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Matrix metalloproteinases: Regulation and biological functions

SHRAVAN K CHINTALA and JASTI S RAO*
Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
e-mail: jrao@notes.mdacc.tmc.edu

Abstract. Remodeling of the extracellular matrix occurs during many physiological and pathological conditions. The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteases that are responsible for the proteolytic degradation of number of extracellular matrix (ECM) components. Regulation of MMP activity both at transcriptional and translational levels modulates the degradation of ECM components. Although most MMPs have similar regulatory mechanisms, certain MMPs have unique mechanims of activation at the cell surface. Understanding the processes that regulate the expression of MMPs is important since an imbalance between MMPs and their natural inhibitors, tissue inhibitors of metalloproteinses, leads to a pathological situation such as cancer. In this review, we will briefly discuss various MMPs, their structure, regulation and function.

Keywords. Collagenase; gelatinase; stromelysin; MT-MMP.

1. Introduction

Matrix metalloproteinases (MMPs) are a family of structurally related enzymes produced by a variety of cell types during both physiological and pathological conditions. MMPs play a major role in tissue remodeling and differentiation during such development as uterine and mammary gland involution. MMPs also degrade extracellular matrix components, including fibrillar and nonfibrillar collagens, fibronectin, laminin, and basement membrane proteoglycans (table 1), during cartilage degeneration and tumor cell invasion to distant places [1]. Normal degradation of the extracellular matrix by MMPs is a highly regulated process. Uncontrolled proteolysis leads to abnormal tissue degradation or a lack of degradation in certain pathological conditions [1]. In this work, we briefly describe the details of the MMPs' structure, function, activity and substrate preferences and the regulatory mechanisms of these proteases.

2. Matrix metalloproteinases

MMPs are a group of structurally related enzymes that degrade several components of the ECM, including fibrillar and nonfibrillar collagens, fibronectin, laminin, and basement membrane proteoglycans (table 1). At least 17 members of the human MMP family have been identified [2]. Most MMPs are secreted as zymogens, and their activity is controlled by activators and inhibitors, the latter termed tissue inhibitors of metalloproteinases (TIMPs). Common characteristics of MMPs include Zn^{+2} atoms at their active sites, requiring Ca^{+2} ions for enzyme activity, and having highly conserved N-terminal propeptide and catalytic

* For correspondence

Table 1. Matrix metalloproteinases (MMPs).

Subgroup	Enzyme	Substrate
Collagenases	MMP-1 (Interstitial collagenase)	Collagen I, II, III, VII, VIII, X, gelatin, proteoglycans
	MMP-8 (Neutrophil collagenase)	Same as MMP-1
	MMP-13 (Collagenase-3)	Same as MMP-1
	MMP-18 (Collagenase 4, Xenopus)	Collagen I
Gelatinases	MMP-2 (Gelatinase A)	Collagen I, IV, V, VII, X, gelatin, elastin, fibronectin proteoglycans
	MMP-9 (Gelatinase B)	Same as MMP-2
Stromelysins	MMP-3 (Stromelysin-1)	Collagen IV, IX, X, elastin, fibronectin, laminin, proteoglycans, pro-interstitial collagenase
	MMP-10 (Stromelysin-2)	Same as MMP-3
	MMP-11 (Stromelysin-3)	Alpha-1-antitrypsin
Others	MMP-7 (Matrylysin)	Collagen IV, fibronectin, laminin, gelatin, pro-interstitial collagenase, proteglycans
	MMP-12 (Metalloelastase)	Elastin
	MMP-19	Unknown
	MMP-20 (Enamelysin)	Unknown
Membrane-type MMPs	MMP-14 (MT1-MMP)	Activates MMP-2
	MMP-15 (MT2-MMP)	Unknown
	MMP-16 (MT3-MMP)	Activates MMP-2
	MMP-17 (MT4-MMP)	Unknown

domains[2,3]. MMPs have been grouped according to their substrate specificities i.e, as collagenases, gelatinases, stromelysins, and the membrane-type MMPs (table 1).

