surface by a transmembrane segment that terminates in a short, 20-amino acid cytoplasmic domain [3,5]. The simultaneous presence of an extracellular protease domain and of an intracellular juxtamembranous sequence within the structure of MT1-MMP may thus confer this enzyme with the unique ability to modify the surrounding extracellular matrix environment by proteolysis as well as to closely interact with the intracellular transduction machinery involved in the cell response to extracellular signals.

Several studies performed during the last decade have provided strong evidence that MT1-MMP plays an essential role in both physiological and pathophysiological processes. In mice, MT1-MMP deficiency resulted in craniofacial dysmorphism, arthritis, osteopenia, dwarfism, fibrosis of soft tissues and premature death [6,7], emphasizing the importance of MT1-MMP-mediated ECM degradation during development. The activity of MT1-MMP is however stringently regulated during subsequent maturation due to the existence of multiple levels of transcriptional and posttranscriptional regulatory mechanisms that control the expression, activity as well as the subcellular localization of the enzyme [8–10]. The importance of these regulatory mechanisms for the maintenance of normal cell function is well illustrated by several studies showing elevated expression of MT1-MMP in a wide variety of human cancers, including those of the lung, colon, liver, breast, brain, head and neck, ovary, and uterine cervix [11–13], reflecting the crucial role of MT1-MMP for efficient invasion of ECM barriers by tumor cells [14,15]. The understanding of the molecular and cellular mechanisms involved in the up-regulation of MT1-MMP expression and activity by cancer cells as well as of those by which this proteolytic activity is integrated in order to sustain their invasion and proliferation within surrounding ECM may thus provide important new insights for the development of novel anticancer therapeutics that control tumor progression through interference with MT1-MMP function.

Herein, we review emerging evidence indicating that in addition to the essential role of MT1-MMP-mediated pericellular proteolysis in the invasion of ECM by tumor cells, the cytoplasmic domain of the enzyme exerts an important influence on these properties by regulating its cell surface localization as well as the activation of intra-

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cellular signal transduction cascades. Interference with these intracellular pathways may thus represent an attractive alternative strategy to inhibit tumor progression triggered by this crucial enzyme.

2. Role of the cytoplasmic domain in the cell surface localization of MT1-MMP

2.1. Membrane anchoring of MT1-MMP is essential for its proinvasive properties

MT1-MMP displays an unusually broad substrate diversity for ECM proteins, hydrolyzing proteins as diverse as collagen types I, II and III, fibrin, laminins-1 and -5, fibronectin, vitronectin and aggrecan [16,17] as well as tumor cell surface-associated receptor molecules such as CD44 [18], tissue transglutaminase [19], the low density lipoprotein receptor-related protein (LRP) [20], α v integrin subunit [21] and the integrin-associated protein syndecan-1 [22] (for a review, see [23]). Thus, the expression of MT1-MMP at the cell surface creates a broad proteolytic repertoire in the pericellular space that results in the modification of a number of tumor cell functions, including migration [24–26], invasion of ECM barriers [14,27] as well as sustained proliferation in otherwise growth-restrictive three-dimensional (3D) type I collagen or fibrin matrices [28].

In spite of this large proteolytic repertoire and of the robust proteolytic activity of engineered soluble forms of the enzyme [16,17], several studies indicate that MT1-MMP-mediated proteolysis is not sufficient *per se* for efficient penetration of ECM barriers and that localization of the enzyme at the cell surface is essential to translate its proteolytic activity into a modification of cell function. For example, while overexpression of wild type MT1-MMP confers cells with the ability to invade collagen of fibrin gels, constitutive secretion of the enzyme following deletion of its transmembrane domain completely abolishes this process [27,29]. In a similar manner, whereas a plethora of studies indicate that membrane-tethered MT1-MMP induces migration of a variety of cell types [18,30–39], the soluble version of the enzyme failed to do so [32]. The importance of cell surface-associated MT1-MMP activity is also well illustrated by the observation that cells overexpressing MT1-MMP mutants in which the transmembrane domain is replaced with the GPI anchor of MT6-MMP retain the ability to invade 3D collagen gels [40] and to sustain cell proliferation within these matrices [41]. Unexpectedly, the expression of a MT1-MMP chimera in which the catalytic domain of the enzyme is replaced with that of an unrelated collagenase (MMP-1) also confers cells with significant collagen-invasive properties [40], indicating that the central function of MT1-MMP in tumor cell invasiveness is not solely due to its proteolytic activity but also strongly relies on its appropriate localization at the plasma membrane of migrating cells.

