Membrane associated matrix metalloproteinases in metastasis

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Summary

Hematogenous metastasis is postulated to involve tumor cell-initiated degradation of basement membrane barriers and underlying connective tissue matrices. Matrix metalloproteinases (MMP) are zinc-dependent endopeptidases that have been implicated in the proteolytic events of tumor cell invasion. Research has revealed a class of membrane-anchored metalloproteinases (MT-MMPs) and has provided convincing evidence that these enzymes activate latent MMP-2 (72 kDa gelatinase A) on the cell surface. The activation of plasma membrane associated MMP is a potential mechanism for facilitation of cellular metastasis and requires consideration when addressing potential roles of MMPs in tumor progression. This review focuses on potential in vivo regulatory mechanisms of membrane-associated MMP activity in the context of tumor cell interaction with matrix macromolecules. *BioEssays* 1999;21:940–949. © 1999 John Wiley & Sons, Inc.

Introduction

A hallmark of solid tumor progression to malignant neoplasia is the ability to invade and metastasize.[1] In both lymphatic and hematogenous metastases, cancer cells intravasate into the lymphatics, venules, or capillaries through enzymatic digestion of basement membrane in combination with mechanical pressures stemming from aberrant growth of the primary tumor mass. After surviving transport, hematogenously metastasizing tumor cells temporarily arrest and adhere to endothelial cells, then extravasate by infiltrating and invading the underlying denuded basement membrane. The capacity to form metastatic foci appears to correlate with the ability of a tumor cell to degrade basement membrane barriers and to proteolytically modify its target microenvironment to one that supports cell proliferation. The matrix metalloproteinases (MMPs) are among the principle classes of proteinases that facilitate these events.[2–6] In addition to the role of MMPs in metastasis, MMP-mediated matrix turnover is important to such biological processes as embryogenesis, wound healing, connective tissue remodeling, inflammatory responses, and in pathologies such as arthritis and cancer.

The MMP family is composed of at least 20 zinc-dependent extracellular endopeptidases whose activities are regulated predominantly by expression as inactive precursors, or zymogens.[7,8] MMP family members contain a HEXGH motif in the active site that chelates a catalytically essential zinc atom, and a PRCGVPD sequence in the pro-domain that maintains enzyme latency (Fig. 1). The currently accepted mechanism of pro-MMP activation is the "cysteine switch" model.[9] In this model, cleavage of the pro-domain destabilizes the inhibitory interaction between the unpaired cysteine in the PRCGVPD sequence and the active site zinc atom. Although the precise physiological activators of specific MMPs are unknown, the initial cleavage event can be carried out in vitro by other MMPs, as well as by a variety of serine proteinases in the extracellular milieu, including plasma kalikrein, trypsin, plasmin, and neutrophil elastase. An apparent exception to serine protease activation is pro-MMP-2 (72 kDa gelatinase A), which may not contain the necessary basic amino acid cleavage sites in its pro-domain. Following

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Abbreviations: MMP, matrix metalloproteinase; MT-MMP, membrane type-matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase.

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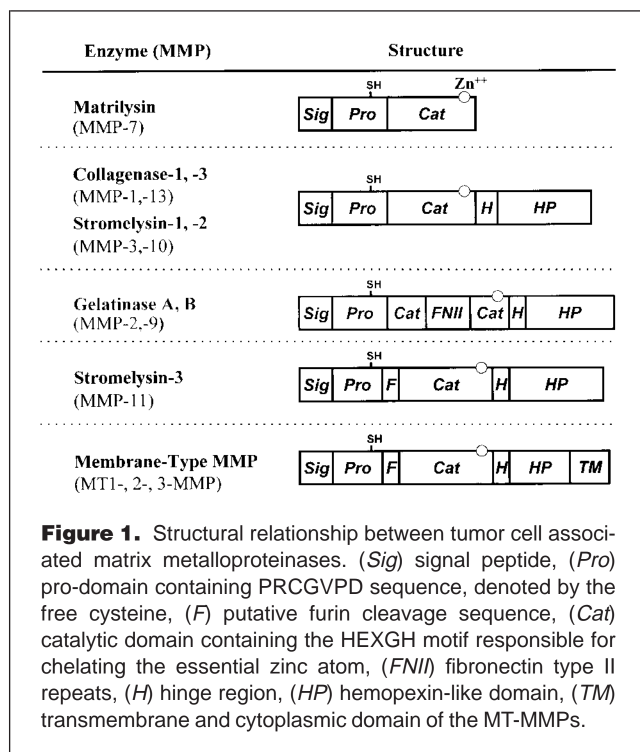

Figure 1. Structural relationship between tumor cell associated matrix metalloproteinases. (*Sig*) signal peptide, (*Pro*) pro-domain containing PRCGVPD sequence, denoted by the free cysteine, (*F*) putative furin cleavage sequence, (*Cat*) catalytic domain containing the HEXGH motif responsible for chelating the essential zinc atom, (*FNII*) fibronectin type II repeats, (*H*) hinge region, (*HP*) hemopexin-like domain, (*TM*) transmembrane and cytoplasmic domain of the MT-MMPs.