One MMP, MMP-2 or gelatinase A, is unusual in its constitutive expression by many cells, its ubiquitous tissue distribution, and its mode of activation which differs from that of any other MMP. Moreover, unlike other MMP proenzymes, progelatinases A and progelatinase B (also known as MMP-9) are usually isolated in complex with their endogenous inhibitors, TIMP-2 and TIMP-1 respectively. Both the free enzyme and the enzyme-inhibitor complex can be activated on the cell surface by membrane-type MMPs (MT-MMPs).

Commonalities in the structural domains of MMPs are shown in figure 1. A 17 to 20-residue signal peptide, rich in hydrophobic amino acids, directs the translational product to the endoplasmic reticulum in all but MMP-17, which lacks this peptide[4]. Other common domains include an 80-amino acid propeptide domain that contains the highly conserved PRCXXPD sequence, which is cleaved during activation; a catalytic domain of about 160–170 amino acids, that contains the highly conserved HEXGHXXGXXHS/T region, a thermolysin-type zinc-binding region, and a calcium-binding site; a 200-residue carboxy-terminal domain homologous to hemopexin and vitronectin; a hinge region of about 75-amino acids, that connects the catalytic and hemopexin domains. Two MMPs (MMP-2 and MMP-9) have additional three fibronectin type II repeats in the catalytic domain and seem to aid in binding to the substrate. Membrane-type MMPs also have an additional 80- to 110-residue transmembrane domain. The presence of the furin cleavage

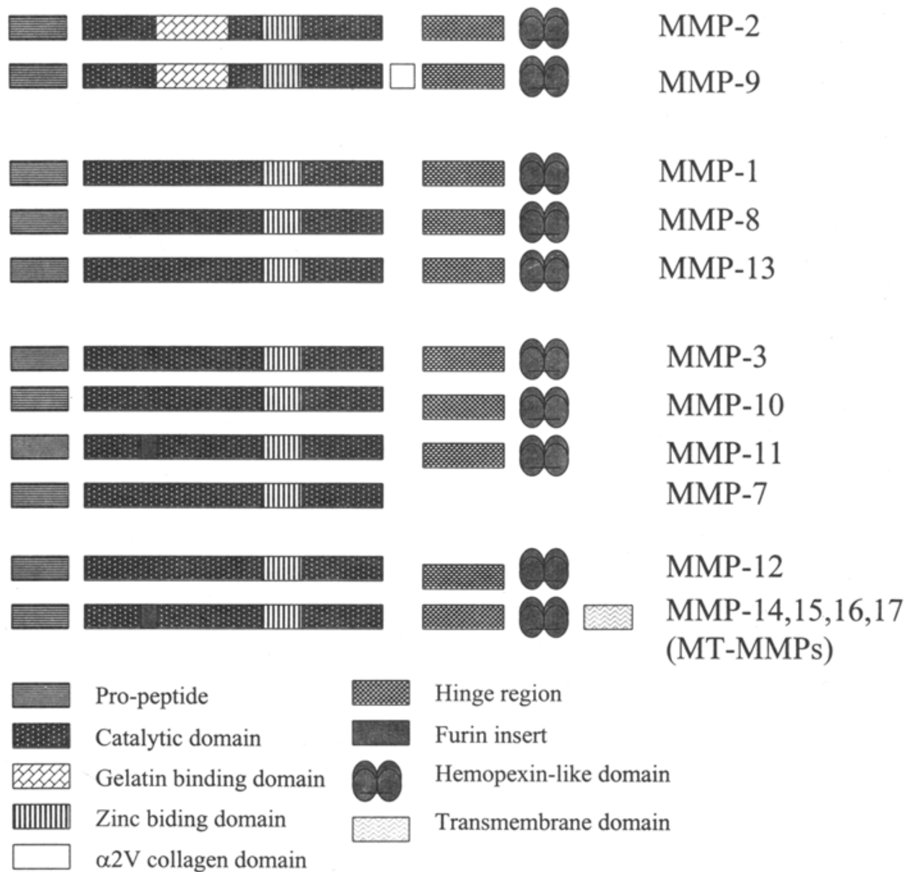

Figure 1. Structural domains of MMPs.

site RXKR/RRKR between the prodomain and catalytic domains in stromelyin-3 and all known MT-MMPs suggest that these MMPs may be activated while they are still in the cell, whereas the other enzymes require proteolytic cleavage after they are secreted as zymogens.