Although the above-mentioned studies may suggest at first sight that the participation of the transmembrane and cytoplasmic domains of MT1-MMP to the proinvasive properties of the enzyme is solely related to their role in the tethering of the protease to the cell surface, numerous studies indicate that these domains may play a more complex role in the regulation of the proteolytic activity of MT1-MMP. For example, a MT1-MMP chimera in which the expression of the enzyme at the cell surface is achieved by fusing its extracellular sequence to the transmembrane and cytoplasmic segments of the interleukin-2 receptor retains the ability to activate exogenous proMMP-2 [42] but showed impaired cell surface localization and degradation of the ECM [43]. The cytoplasmic domain of MT1-MMP seems to be of prominent importance in linking the enzyme proteolytic activity to efficient cell migration and invasion since several studies have shown that deletion of this domain has no inhibitory effect on the cell surface proteolytic activity of the enzyme but markedly reduces cell migration triggered by the enzyme [32,37,39,44]. As discussed below, this regulatory function of

the cytosolic sequence of MT1-MMP stems from the participation of this domain to processes governing the polarized localization of the enzyme at the cell surface.

2.2. Cytoplasmic domain-dependent endocytosis of MT1-MMP

The initial response of cells to migration-promoting agents is to polarize and extend membrane protrusions in the direction of migration [45]. These protrusions, such as lamellipodia or invadopodia, contain a diversified arsenal of proteins involved in cell adhesion, proteolytic degradation of the ECM as well as signal transduction that, collectively, confer cells with the ability to migrate and invade ECM barriers [46]. Interestingly, studies have shown that migrating cells relocalize MT1-MMP to lamellipodia and invadopodia [43,47–50] where the enzyme interacts with key adhesion receptors, such as CD44 [47], or with signal-transducing intermediates such as cortactin [51] or the adaptor protein p130Cas [52].

There is now considerable evidence that the redistribution of MT1-MMP to these sites of ECM degradation is highly complex and involves a dynamic interplay of endocytic [53–55] and exocytic [56,57] trafficking events. Following its synthesis and maturation in the trans-Golgi complex [58], MT1-MMP is rapidly translocated to the plasma membrane [59] through Rab8- [57], VAMP7- [56] and microtubule-dependent [50,53] processes, resulting in the polarized localization of the protease to invasive structures [56,57]. However, cell surface expression of MT1-MMP is usually very weak in most cell types due to the rapid endocytosis of the enzyme from the plasma membrane that results in its intracellular localization to early and late endocytic structures [53,55,59]. This dynamic regulation of cell surface expression and localization of MT1-MMP in migrating cells thus suggests that the spatiotemporal regulation of the recruitment of the protease to specialized domains involved in motility and invasion plays an essential role in its proinvasive properties.

There is mounting evidence that the cytoplasmic domain of MT1-MMP plays an important role in the regulation of its internalization and redistribution at the plasma membrane. Numerous studies have shown that overexpression of cytoplasmic tail-deleted forms of MT1-MMP results in higher expression levels of the enzyme at the cell surface, as reflected by enhanced activation of the MMP-2 zymogen [39,55], this behavior being related to a markedly reduced internalization rate compared to the wild type enzyme [40,54,55]. Importantly, defective endocytosis of MT1-MMP induced by C-terminal truncation impairs the localization to the leading edge of migrating cells [39,43,50]. The requirement of the cytoplasmic domain for efficient MT1-MMP endocytosis is related, at least in part, to an interaction of the ⁵⁷¹LLY⁵⁷³ sequence of this domain with the μ 2 subunit of adaptor protein 2, a key component of clathrin-coated vesicles [54]. Most interestingly, disruption of this interaction following site-directed mutagenesis of this structural region or through deletion of the entire cytoplasmic domain results in decreased cell migration, suggesting that regulated endocytosis of MT1-MMP is essential for efficient cell locomotion triggered by this enzyme [54]. Thus, although endocytosis has been traditionally considered as a mechanism that can achieve attenuation of the function of cell surface membrane proteins by committing the internalized proteins to degradation, regulated internalization of MT1-MMP appears to be of prominent importance for the spatiotemporal regulation of the enzyme's activity at the cell surface. Since exocytosis of the enzyme seems unaffected by the truncation of the cytosolic sequence [40], the control of cell surface expression and localization of MT1-MMP by its cytoplasmic domain occurs predominantly through regulation of endocytosis-mediated internalization of the enzyme.