initial cleavage, autolysis of the remaining pro-domain ensues, resulting in a mature species that is approximately 10 kDa smaller than the latent enzyme. The presence of a putative furin or furin-like convertase recognition site (RXKR) downstream of the pro-domain in MMP-11 (stromelysin-3) and the MT-MMPs suggests that these enzymes can be activated by a unique cellular mechanism during post-translational processing.[10,11]

Once activated, MMPs can cleave a variety of extracellular matrix proteins (Table 1). MMP activity is inhibited in vivo through the formation of a tight, noncovalent complex with tissue inhibitors of metalloproteinases (TIMPs).[12,13] TIMP-1 (28 kDa) and TIMP-2 (21 kDa) are found in the soluble form, while TIMP-3 (24 kDa) is regarded as insoluble. TIMP-2 and TIMP-3 can inhibit all MMPs to varying degrees, while TIMP-1 is a poor inhibitor of MT1-MMP (MMP-14). A recently cloned TIMP family member, TIMP-4 (22 kDa), has been reported to inhibit MMP-1, -2, -3, -7, and -9.[14] Expression studies demonstrate that TIMPs exert a suppressive function in tumorigenesis. For example, recombinant TIMP-1 reduces extravasation and invasion in mouse models and expression of antisense TIMP-1 mRNA confers invasive, tumorigenic properties to normally noninvasive cells.[15–17] Overexpression of TIMP-2 through transfection downregulates local invasion and reduces tumor cell growth and neoangiogenesis in vivo.[18,19] This work provides strong support for a role of MMPs in tumor cell progression. It remains unclear whether TIMP represses invasion and metastasis solely through MMP inhibition, as TIMPs have also been reported to affect cellular apoptosis, proliferation, and differentiation.[20–22]

TABLE 1. Selected Tumor-Associated Matrix Metalloproteinases and Their Potential In Vivo Substrates*

Enzyme (MMP)	Size (latent/active)	Reported substrates[a]
Matrilysin (MMP-7)	28,000/19,000	Collagen IV, gelatin, elastin, fibronectin, laminin, pro-MMP-1, -2, and -9
Collagenase-1 (MMP-1)	55,000/45,000	Collagens, gelatin, aggrecan, pro-MMP-2 and -9
Collagenase-3 (MMP-13)	60,000/48,000	Collagens I, II, III, and IV, gelatin
Stromelysin-1 (MMP-3)	57,000/45,000	Collagens III and IV, gelatin, proteoglycans, fibronectin, pro-MMP-1, -7, -9, and -13
Stromelysin-2 (MMP-10)	57,000/44,000	Collagens III, IV, V, gelatin, fibronectin, pro-MMP-1
Stromelysin-3 (MMP-11)	51,000/44,000	Laminin, fibronectin (weakly)
Gelatinase A (MMP-2)	72,000/66,000	Collagens, gelatin, elastin, fibronectin, laminin, pro-MMP-9, -13
Gelatinase B (MMP-9)	92,000/86,000	Collagens IV, V, and VII, gelatin, fibronectin
MT1-MMP (MMP-14)	66,000/60,000	Collagens I, II, and III, gelatin, elastin, fibronectin, vitronectin, proteoglycans, pro-MMP-2, -13
MT2-MMP (MMP-15)	72,000/?	Gelatin, fibronectin, tenascin, pro-MMP-2
MT3-MMP (MMP-16)	64,000/52,000	Pro-MMP-2, type III collagen, fibronectin

*Not an inclusive list.
[a]Compiled from References 3, 7, 8, 39–41, 52, and 79, and references therein.

MMPs can be categorized according to the presence of various structural domains that confer an array of different physiological properties on the enzymes (Fig. 1). For example, MMP-2 and MMP-9 (92 kDa gelatinase B) possess three fibronectin type II repeats that promote binding to gelatin and native type I collagen, while the MT-MMPs (MMP-14, -15, -16, and -17) contain a unique transmembrane domain that anchors the enzymes to the cell surface. With the exception of MMP-7 (matrilysin), all MMPs possess a carboxyl-terminal domain that has strong sequence homology to the hemopexin family of proteins.[23] In MMP-2, -9, and -13, the hemopexin domain provides a docking site for the carboxyl-terminal domain of a TIMP molecule, forming a zymogen/inhibitor complex. This interaction facilitates intermolecular

rearrangement, resulting in an inhibitory interaction between the TIMP amino-terminus and the enzyme catalytic domain.[24] Alternatively, it is hypothesized that the amino-terminus of TIMP in the zymogen/inhibitor complex may simultaneously inhibit a second MMP catalytic domain, forming a trimolecular complex (e.g., MMP-9/TIMP-1/MMP-1).