3. Collagenases

Each of the three distinct collagenases has specificity for different substrates. MMP-1 preferably cleaves type III collagen [5], MMP-8 is most active against type I collagens [6–8], and MMP-13 is most efficient in degrading type II collagen unlike other collagenases [9]. MMP-13 also has gelatinolytic and peptidolytic activity [10,11].

4. Stromelysins

Stromelysin family includes MMP-3, MMP-7, and MMP-10, which have broad substrate specificity and MMP-11, which is specific for α-1-antitrypsin. The patterns in which individual stromelysins are expressed are often distinct [12]. MMP-3 is expressed mostly by mesenchymal and connective-tissue fibroblasts in a wide variety of tissues [13]. The presence of elevated MMP-3 and MMP-11 levels in involuting mouse mammary glands [14–16] suggest that these MMPs play a role in mammary gland morphogenesis and involution. Osteoblasts in culture produce MMP-3 and the collagenase MMP-1 in response to bone resorptive agents [17]; the removal of nonmineralized collagen by these enzymes prevents the adherence and activation of osteoclasts [18]. Stromelysins are also involved in the degradation of type II collagen in cartilage matrix associated with arthritis. Serum levels of MMP-3 were elevated in patients with osteoarthritis [19,20]. MMP-3 immunostaining in osteoarthritic cartilage correlated with histologic score and proteoglycan degradation [21].

Stromelysins also are expressed during the tumor invasion process. The type of MMP expressed in the tumor cells and in the surrounding stromal cells depends on the type of tumors. MMP-11 is expressed by breast adenocarcinoma cells [22,23] and other invasive carcinomas [24]. MMP-3 is expressed in the tumor stroma of invasive breast cancer [25]. Head and neck carcinomas express both MMP-3 and MMP-10 [26]. Esophageal squamous-cell carcinomas express MMP-3 and MMP-2 [27]. Elevated MMP-3 levels have been detected in normal mucosa adjacent to bronchial, interepithelial lesions, and in invasive foci of lung carcinoma [28].

Distinct patterns of expression of MMP-3 and MMP-10 were observed in different types of basal keratinocytes in chronic cutaneous ulcers [29]. MMP-3 mRNA and protein were detected in proliferating basal keratinocytes adjacent to but distal from the wound edge, whereas MMP-10 mRNA was found only in the basal keratinocytes at the migrating front. MMP-3 mRNA also found in granulation tissues in gastrointestinal ulcers, peptic ulcers, and ulcerative colitis [30].

5. Gelatinases

The gelatinase subgroup of MMPs has two members, MMP-2 (gelatinase A) and MMP-9 (gelatinase B). MMP-2, perhaps the most widespread of all MMPs, is produced by a variety of cells and is frequently elevated in malignant tumors [31]. MMP-2 is often secreted as a complex with TIMP-2 [32]. The other gelatinase, MMP-9, is expressed by transformed tumor cells, neutrophils, monocytes, and alveolar macrophages [12], and is often secreted as a complex with TIMP-1 [33,34]. MMP-2 has a unique mechanism of activation [35] that involves a trimolecular complex consisting of MT-MMP [36], TIMP-2, and the C-terminal domain of MMP-2 [35]. Details of these two MMPs are given below.

MMP-2, a 72-kDa gelatinase (also known as gelatianase A, 72 kDa type IV collagenase, and EC3.4.24.24) was first identified by Liotta *et al* in 1979 [37]. Constitutively expressed by many cells, MMP-2 not only degrades ECM but also regulates cell proliferation, adhesion, and migration [38]. MMP-2 is not transcriptionally activated by TPA (12-0-tetradecanoylphorbol-13-acetate) or IL-1 (interleukin-1) and lacks the typical transactivation sequences such as AP-1, PEA-3, or TATA boxes that play pivotal roles in the transcriptional regulation of most promoters. Moreover, unlike other MMPs it lacks an upstream transforming growth factor-beta-inhibitory element (TIE) [39].