Although endocytosis of MT1-MMP occurs primarily through clathrin-dependent mechanisms [53,55], a significant proportion of the enzyme is associated with caveolae [53,60–62] which are small,

flask-shaped microdomains of the plasma membrane that play important roles in signal transduction processes [63] as well as in the endocytosis of a variety of proteins [64]. A number of studies have suggested that cell surface relocalization of the enzyme mediated by caveolae-dependent mechanisms may contribute to the regulation of its promigratory properties [53,65]. However, transient and regulated association of MT1-MMP with these membrane domains seems to be important for appropriate invasion mediated by the enzyme since overexpression of the cytoplasmic tail-deleted form of MT1-MMP resulted in an increased association of this mutant with caveolae that correlates with impaired internalization and reduced cell migration *in vitro* and tumor growth *in vivo* [66]. Such an inhibitory function of caveolae in the regulation of MT1-MMP function is also suggested by the predominant localization of the 44-kDa proteolytically inactive form of the enzyme in these domains [53,60]. Interestingly, recent studies suggest that this 44-kDa form may regulate the endocytosis rate of the active enzyme, possibly by competing for components of the endocytic machinery [67]. It is thus plausible that the cytoplasmic domain-dependent interaction of MT1-MMP with other caveolae-resident protein would be required to allow appropriate internalization of the enzyme. In this respect, it is noteworthy that endocytosis via caveolae is critically dependent on stimulation of tyrosine kinase signaling, including Src-induced phosphorylation of caveolin-1 at Tyr14 [68]. Since MT1-MMP specifically interacts with tyrosine phosphorylated caveolin-1 in Src-expressing cells (see below) [62], this interaction could thus participate in regulated caveolae-mediated internalization of the enzyme.

3. MT1-MMP as a signal-transducing metalloproteinase

3.1. Activation of signal transduction cascades by MT1-MMP

There is increasing evidence that the participation of the cytoplasmic sequence of MT1-MMP in the proinvasive properties of the enzyme may also involve a role of this domain in the activation of a number of signal transduction events. Insights for such a role were initially provided by studies showing that overexpression of MT1-MMP induced the activation of the ERK cascade [32]. Pharmacological blockade of ERK activation was correlated with the inhibition of MT1-MMP-dependent cell migration induced by serum [32] or the chemoattractant sphingosine-1-phosphate [37,69], strongly suggesting the participation of this signaling cascade to cell locomotion triggered by the enzyme.

The adhesion of cells to the ECM appears important for this process since ERK activation was observed in cells plated on denatured type I collagen but abolished in cells grown in suspension or on poly-L-lysine-coated culture dishes [32]. In a similar manner, adhesion of HT1080 cells to type I collagen was found to induce MT1-MMP-dependent ERK activation, leading to subsequent cell migration [70]. This latter group also showed that lysis of the extracellular matrix by MT1-MMP induced focal adhesion turnover and subsequent ERK activation, which in turn stimulates cell migration [71]. This matrix requirement, as well as the well described activation of the ERK cascade in response to integrin-mediated cell adhesion [72], suggests a potential role of integrins in the regulation of MT1-MMP activity. This is supported by the colocalization of some integrins with the protease in migratory structures [48] and their regulation of MT1-MMP function [73,74], possibly via the activation of the MT1-MMP/integrin complex by Mss4 (mammalian suppressor of Sec4), a guanine nucleotide exchange factor specific for exocytic Rab GTPases [75].

The structural determinants of MT1-MMP responsible for its stimulatory effect on the ERK cascade remain to be firmly established. Initial experiments showed that ERK activation was abolished in cells overexpressing the catalytically-inactive E240A mutant or the cytoplasmic domain-deleted forms of the enzyme, suggesting the

participation of both domains in this process [32]. In MCF-7 cells, the addition of physiological concentrations of TIMP-2 resulted in a protease-independent MT1-MMP-dependent activation of the Ras-MEK-ERK signaling cascade that was strictly dependent on the presence of the cytoplasmic sequence of the enzyme [76]. Such a requirement of the intracellular domain is interesting since it would suggest that defective activation of ERK observed following deletion of this sequence could be related to alteration of the internalization of the enzyme, as described for several growth factor receptors [77]. In this respect, it is noteworthy that the structural region of MT1-MMP previously reported to participate in endocytosis (⁵⁷¹LLY⁵⁷³) overlaps with that involved in ERK activation ⁵⁷³YCQR⁵⁷⁶ [76].