Matrix metalloproteinases and metastasis

The observation that extracellular matrix breakdown is vital to cellular invasion indicates that matrix-degrading proteinases are essential for tumor cell metastasis. The initial support for this hypothesis was the demonstration that metastatic competence of B16 melanoma cells correlated with type-IV collagenase activity.[25] Subsequent studies have revealed, with few exceptions, that enhanced or de novo production of MMPs correlates with advanced stages of many human cancers.[3,4] For example, MT1-MMP mRNA and protein were not present in normal human brain, but readily detectable in malignant astrocytoma.[26] Similarly, analysis of human brain tissues demonstrated activated MMP-2 in malignant astrocytomas and glioblastomas, while only the latent form of the enzyme was detected in low-grade gliomas and normal brain tissue. Parallel findings were reported in cervical cancer specimens, which exhibited high-level MT1-MMP expression in both invasive cervical carcinoma and lymph node metastases relative to noninvasive cervical lesions.[27] MT1-MMP expression functions as an independent prognostic factor in gastric cancer patients, as patients whose tumors contain MT1-MMP immunoreactivity have a worse prognosis than those with MT1-MMP-negative tumors.[28] In addition to evidence from biopsy specimens, a preliminary study has demonstrated that urinary levels of MMP-2 and MMP-9 are independent predictors of organ-confined cancer, suggesting that urinary MMP analysis may be of value in determining disease status.[29]

While such studies successfully link MMP expression with disease progression, the functional contributions of these enzymes to metastasis are not firmly established. As previously reported in many other disease models, unbalanced expression of MMPs and inhibitors may contribute to the invasive or metastatic phenotype. This is evident in renal cell carcinoma samples evaluated for expression of MMP-2, MMP-9, TIMP-1, and TIMP-2 by reverse transcriptase PCR. While the MMP:TIMP ratio in normal kidney was reported as 1, the ratio was increased to 2.4 in locally confined carcinoma and to 4.9 in advanced carcinoma, suggesting that the MMP:TIMP balance is predictive of disease aggressiveness.[30] The requirement for MMP *activity* in cancer metastasis is evident when considering the ability of both synthetic enzyme inhibitors (i.e., peptide hydroxamic acids and tetracyclines) and TIMPs to block the invasive properties of tumor cells and reduce experimental metastasis in animal models.[31] Numerous clinical trials using MMP inhibitors against a variety of human cancers are under way, but data on their clinical efficacy are still limited.[31]

The contribution of host tissue-generated MMPs and TIMPs to tumor cell invasion is often neglected in expression studies. For example, although MMP-2 immunolocalizes to the surface of colorectal tumor cells, in situ hybridization analysis reveals that the surrounding stromal tissue is responsible for its production.[32] Similarly, MT-MMP transcripts have been localized to stromal cells in close proximity to both lung and breast tumor cells.[33] These studies suggest cooperation between tumor and host for expression of matrix-degrading MMPs. This observation is supported by in vitro studies which show that cancer cell remodeling of a three-dimensional collagen lattice is dependent on cell surface association and activation of MMP-2, while the amount of soluble activated enzyme in the matrix is negligible.[34] Moreover, membrane association of MMP-2 has been correlated with metastatic potential and increased catalytic efficiency of the metalloproteinase.[35–38] In such cases, tumor cell invasion may be strongly enhanced by the ability of cancer cells to bind host-produced enzymes and direct their activity towards creating a pericellular environment that supports effective migration or cellular proliferation.

The cloning and characterization of MT-MMPs that bind and activate pro-MMP-2 at the membrane surface has revealed a novel mechanism for tumor cell utilization of pericellular MMP.[11,39,40] Furthermore, MT-MMPs exhibit broad proteolytic capacities, including activity against collagens, fibronectin, and proteoglycans.[41] The ability to cleave matrix directly as well as produce MMP-2 activity at the cell surface suggests that MT-MMPs can facilitate invasion by promoting cleavage of matrix protein barriers proximal to infiltrating cellular membrane extensions. Thus, a detailed understanding of the regulation of cell membrane associated MMP activity may further elucidate the complex role of MMPs in cellular metastasis.

MMP-2 cell surface binding and activation

Matrix metalloproteinase localization to the cell surface was initially demonstrated through the purification of gelatinolytic activity from plasma membrane preparations of cancer cells.[42] Subsequent reports revealed that activation of pro-MMP-2 by concanavalin A-stimulated fibroblasts is inhibited by TIMP-2 and requires the plasma membrane fraction, indicating the existence of an uncharacterized cell surface MMP(s).[43] MT-MMPs have since been cloned and identified as the predicted TIMP-2 inhibitable cell surface enzymes.[11,39,40] Of the MT-MMP family members, the role of MT1-MMP in tumor cell progression has been the most extensively characterized.