MMP-9, also known as gelatinase B, type V collagenase, and EC3.4.24.25, cleaves native type-I collagen, proteoglycans and laminins. MMP-9 was first identified as a neutral protease isolated from human neutrophils that can degrade denatured collagens [40]. Proteases that degrade type IV and V collagens subsequently identified in human neutrophils [41] were characterized as 90 to 110 kDa metalloproteinases. A gelatinolytic substance obtained from simian virus 40 (SV40)-transformed human lung fibroblasts shows genetic homology to MMP-2 [42]. The MMP-9 gene in human is 7·7 kb and contains 13 exons [43]. Unlike MMP-2, MMP-9 has a TATA box, an AP-1 element, an SP1 transcriptional factor, a 12-O-tetradeconyol phorbol 4-acetate responsive element (TRE) and an NF-κB binding site.

The expression of MMP-9 can be influenced by many agents, including growth factors, cytokines, ECM molecules, cell-cell and cell-ECM adhesion molecules and agents that alter cell shape. For example transfecting glioma cells with NCAM-B downregulate their MMP-9 secretion, but transfection with NCAM-C, which is expressed at the cell surface through GPI linkage, had no effect on MMP-9 secretion [44]. On the other hand, plating cells on a mixture of tenascin and fibronectin upregulated their production of MMP-9 [45]. Moreover, cytoskeletal reorganization with cytochalasin D, but not with colchicine B decreased MMP-9 expression by human glioma cells [46] and in melanoma cells [47]. Cytochalasin D also abolished the induction of MMP-9 in HL-60 cell lines treated with TPA [48].

Cell-to-cell contact also influences MMP-9 activation. Co-cultures of fibroblasts with colon carcinoma cells resulted in MMP-9 induction [49]. MMP-9 is not often expressed in normal cells, but is expressed in melanomas, lung tumors, breast adenocarcinoma, hepatocellular carcinoma, and gliomas [50–54].

Although MMPs are required under physiological conditions, an imbalance between MMP and TIMP activities results in excessive degradation of the extracellular matrix in conditions such as tumor formation and metastasis [55]. Evidence to date indicates that the expression of gelatinase A is increased in many human tumors, including squamous and basal cell carcinomas [52] and cancers of the colon [56], pancreas [57], prostate [58], bladder [59], skin [52], breast [60,61], and ovary [62]. Studies of tumors of the central nervous system, particularly gliobastoma, have shown that glioblastoma multiformes express elevated levels of MMPs compared with low-grade glioma and normal brain tissues both *in vitro* [63–66] and *in vivo* [67]. A study of MMP and TIMP gene expression by Nakano *et al* [68] showed that gelatinase A (MMP-2) and TIMP-2 genes are overexpressed in glioblastoma multiformes, and a study by Lampert *et al* [69] showed upregulation of MT1-MMP mRNA and gelatinase A expression.

6. Membrane-type MMPs (MT-MMPs)

A different type of MMP, one having a membrane-binding domain rather than being secreted in proenzyme form, was first described by Sato *et al* [36] MT-MMPs are distributed widely, and have been found in colon, breast, and head and neck carcinomas [70] and in gastric carcinomas [71]. The MT-MMP family has at least 4 members, MMP-14 [36], MMP-15 [72], MMP-16 [73], and MMP-17 [74]. MMP-14 and MMP-16 specifically activate MMP-2 [38]. Structurally all MT-MMPs resemble the other MMPs, but have three additional inserts: an 11-amino-acid furin recognition site, located between the propeptide and the N-terminal catalytic domains [75]; an 8-amino-acid insertion in the N-terminus; and a

72-amino-acid hydrophobic insert in the C-terminus that can pass through the plasma membrane and thus act as a potential transmembrane domain.