The importance of the cytoplasmic domain for activation of the ERK cascade remains however unclear since other studies have reported that overexpression of cytoplasmic domain-deleted MT1-MMP activates ERK and sustains VSMC proliferation [78]. Under these conditions, stimulation of ERK phosphorylation was shown to involve activation of the PDGF receptor, a requirement that is related to MT1-MMP-mediated proteolysis of the multifunctional LDL receptor-related protein LRP1 and subsequent internalization of a PDGFR-β3-integrin-MT1-MMP-LRP1 complex [79]. Such a participation of receptor tyrosine kinases to MT1-MMP-mediated ERK activation is also suggested by studies showing that overexpression of MT1-MMP induces transactivation of the EGF receptor and that this process is essential to cell migration [80]. By contrast to the requirement for the PDGF receptor, however, MT1-MMP- and EGFR-dependent activation of ERK, as well as of cell migration, were found to require the cytoplasmic domain of the enzyme. The reasons for the differential requirement of proteolysis, cytoplasmic signaling as well as receptor tyrosine kinase transactivation for efficient ERK activation by MT1-MMP in different cell systems remain unclear but possibly reflect the complexity of this process and its sensitivity to the very distinct experimental conditions used in these cellular assays.

Although most studies performed so far on the signaling pathways that are activated downstream of MT1-MMP have focused on the ERK cascade, it should be noted that recent studies have suggested that the enzyme may have the ability to trigger the activation of additional signaling pathways. For example, stimulation of corneal fibroblast cell lines deficient for MT1-MMP with bFGF markedly inhibits the activation of the JNK and p38 MAP kinases as well as secretion of VEGF by these cells, implying a role of the enzyme in the modulation of the bFGF signaling pathway [81]. Given the ubiquitous role of ERK, p38 and JNK pathways in signal transduction events, the stimulation of these cascades by MT1-MMP strongly suggests that the enzyme actively participates in these processes.

3.2. MT1-MMP-interacting proteins

Since MT1-MMP lacks known recognition motifs for tyrosine kinases (R/K/Q)X₂₋₄(D/E)X₂₋₃pY or binding motifs for either Src homology(SH)2 (pYXX(I/L)) or SH3 (PXXP) domains that would facilitate recruitment of tyrosine kinases and signaling proteins, the mechanisms involved in the activation of signaling cascades by the enzyme remain obscure. However, in spite of this lack of signal-transducing domains, several studies indicate that MT1-MMP associates with a variety of membrane-associated and cytosolic proteins [47,48,52,62,78,82–86]. The best documented example of these interactions is the association of MT1-MMP with the hyaluronan receptor CD44 at migrating structures of tumor cells [47]. MT1-MMP physically interacts with this receptor through its hemopexin domain, leading to the cleavage of CD44 and enhanced cell migration [18]. Since CD44 interacts with filamentous actin through its cytoplasmic domain, a process that is mediated by ERM (ezrin/radixin/moesin) proteins, the binding of MT1-MMP to the receptor indirectly links the protease to the cytoskeleton and thus enables its focal localization to lamellipodia [47]. Recently, *in vitro* surface plasmon resonance analysis

revealed a direct interaction of a synthetic peptide corresponding to the MT1-MMP cytoplasmic domain with the N-terminal FERM domain of radixin [87], raising the possibility that the protease may also have the intrinsic ability to directly interact with the cytoskeleton.

Given the crucial role of the cytoplasmic domain of MT1-MMP in the regulation of its cell surface expression, the identification of proteins that specifically associate with this domain is of particular interest. In addition to the $\mu 2$ subunit of adaptor protein 2 [54] discussed above, pull down and two hybrid techniques subsequently showed that the intracellular domain of MT1-MMP interacts with gC1qR [82], the receptor for the C1 complex of the classical pathway of complement activation and MTCBP-1, a 19-kDa protein that possibly acts as a regulator of the methionine salvage pathway [83]. However, the multiple cellular functions performed by these proteins make their contribution to MT1-MMP-dependent cell migration and/or invasion still elusive. More recently, it was reported that in Src-overexpressing cells, MT1-MMP specifically associates with the tyrosine phosphorylated form of caveolin-1 [62], a 21-kDa membrane protein that acts as the predominant structural component of caveolae membrane domains. Caveolin-1 was first identified as a major tyrosine phosphorylated protein in v-Src transformed embryo fibroblasts [88] and has since emerged as an important scaffolding protein that interacts with multiple proteins involved in signal transduction, cell adhesion and migration as well as in cell survival [89]. Interestingly, the phosphorylated form of caveolin-1 is associated with integrins at focal adhesions [90], raising the possibility that phosphorylated caveolins contribute to cell attachment and migration [91]. Accordingly, phosphorylation of caveolin-1 leads to its polarized accumulation at the leading edge of migrating endothelial [92] as well as tumor cells [93]. Since the association of MT1-MMP with phosphorylated caveolin-1 was also observed following stimulation of endothelial cells with chemoattractants such as S1P or VEGF, this suggests that this molecular interaction may contribute to cell locomotion triggered by these angiogenic cytokines.