The detailed molecular mechanism of MT1-MMP-mediated activation of pro-MMP-2 is not fully understood. Cross-

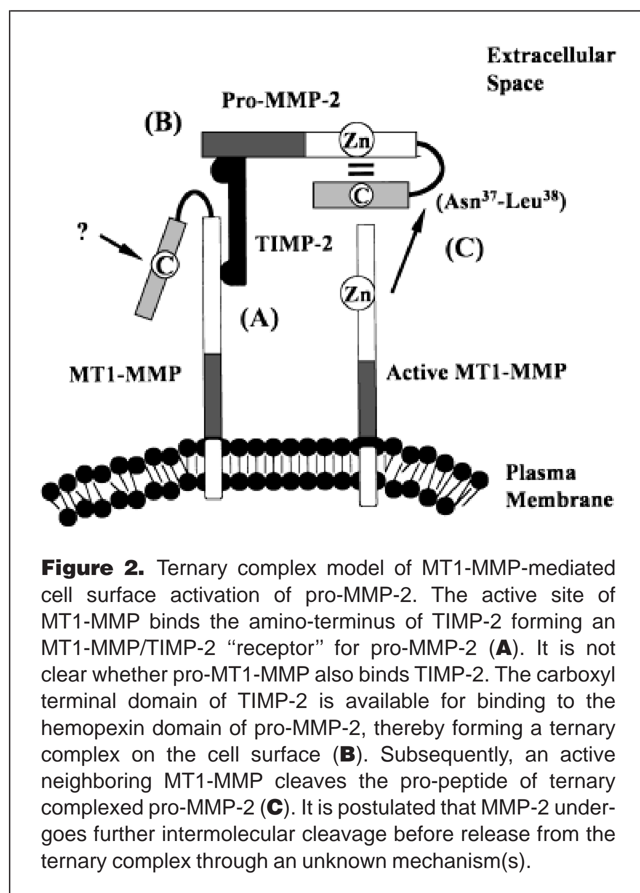

Figure 2. Ternary complex model of MT1-MMP-mediated cell surface activation of pro-MMP-2. The active site of MT1-MMP binds the amino-terminus of TIMP-2 forming an MT1-MMP/TIMP-2 "receptor" for pro-MMP-2 (**A**). It is not clear whether pro-MT1-MMP also binds TIMP-2. The carboxyl terminal domain of TIMP-2 is available for binding to the hemopexin domain of pro-MMP-2, thereby forming a ternary complex on the cell surface (**B**). Subsequently, an active neighboring MT1-MMP cleaves the pro-peptide of ternary complexed pro-MMP-2 (**C**). It is postulated that MMP-2 undergoes further intermolecular cleavage before release from the ternary complex through an unknown mechanism(s).

linking experiments, domain deletion analysis, utilization of inhibitors (i.e., peptide hydroxamic acid compounds) that preclude TIMP-2/catalytic domain interactions, and the recent elucidation of the TIMP-2/MT1-MMP catalytic domain crystal structure have demonstrated that MMP-2 interacts with MT1-MMP through a mutual TIMP-2 binding partner (Fig. 2).[43–47] This trimolecular complex is believed to form through binding of the TIMP-2 amino-terminus to the MT1-MMP catalytic domain (K_i in the subnanomolar range) (Fig. 2A), while the carboxyl-terminus of the inhibitor interacts with the pro-MMP-2 hemopexin domain (apparent K_d 6.6×10^{-8} M) (Fig. 2B).[48] The observation that pro-MMP-2, but not preformed pro-MMP-2/TIMP-2 complexes, readily interacts with and is activated by membrane preparations suggests that the macromolecular ordering of trimer formation involves TIMP-2 binding to MT1-MMP prior to association with pro-MMP-2.[44] Pericellular TIMP-2 concentration is critical for MT1-MMP-mediated activation, such that insufficient TIMP-2 results in failure to localize pro-MMP-2 to the cell surface, while excess inhibitor reduces the population of free MT1-MMP needed to activate pro-MMP-2.[45,49] TIMP-1 does not bind MT1-MMP, and the possible roles of TIMP-3 and TIMP-4 in this system have not been reported. TIMP-4 binds to the MMP-2 carboxyl terminus with affinity similar to that of TIMP-2 (apparent K_d 1.7×10^{-7} M),[48] and may thus influence trimolecular complex formation in an as-yet undescribed manner.

Once the ternary complex is formed, a neighboring MT1-MMP molecule may cleave the anchored pro-MMP-2 at Asn^{37}-Leu^{38} in the pro-domain (Fig. 2C). Following the initial proteolysis, MMP-2 undergoes further intermolecular cleavage to generate the mature enzyme and is "released" from the cell surface.[50] The biochemical mechanism of MMP-2 release and whether it precedes or follows enzyme maturation is not clear. Further, the ability of the trimolecular complex to release active enzyme is perplexing when one considers the dissociation kinetics; binding affinities, however, may shift following pro-MMP-2 activation. Recent findings indicate that membrane-bound TIMP-2 is released with and inhibits MMP-2 activated on the surface of human cervical fibroblasts.[51] Yet membrane preparations treated with an hydroxamic acid inhibitor to remove TIMP-2 bound to MT1-MMP catalytic domains retained the pool of amino-terminally associated TIMP-2 (40–50% of total) responsible for the inhibition of released MMP-2. This observation suggests that either a TIMP-2 binding protein (distinct from MT1-MMP) exists on the cell surface or that TIMP-2 can associate with MT1-MMP in a hydroxamic acid inhibitor-insensitive fashion.[51] Nonetheless, this deters from a firm conclusion that MT1-MMP-associated TIMP-2 molecules are released to inhibit MMP-2 following its activation by MT1-MMP.