All MT-MMPs share 30–50% sequence homology and a common multidomain structure [36,74]. The potential furin/prohormone cleavage site at the end of the propeptide domain, common to all MT-MMPs is also conserved in stromelysin-3, a soluble MMP. The function of an 8-amino-acid insertion within the catalytic domain of MT-MMPs is not clear but may be related to substrate specificity or impaired TIMP-1 binding of MT1-MMP and MT2-MMP. The cytoplasmic tail at the carboxyl end of three of the MT-MMPs contains an additional conserved cysteine residue that is flanked by tyrosine and serine residues, which may be a potential phosphorylation site. However these tyrosine and serine residues are absent in MT4-MMP [74].

MT-MMP expression is tightly regulated at the transcriptional level by growth factors and cytokines. MMP-14 expression is upregulated in synovial fibroblasts by tumor necrosis factor-alpha (TNF-α), interleukin-1B (IL-1B), epidermal growth factor (EGF), and by basic fibroblast growth factor (bFGF) [76,77]. Little is known about the regulatory elements of the MT1-MMP promoter sequence. The lectin concanavalin A (ConA) induces MT1-MMP activity in a c-Ras-dependent manner [78] and usually induces proMMP-2 activation as well [79,80]. However, ConA could also be responsible for cross-linking of cell surface MT1-MMP or concentrating the enzyme at the surface, thereby facilitating rapid activation of proMMP-2. In some cell types, MT1-MMP is modulated by phorbol esters and proMMP-2 processing [77,81]. MT1-MMP synthesis is also influenced by cytoskeleton; treatment of fibroblasts with cytochalasin-D, which disrupts stress fibers, leads to increase in MT1-MMP mRNA levels and proMMP-2 activation [48]. In addition to their ability to activate proMMP-2, MT-MMPs can degrade denatured interstitial collagenase, cartilage aggrecan, perlecan, fibulina 1 and 2, fibronectin (FN), vitronectin (VN), nidogen, and large tenascin-C [82–85]. We recently showed that MT1-MMP mRNA was expressed at high levels in human gliomas both *in vitro* and *in vivo* [67].

7. Regulation of proteases

MMPs expression is controlled at several levels including transcription, translation, secretion, and activation of proenzymes. In the following sections, we will briefly address each of these regulatory mechanisms.

7.1 Transcriptional regulation

Transcriptional regulation of MMPs is complex, but a number of similarities exist in the expression patterns among the members of the MMP family. Promoter sequence analysis of various MMPs have shown a few common elements among MMP promoters, such as AP-1 protein, TPA-responsive element (TRE), TGF-β-inhibitory element (TIE) which modulate the expression of MMPs by various cytokines and growth factors. The expression of MMPs is induced by tumor necrosis factor, interleukin-1, tPA, phorbol-12-myristate 13-acetate (PMA), epidermal growth factor, platelet-derived growth factor, transforming growth factor-beta, and a variety of other agents [86,87]. Although tumor necrosis factor, interleukin-1 and PMA generally induce MMP expression, MMP induction is cell type- and inducer-specific; thus, all agents will not induce MMP expression in all cells. As said above, the induction of gelatinase A is distinct in that gelatinase A lacks a TATA box and the common AP-1 element critical for the induction

by PMA, growth factors, and cytokines. Another exception is human stromelysin-3 which lacks an AP-1 site but is upregulated in response to PMA [88]. Ets protein products interact with AP-1 and PEA-3 and may work in conjunction with AP-1 to modulate the induction of MMPs. Interestingly, mouse MMP-9 and human MMP-2 promoters lack Ets binding sites. TGF-β inhibit the expression of some MMPs through TIE which is found in human interstitial collagenase, human MMP-9 and human matrilysin. Interestingly, although MMP-9 has TIE and MMP-2 lacks this, TGF-β in general induced MMP-2 and MMP-9 expression rather than inhibition [89].