It is also noteworthy that MT1-MMP is among the few proteins identified to date that specifically interact with the tyrosine phosphorylated form of caveolin-1 and, by analogy to the involvement of these phosphocaveolin-binding proteins in signal transduction processes, it is tempting to speculate that this interaction may be important for the proinvasive properties of MT1-MMP. For example, interaction of phosphocaveolin-1 with the C-terminal Src kinase (Csk), a negative-regulator of Src family kinases, leads to its translocation into caveolae and may thus be involved in the inactivation of the caveolar forms of these enzymes [94]. Tyrosine phosphorylated caveolin-1 also specifically associates with growth factor receptor-bound protein 7 (Grb7) [95], an SH2-domain adaptor protein that links receptor tyrosine kinases to the Ras pathway [96] and that was reported to be involved in growth factor-induced cell migration [97]. Since MT1-MMP interacts with the phosphorylated but not with the native form of caveolin-1, it is possible that this specific association reflects a similar participation to intracellular signaling cascades underlying cell locomotion.

MT1-MMP lacks consensus caveolin-1 binding motifs (ψ X ψ XXXX ψ and ψ XXXX ψ XX ψ , where ψ is an aromatic amino acid Trp, Phe or Tyr) [98] in its cytoplasmic domain, suggesting that its association with phosphocaveolin is likely to involve additional proteins. In this respect, preliminary evidence from our laboratory indicate that upon overexpression of Src in COS-7 cells, the cytoskeletal associated protein paxillin associates with tyrosine phosphorylated caveolin-1 and that this complex could be involved in the recruitment of MT1-MMP (D. Gingras, E. Béliveau and R. Béliveau, unpublished results). Alanine scanning mutagenesis indicates that the formation of the MT1-MMP/phosphocaveolin-1 complex involves the cysteine residue as well as the C-terminal DKV motif located in the cytoplasmic domain of the enzyme, suggesting that these regions contain structural information that enable the interaction of MT1-MMP with these

intermediary proteins [62]. Interestingly, the DKV sequence present at the C-terminus of several proteins, including MT1-MMP, was previously identified as a ligand for the PDZ domain of neuronal nitric oxide synthase [99]. Since PDZ domains that recognize these (D/E)X ψ motifs (where ψ is an hydrophobic amino acid) have been shown to mediate several protein-protein interactions [100], this suggests that this region could be involved in the formation of molecular complexes containing MT1-MMP. This hypothesis is supported by the observation that the C-terminus of MT5-MMP, that also contain this binding motif (EWW), interacts with X11 γ /Mint3 [101], a PDZ domain-containing adaptor protein that mediates the assembly of functional multiprotein complexes [102], as well as with two PDZ-containing proteins of the postsynaptic density in neurons [103] and in both cases these interactions may participate to the trafficking of the enzyme [101,103].

It is also noteworthy that posttranslationally modification of juxtamembranous cysteine residues by palmitoylation have been proposed to trigger the specific association of transmembrane proteins lacking signaling motifs with proteins involved in signal transduction [104]. Since MT1-MMP was reported to be palmitoylated on Cys574 [105] and that mutation of this residue reduces the association of the protease with phosphocaveolin-1 [62], this hypothesis also warrants further investigation.

Overall, the recognition of MT1-MMP as a cell surface protease that associates with both cell surface ECM receptors [18,48], receptor tyrosine kinases [78] and various tetraspanins [84,85,106] as well as with intracellular signaling proteins such as phosphocaveolin-1 and p130Cas [52] lends credence to the hypothesis that the transmembrane topology of the enzyme imbues the protease with the unique ability to influence both the extracellular remodeling of the matrix surrounding tumor cells as well as intracellular signaling events involved in cell invasion.