The soluble recombinant catalytic domain of MT1-MMP can also initiate activation of the interstitial collagenase pro-MMP-13 (collagenase-3) by cleaving the zymogen at Gly^{35}-Ile^{36}.[52] The mechanism of MT1-MMP mediated pro-MMP-13 activation remains to be fully addressed; but it is important to note that MMP-2 efficiently activates pro-MMP-13 as well.[52] A potential MMP-2/MMP-13 activation cascade at the cell surface may work synergistically to drive an efficient matrix hydrolytic response (Fig. 3).

It has been suggested that the hemopexin domain of MMP-2 binds $\alpha v\beta 3$ integrin on the surface of melanoma cells.[53] The precise role of an MMP-2/$\alpha v\beta 3$ integrin complex in the context of MT1-MMP-mediated binding and activation, however, has not been explored. Another potential mechanism of cell surface localization (indirect) is MMP-2 binding to $\alpha 2\beta 1$ integrin-associated collagen.[54] It was recently reported that pro-MMP-2 associated with integrin-bound collagen may act as a store of latent MMP-2 that can potentially feed into the MT1-MMP activation pathway upon release (Fig. 3). The mechanism(s) of pro-MMP-2 release from pericellular collagen is not known but the destabilization of collagen fibrils through collagenase activity may play a pivotal role.

Regulation of MT1-MMP activity

The presence of a putative pro-domain in the amino-terminus of MT1-MMP indicates that the enzyme is expressed as a zymogen and requires proteolytic activation. Pro-MT1-MMP,

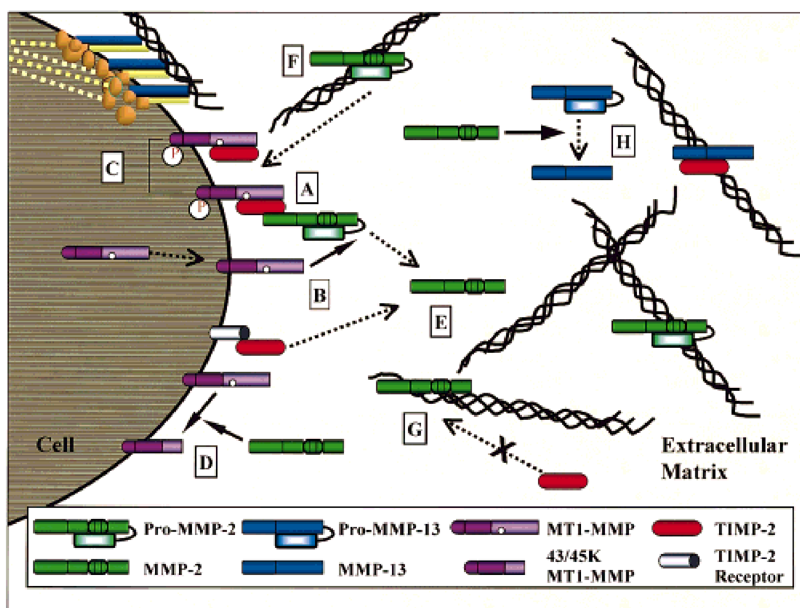

Figure 3. Regulation of cell surface MMP activity. Plasma membrane anchored MT1-MMP binds pericellular TIMP-2, forming a MT1-MMP/TIMP-2 "receptor" for pro-MMP-2 (**A**). It is unclear whether TIMP-2 receptors other than MT-MMP exist on the cell surface. An active proximal MT1-MMP cleaves the pro-domain of ternary complexed pro-MMP-2 (**B**). The cytoplasmic tail of MT1-MMP contains potential phosphorylation sites that may facilitate deposition of MT1-MMP to integrin rich invadopodia (**C**). The appearance of a 43/45 kDa specie of MT1-MMP correlates with pro-MMP-2 processing activity and has been demonstrated to be the product of MMP-mediated hydrolysis of an active MT1-MMP intermediate (**D**). Although the 43/45 kDa specie is predicted to be inactive, it appears to retain the invadopodia localization signals within the carboxyl domain and may, thus, negatively influence MT1-MMP activity by diluting the concentration of active enzyme. Following cleavage by MT1-MMP, MMP-2 undergoes further intermolecular cleavage to generate mature enzyme and is subsequently released from the cell surface (**E**) where it can be readily bound and inhibited by TIMP-2. Both latent and active MMP-2 bind pericellular collagen through the fibronectin type II repeats (**F**). Pro-MMP-2 bound to type I collagen may provide a pool of zymogen available for release and subsequent activation by MT1-MMP. Further, binding of active MMP-2 to type I collagen may influence the ability of TIMP-2 to bind and/or inhibit the enzyme (**G**). Both MT1-MMP and MMP-2 (**H**) can activate pro-MMP-13. Thus, an MMP activation cascade initiated at the cell surface may work synergistically to promote an efficient proteolytic response.

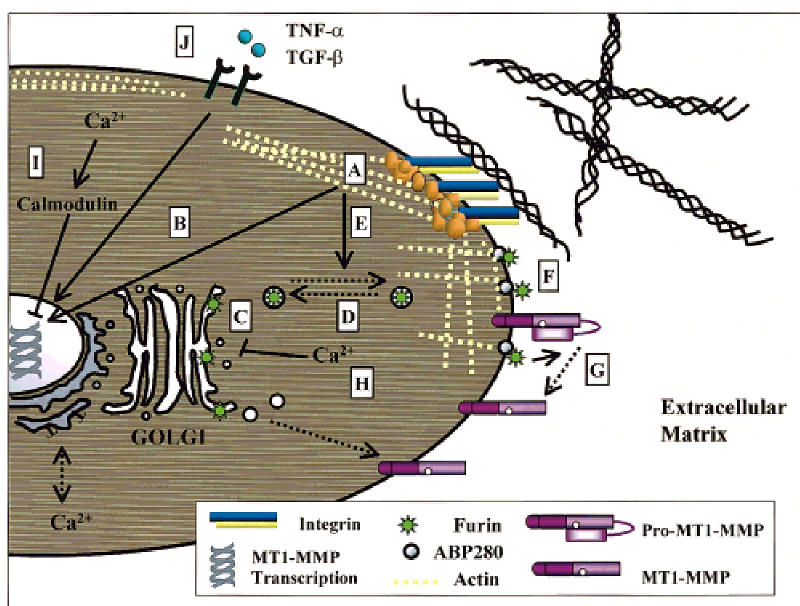

Figure 4. Cellular regulation of MT1-MMP expression and processing. β1 integrin clustering by type I collagen (**A**) stimulates MT1-MMP gene expression and/or stabilization of MT1-MMP mRNA (**B**). Spherical structures at the cytoplasmic face of integrins denote focal adhesion complex proteins. Furin is postulated to activate pro-MT1-MMP in the trans-Golgi network (**C**). Furin traffics between the trans-Golgi and plasma membrane through the late endosomes in a kinase/phosphatase-dependent fashion (**D**). Potential modulation of the furin cycling pathway by integrin signaling may indirectly influence intracellular processing of pro-MT1-MMP by disrupting furin trafficking (**E**). Tethering of furin to cortical actin through actin-binding protein 280 (ABP280) (**F**) could influence MT1-MMP activity by affecting furin return to the trans-Golgi or by effectively concentrating the low abundance convertase to cortical actin-rich membrane extensions. Furthermore, aggregating the convertase on the cell membrane (**G**) may increase the efficiency of pro-MT1-MMP activation. Experimental data using calcium ionophores (**H**) and calmodulin antagonists (**I**) suggests that calcium negatively influences MT1-MMP at multiple levels. While MT1-MMP mRNA expression appears refractory to treatment with growth factors such as bFGF and EGF, MT1-MMP mRNA expression can be upregulated in fibroblasts by TGF-β or TNF-α (**J**).

like pro-MMP-11 (stromelysin-3), contains a furin recognition motif (Arg^{108}-Arg^{109}-Lys^{110}-Arg^{111}) between the pro- and catalytic domains, and appears to be activated through furin or a furin-like enzyme. In support of this idea, furin has been demonstrated to activate pro-MMP-11.[10] Moreover, amino-terminal Tyr^{112}-MT1-MMP protein has been isolated from fibrosarcoma cell membranes, and generation of this form appears to be reduced upon treatment with a synthetic furin inhibitor.[55,56] Work with MT1-MMP-transfected COS-1 cells, however, suggests that furin processing may not be an absolute requirement for MT1-MMP-mediated activation of pro-MMP-2 and that the MT1-MMP pro-domain may play a functional role in this event.[57,58] Clearly, further research is necessary to address the requirement of furin processing in the context of both pro-MMP-2 activation and matrix degrading potential of the metalloproteinase.

The subcellular localization of furin is not static, as demonstrated by studies which demonstrate that furin normally cycles between the trans-Golgi and the cell surface in a kinase- and phosphatase-dependent fashion [59] (Fig. 4). Assuming that furin is the cellular activator of pro-MT1-MMP, zymogen activation may be regulated by furin trafficking events. It was recently demonstrated that actin-binding protein 280 (ABP280) tethers furin at the plasma membrane.[59] It is interesting to speculate that ABP280-mediated association of furin with the actin cytoskeletal network may serve to concentrate the furin protein for efficient pro-MT1-MMP processing. Although activation of pro-MT1-MMP by furin on the external membrane surface has not been demonstrated, furin, or a furin-like enzyme, is active on the plasma membrane.[60]

Activation of pro-MMP-2 has been correlated with the appearance of 60 kDa and 43/45 kDa processed forms of MT1-MMP on the surface of HT1080 fibrosarcoma cells.[61,62] The 60 kDa intermediate MT1-MMP specie has an amino-terminus of Tyr^{112}, consistent with furin activation, and is an active enzyme as determined by gelatinolytic activity.[55] The 43/45 kDa form is generated by MMP-dependent proteolysis of the 60 kDa intermediate and is predicted to be an inactive protein, suggesting that increased cell surface MMP activity may downregulate MT1-MMP activity through proteolysis (Fig. 3). It is difficult to determine whether the 43/45 kDa form is generated through MT1-MMP intermolecular cleavage or through the activity of another MMP, such as MMP-2. Furthermore, although the 43/45 kDa form is predicted to be catalytically inactive, it may have important biological properties, since it retains both the hemopexin domain and the cytoplasmic tail.