3.3. Modulation of gene transcription by MT1-MMP

There is increasing evidence that cytoplasmic domain-dependent activation of signaling cascades by MT1-MMP may influence cell function through modulation of gene transcription. Reporter assays performed on cells overexpressing MT1-MMP showed that activation of the ERK cascade was correlated with increased transcription under the control of the serum response element (SRE) [32]. Similar to the activation of the ERK cascade, this stimulatory effect required the catalytic activity as well as the cytoplasmic domain of the enzyme, suggesting that activation of signal transduction pathways by the enzyme may lead to gene transcription.

Activation of the transcription program by MT1-MMP is also suggested by the marked increase in the production of VEGF by tumor cells overexpressing the enzyme [107–109], an up-regulation that is correlated with enhanced tumor growth rates in animals as well as elevated levels of angiogenesis [107,108]. Increased VEGF synthesis requires the catalytic activity as well as the cytoplasmic region of the enzyme since deletion of the six C-terminal amino acids of the cytoplasmic domain or mutation of Cys574 completely abolished MT1-MMP-dependent up-regulation of the VEGF gene [109]. Pharmacological blockade of tyrosine kinase activity suggests that the signaling pathway linking the cytoplasmic domain of MT1-MMP to VEGF gene transcription may involve Src activity, raising the interesting possibility that Src-dependent association of MT1-MMP with caveolin-1 [62] and/or phosphorylation of the unique tyrosine residue located in its cytoplasmic domain (see below) [110] may participate in activation of VEGF synthesis by the enzyme. Since endothelial cell-associated MT1-MMP also actively participate in angiogenesis [29,37,111–113] the transcriptional activation of VEGF by the enzyme in tumor cells suggests that the modulation of neovascularization by MT1-MMP represents a key mechanism underlying the crucial role of this enzyme in tumor progression.

In addition to VEGF, siRNA-mediated silencing of MT1-MMP expression suggests that the transcription of several additional genes is under the control of the enzyme. For example, ablation of MT1-MMP resulted in a marked down-regulation of Smad1 that was correlated with reduced tumor growth [114]. In a similar manner, silencing of MT1-MMP expression in human urothelial cell carcinoma cell lines resulted in a marked increase in the levels of the tumor suppressor Dickkopf-3 [115], suggesting that repression of this protein by MT1-MMP may participate in the invasive properties of these cells. Recently, a genome-wide expression profiling of MT1-MMP-overexpressing versus MT1-MMP-silenced cancer cells revealed the existence of a gene network whose expression is strongly associated with MT1-MMP levels [116]. These genes included regulators of energy metabolism, trafficking and membrane fusion, signaling and transcription, chromatin rearrangement, cell division, apoptosis and mRNA splicing [116]. The identification of the signaling pathways linking MT1-MMP activity to the transcription of these genes should provide crucial information on the mechanisms by which pericellular proteolysis by the enzyme modulates the invasive properties of tumor cells.

3.4. Tyrosine phosphorylation of the cytoplasmic domain of MT1-MMP

Additional insights for a role for the cytoplasmic domain of MT1-MMP in the regulation of the proinvasive properties of the enzyme may come from the observation that the unique tyrosine residue in the MT1-MMP's cytoplasmic sequence is phosphorylated in a Src-dependent manner and that this event likely plays an important role in cell migration [110]. Overexpression of a non phosphorylatable MT1-MMP (Y573F) mutant markedly reduced cell migration induced by serum or by S1P in cells that endogenously express the enzyme, suggesting that the mutant acts in a dominant-negative manner. Interestingly, upon stimulation of EC with S1P, MT1-MMP is relocalized to actin-rich membrane ruffles where it interacts with the tyrosine phosphorylated form of the adaptor protein p130Cas [52]. Although the participation of phosphorylation of MT1-MMP to this interaction remains to be firmly established, the large majority of tyrosine phosphorylated MT1-MMP was found to colocalize with p130Cas at cell protrusions under these migratory conditions, strongly suggesting a crucial role of phosphorylation of the intracellular domain of MT1-MMP in this process.

The importance of tyrosine phosphorylation of MT1-MMP is however not restricted to cell migration since fibrosarcoma cells stably transfected with the non phosphorylatable MT1-MMP point mutant showed markedly impaired proliferation activities within 3D but not on 2D type I collagen gels [117]. This inhibitory effect was not related to defective proteolytic activity of MT1-MMP since cells overexpressing the mutant showed slightly higher cell surface expression of the enzyme as well as an increased proteolytic activity towards proMMP-2 and fibrillar collagen [117]. This raises the interesting possibility that the proteolytic activity of MT1-MMP, although essential for the degradation of collagen barriers, may not be sufficient *per se* to sustain the proliferation of tumor cells within a 3D environment and that this process would also require signals originating from the phosphorylated intracellular domain of the enzyme. The reduction of cyclin D3 and CDK4 levels as well as the increase in p16^{INK4a} induced by the expression of the non phosphorylatable MT1-MMP mutant may suggest an involvement of the tyrosine phosphorylated form of the protease in cell cycle progression [117].