Recent evidence suggests that MT1-MMP is not randomly distributed on the cell surface. MT1-MMP localizes to invadopodia through the cytoplasmic tail of the enzyme, though the precise mechanism is unknown.[63] It is interesting to note that the MT1-MMP cytoplasmic tail contains potential phosphorylation sites (Thr^{567}, Tyr^{573}, and Ser^{577}) that may be involved in the recruitment of intracellular protein(s) and which could facilitate localization of MT1-MMP to specific cellular structures (Fig. 4). Furthermore, MT1-MMP colocalizes with clustered $\beta1$ integrin complexes, providing an additional mechanism for recruitment of the proteinase to cell-matrix contact sites (Ellerbroek and Stack, unpublished observation and L. Yan, Harvard University, personal communication). The accumulation of MT1-MMP at invadopodia or integrin complexes may promote pro-MMP-2 activation by facilitating the ability of a neighboring MT1-MMP to cleave pro-MMP-2 in a MT1-MMP/TIMP-2/pro-MMP-2 trimolecular complex. In addition, the recruitment of MT1-MMP to invadopodia or integrin complexes could potentially promote cell surface MMP activity by localizing MT1-MMP near pro-MMP-2 bound to putative cellular binding proteins such as integrin-associated collagen. Moreover, it is postulated that the 43/45 kDa form of MT1-MMP retains its ability to localize to invadopodia or focal complexes and thereby diminish subsequent pro-MMP-2 processing by diluting the concentration of the active, furin-processed MT1-MMP intermediate(s).

Although MT1-MMP activity is nominal under standard cell culture conditions, culturing a variety of cells in three-dimensional collagen gels stimulates pro-MMP-2 activation and pro-MT1-MMP expression.[64–67] Matrix regulation of MT1-MMP has been further illustrated through culturing HT1080 fibrosarcoma on fibronectin and GCT23 giant tumor cells on matrices containing the Arg-Gly-Asp integrin recognition motif.[62,68] It has previously been demonstrated that matrix-induced focal contact formation and integrin-mediated signaling may require integrin clustering.[69] Experiments addressing the mechanism of matrix regulation of pro-MMP-2 activation have established that neither occupation of integrins (with soluble peptide ligand) nor ligation of integrins (with soluble integrin subunit-specific antibodies) are sufficient to mimic the induction of pro-MMP-2 activation.[70,71] Additional clustering of integrins, however, leads to formation of active MT1-MMP, suggesting that focal contact-generated signaling may be involved in matrix stimulation of MT1-MMP and subsequent pro-MMP-2 activation.[62,72] The ability of integrin clustering events to enhance MT1-MMP activity raises the hypothesis that activation of a cell surface MMP proteolytic cascade by migratory tumor cells may be influenced not only by the type of matrix encountered, but its proteolytic status. For example, if a cell encounters an intact interstitial collagen barrier, collagen-binding integrins may cluster along the length of intact fibrils, thereby promoting an MT1-MMP-mediated proteolytic response (Fig. 4). If a cell encounters a degraded matrix, however, occupied integrins may not be sufficiently clustered, such that the fragmented matrix would fail to stimulate additional proteolysis. The ability of a tumor cell to regulate MT1-MMP activity in response to matrix status may be essential for effective invasion, since

uncontrolled extracellular matrix breakdown will likely decrease the efficiency of cellular motility.

Collagen gel stimulation of MT1-MMP-mediated pro-MMP-2 processing may involve mechanisms other than matrix signaling through integrin receptors. It has recently been reported that a type I collagen-associated protein, SPARC/osteonectin, can stimulate pro-MMP-2 activation by breast cancer cells grown on culture plastic and can enhance three-dimensional collagen induction of pro-MMP-2 activation.[73] The mechanism of SPARC stimulation of pro-MMP-2 activation appears to involve reduction of pericellular TIMP-2 protein levels, thereby increasing the population of free MT1-MMP on the cell surface.

In many tumor cell models, matrix stimulation of MT1-MMP consistently correlates with cell shape changes, prompting the hypothesis that MT1-MMP activity is influenced by cytoskeletal rearrangements. Indeed, treatment of fibroblasts with an actin-destabilizing agent such as cytochalasin D or mechanical stretching can stimulate MT-MMP activity.[74,75] Although mechanistically unclear, it is possible that actin rearrangements may stimulate MT1-MMP activity by influencing MT1-MMP or furin aggregation on the cell surface through ABP280.[59] Also, as integrin clustering can trigger cell shape changes, it is difficult to discern whether integrins stimulate MT1-MMP through focal contact-mediated signal transduction and/or nucleated actin cytoskeletal rearrangements that lead to cell shape changes.