It should be noted however that these observations stand in contrast to several studies showing that overexpression of a cytoplasmic domain-deleted MT1-MMP mutant in otherwise invasion-incompetent cells induces proliferation and invasion activities similar to those achieved following expression of the wild type enzyme [15,27,28,40]. Although this discrepancy remains to be solved, it may be related to differences in the intrinsic invasion program of the

recipient cells used in these studies. For example, in cells that endogenously express MT1-MMP (such as fibrosarcoma cells), the presence of a non phosphorylatable dominant-negative MT1-MMP mutant impairs tyrosine phosphorylation of the endogenous MT1-MMP pool, inducing competition for the activation of the cell autonomous invasion program. This competition would lead to reduced cell cycle progression and proliferation, notwithstanding adequate proteolysis of the surrounding 3D matrix. By contrast, in invasion-incompetent cells, pericellular proteolysis induced by overexpression of the cytoplasmic domain-deleted mutant would be sufficient to release the growth constraints induced by the 3D environment and confer cells with a 3D growth advantage. Whether this cell type specificity may also reflect differences in the extent of tyrosine phosphorylation of MT1-MMP or its modification by other posttranslational modifications such as palmitoylation [105] of threonine phosphorylation [118] remains to be established.

4. Future directions

Merely 15 years following its identification as a cell surface receptor and activator of the proMMP-2 zymogen [5], MT1-MMP is now considered as one of the most important proteinases among the >180 metalloproteases encoded by the human genome [119]. However, in spite of the remarkable progresses made by several outstanding groups in our understanding of the enzymatic activity, substrate specificity and tissue-invasive properties of this crucial enzyme, a number of key questions remain to be solved in order to fully appreciate the complexity of the cellular mechanisms responsible for the spatiotemporal regulation of MT1-MMP-mediated pericellular proteolysis.

First and foremost, the extent to which the cytoplasmic domain of MT1-MMP contributes to the proinvasive properties of the enzyme must be clarified. As discussed throughout this review, this issue remains a matter for considerable debate and we believe that much of this discrepancy can be attributed to the very distinct cellular assays used to address this question. By analogy to previous studies on the role of tissue factor cytoplasmic domain in angiogenesis [120], the generation of mice from which the MT1-MMP cytoplasmic domain has been deleted would represent an invaluable tool to unequivocally resolve this issue. The availability of such an *in vivo* system would electrify the field and could lead to an in depth understanding of the crucial role of this enzyme in both physiological and pathological processes.

Second, since the internalization and subcellular localization of the enzyme is strongly dependent on its cytoplasmic sequence, it would be extremely interesting to determine how posttranslational modification of this domain by tyrosine phosphorylation influences this process. Given that this unique cytoplasmic tyrosine residue is conserved in all known transmembrane MT-MMPs (Fig. 1), reversible phosphorylation of this domain could constitute a central mechanism by which the regulation of the cell surface expression of these enzymes is achieved. In this respect, it is noteworthy that in Src-transformed cells, FAK-dependent phosphorylation of endophilin A2 inhibited endophilin/dynamin interactions, resulting in reduced endocytosis of MT1-MMP and increased ECM degradation [121].

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MT1-MMP  RRHGT PRRLLYC QRSLLDKV
MT2-MMP  QRKGA PRVLLYCKRSLQEWV
MT3-MMP  KRKGT PRHILYCKRSMQEWV
MT5-MMP  KNKTG P QPVTYYKRPVQEWV
          +RKG PR *LYCKRS*QEWV

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Fig. 1. Alignment of the cytoplasmic sequences of transmembrane MT-MMPs. "+" and "*" indicate basic and hydrophobic residues, respectively.

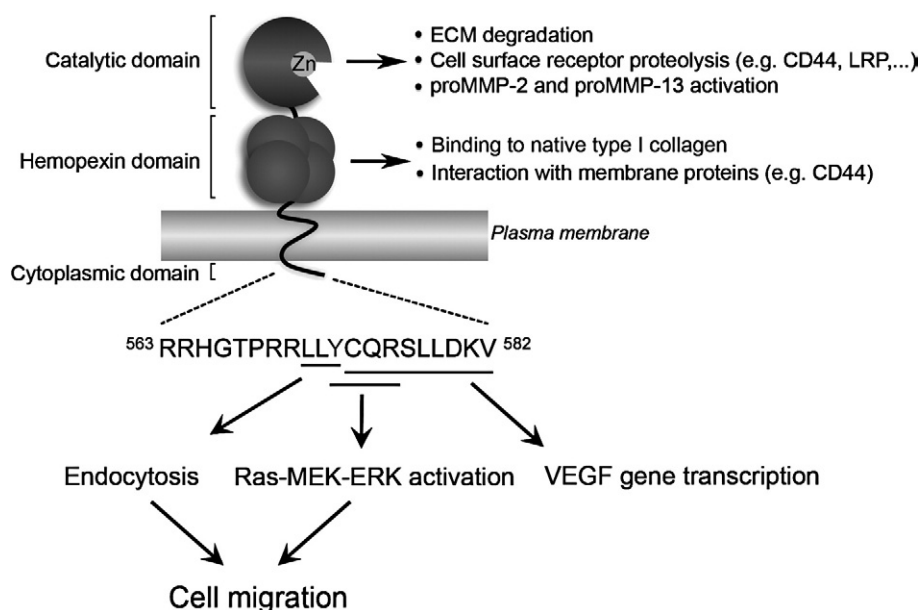


Fig. 2. Schematic representation of the structural domains of MT1-MMP and their involvement in pericellular proteolysis and cell migration. Underlined residues indicate structural regions of the cytoplasmic domain thought to be involved in endocytosis, ERK activation as well as VEGF gene transcription [54,76,109].

Since MT1-MMP is likely to be tyrosine phosphorylated under these conditions [110], this event may also contribute to the regulation of the enzyme internalization rate and to observed proinvasive properties of the Src-expressing cells.

Third, although the relationship existing between endocytosis of the activation of intracellular signaling events and gene transcription by the enzyme remains unclear, structural studies performed so far indicate a significant overlap in the regions of the cytoplasmic domain of MT1-MMP involved in these processes (Fig. 2). It is thus possible that these MT1-MMP-dependent intracellular events are intertwined and share a common dependence on appropriate internalization of the enzyme. Accordingly, trafficking membranes have been suggested to represent an efficient way to deliver messages to biologically relevant locations, an importance reflected by the inhibition of ERK activation by various inhibitors of endocytosis [122]. Elucidation of the relationship existing between the internalization of MT1-MMP, its activation of ERK cascade and its unconventional subcellular localization to centrosome [123] as well as to the cell nucleus [124] could thus provide crucial information on the mechanisms governing the activation of intracellular signaling events by the enzyme. Since MT1-MMP has been shown to be internalized by both clathrin-mediated and raft/caveolar-mediated endocytosis, it is possible that these two routes serve different purposes, as described for a number of plasma-membrane receptors [125]. The merging of modern trends in proteinase biochemistry, cell biology and signal transduction will be required to allow a better understanding of these processes.

5. Conclusions

Transmembrane proteins with at least one transmembrane α -helix segment account for >25% of proteins in almost every genome sequenced so far [126], reflecting the crucial role of these membrane proteins in the integration of chemical and physical signals originating from the extracellular milieu. This importance stems from the contact of these proteins with both the extracellular and intracellular milieus that provide a powerful mean to precisely sense and transduce signals necessary for adequate regulation of cell-to-cell communication, transport of ions across membranes and energy metabolism, among others. In this respect, experimental data available so far indicate that the transmembrane topology of MT1-MMP may confer this enzyme with the unique ability to profoundly influence the extracellular

environment through remodeling of the ECM and, in parallel, actively participate in the signaling events involved in the cell response to these modifications.

Owing to its ECM-degrading activity and important role in tumor progression, MT1-MMP represents an attractive drug target for anticancer therapies. However, broad-spectrum inhibitors of MMP activity described to date have been largely unsuccessful in clinical trials and, in some patients, also induced musculoskeletal side effects [127]. Although the exact mechanisms underlying these effects remain incompletely understood, they may involve an inhibition of MT1-MMP catalytic activity since mice lacking this enzyme present severe skeletal effects [127,128]. In this context, further studies aimed at the molecular characterization of the pathways by which the cytoplasmic domain of MT1-MMP contributes to the proinvasive properties of this enzyme could thus provide interesting new targets for the development of anticancer therapeutics.

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