Direct evidence of MT1-MMP transcriptional regulation is limited. Transformation of epithelial Madin-Darby canine kidney cells with p60(v-src) dramatically increases MT1-MMP mRNA expression.[76] Treatment of cultured cells with tumor necrosis factor-α, transforming growth factor-β, concanavalin A, and phorbol 12-myristate 13-acetate have been reported to upregulate MT1-MMP mRNA in fibrosarcoma cells, while basic fibroblast growth factor and epidermal growth factor have little or no effect on gene expression.[61] Incubation of cervical fibroblasts with a calmodulin antagonist stimulates MT1-MMP expression, consistent with the role of calmodulin as inhibitor of matrix hydrolysis and MMP expression.[77] In addition, calcium ionophores block furin processing of MT1-MMP in fibrosarcoma cells, suggesting a role for intracellular calcium in the negative regulation of MT1-MMP activity (Fig. 4).[61]

Other MT-MMP members

Potential contributions of other MT-MMP members to cancer progression are not well characterized. Like MT1-MMP, MT2-MMP (MMP-15) displays activity against a broad range of matrix molecules and can activate pro-MMP-2 in vitro.[41] MT2-MMP has been found in a variety of carcinomas; however, it has not been linked with disease progression. MT3-MMP (MMP-16) also activates pro-MMP-2; however, it does not appear to be as commonly expressed as MT1-MMP.[40] Furthermore, unlike MT1-MMP, MT3-MMP expression is not influenced by culturing cells in collagen gels and may be differentially regulated.[66] Of interest, soluble MT3-MMP generated from alternatively spliced mRNA was recently reported and found to cleave type III collagen and fibronectin.[78] The discovery of an alternatively spliced transcript is novel to the MMP field and may prove to be an interesting facet of the MT-MMP subfamily. Enzymatic activity of the fourth MT-MMP member, MT4-MMP, has not been reported.[79]

MMP-9 cell surface binding and activation

Recent data describing the localization of MMP-9 to the cell surface suggests that soluble membrane-associated MMP activity is not limited to MMP-2. MMP-9 colocalizes with CD44 in metastatic breast carcinomas and it was recently reported that CD44 and MMP-9 can be coimmunoprecipitated.[80,81] The localization of MMP-9 to the cell surface through CD44 appears to regulate invasion, as disruptions of this association with soluble or truncated surface CD44 inhibits tumor invasiveness in vivo. In addition, MMP-9 also colocalizes with β1 integrins in endothelial cells and associates with β1 integrin-enriched membrane vesicles shed by breast carcinoma cells.[82,83] The ability of MMP-9 to bind pericellular type IV collagen may also influence the localization of the enzyme to the cell surface.[84] Pro-MMP-9 is not a substrate of MT-MMP; however, it can be activated by membrane-associated serine proteases and other MMPs, including MMP-2 and MMP-13. Recently, membrane-anchored 110 kDa glycoprotein, RECK (reversion-inducing-cysteine-rich-protein with Kazal motifs), has been cloned and found to bind and weakly inhibit MMP-9.[85] The observation that restored expression of RECK in malignant cells results in loss of invasiveness in vitro and reduced metastasis in vivo provides additional evidence of a potential role of MMP-9 in cellular metastasis. Elucidation of the mechanism(s) by which MMP-9 interacts with cell membranes will be of considerable interest in future work.

Future directions

The development of well-defined models of in vivo metastasis will promote a more detailed understanding of the role of specific enzymes in tumor progression and metastasis, as well as the functional interplay between MMPs and metastasis-associated proteinases in other mechanistic classes. Initial studies suggest that in vivo findings may differ from results obtained using in vitro approaches. For example, although plasmin has been implicated in pro-MMP-9 activation, this activation appears normal in plasminogen knockout mice.[86] Nevertheless, it will be of considerable importance to discover how proteinases from distinct mechanistic classes interact synergistically to promote the degradation of extracellular matrix.

The role of MMPs in metastasis has historically been limited to proteolysis of matrix barriers. Recent studies with TIMP-1 overexpressing melanoma cells and intravital video-microscopy, however, suggest that MMPs may play a more important role in tumor cell growth following extravasation, rather than the facilitation of the extravasation event itself.[87,88] MMPs may potentially regulate growth through clearance of restricting connective tissue matrix, activation of latent growth factors, proteolysis of existing inhibitory factors, or modulation of angiogenic events.[4]

The discovery of cell membrane-anchored matrix degrading metalloproteinases has shifted the focus of tumor cell proteolysis to the cell surface. Expanding our understanding of known MT-MMPs, as well as new members being cloned at a rapid pace, will be a considerable undertaking. Perhaps one of the most intriguing mysteries is the identification of proteins that interact with the cytoplasmic tail of MT1-MMP and their role in the regulation of MT1-MMP cellular localization and activity. Elucidating the complex biochemical mechanisms controlling cell surface MMP activity will also be of practical importance if MMPs are to become effective therapeutic cancer targets.

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