7.2 Activation of proenzymes

The activation of the zymogen form of MMPs is critical for the efficient ECM degradation. MMP activation *in vitro* has been proposed by Van Wart [90] which is believed to occur via a "cysteine switch mechanism". According to this hypoethesis, an unpaired cysteine residue in the pro-peptide domain of the enzyme (PRCXXPD sequence) maintains the latency of the zymogen by direct coordination with the zinc atom at the active site [90]. Disruption of this interaction by chemical agents such as p-aminophenyl mercuric acetate (APMA) or by chaotropic agents *in vitro*, and proteinases such as trypsin, plasmin, chymotrypsin, neutrophil elastase, and plasma kalikrein *in vivo* [91] leads to a cascade of events that alters the conformation of the protein resulting in activation and cleavage of the propeptide.

Recent data suggest that proMMP-2 has a unique mechanisms of activation at the cell surface that is modulated by MT1-MMP and TIMP-2 [92–94]. The activation process involves the binding of the proenzyme to an MT1-MMP/TIMP-2 complex (which serves as receptor) on the cell surface through interaction between the C-teminal domain of the proenzyme and the C-terminal domain of TIMP-2 [79,95,96]. Cross linking experiments have shown that the trimolecular complexes consisting of proMMP/MT1-MMP/TIMP-2 are concentrated on the cell surface [97]. Once proMMP binds to the MT1-MMP/TIMP-2 receptor complex, processing of proMMP-2 is then initiated by an adjacent free and active MT1-MMP molecule. This initial cleavage destabilizes the structure of the proMMP-2 propeptide domain, and proteolysis then proceeds in an MMP-2 dependent manner, which releases the rest of the propeptide domain and a fully active MMP-2. Also, there is evidence that the integrin $\alpha V\beta 3$ is involved in binding proMMP-2 to the cell surface [98].

In vivo activation of MMP by furin is interesting as the MMP is activated intracellularly prior to secretion [75]. Furin is a trans-Golgi network serine protease that recognizes a specific furin sequence, RXKR [75]. This sequence is present between the propeptide and the catalytic domain of stromelysin-3, and in all the known MT-MMPs. Although activation of stromelysin-3 has been shown to occur by furin, MT-MMP activation by furin has not yet been demonstrated. Interestingly, stromelysin-3 and MT-MMP are not activated by SDS or APMA and as a result of intracellular activation, these MMPs are not present in the ECM as proenzymes.

Recent studies suggest that CD44 serves to anchor MMP-9 on the tumor cells and propose a mechanism for CD44 mediated tumor invasion. It has been shown that CD44 associates with a proteolytic form of MMP-9 on the surface of a broad range of tumor cells. Further, a ternary complex consisting of CD44, hyaluronon and MMP-9 is required for cell surface-associated MMP-9 activity [99].

7.3 Inhibition of proenzyme activation by tissue inhibitors of metalloproteinases (TIMPs)

The balance between MMP and TIMP activities is critical for the normal function of proteases in normal physiological conditions. MMP function is regulated by naturally occurring protease inhibitors, that is, the tissue inhibitors of metalloproteinases (TIMPs). The TIMP family comprises at least four distinct members to date, TIMP-1 (a 28-kDa protein[100], TIMP-2 (a 21-kDa protein)[101,102], TIMP-3 (a 21-kDa protein)[103–105] and TIMP-4 (a 22-kDa protein)[106], all with broad specificity in inhibiting MMPs. TIMPs are expressed by a variety of cells and are present in most tissues and body fluids. TIMP-1 (a 28·5-kDa protein), and TIMP-2 (a 21·0-kDa protein) are present in soluble form, whereas TIMP-3 (21·0 kDa protein) is bound to ECM[107]. All members of the TIMP family share several structural features[3,106,108]. TIMPs bind with high affinity in a 1:1 molar ratio to active MMPs; the binding of TIMP-1 to the proform of MMP-9 inhibits the activation of MMP-9[109], whereas TIMP-2 binding inhibits the acutoactivation of MMP-2[110].

8. Conclusions and future perspectives

We briefly summarized the structural similarities of various MMPs and their role and regulation in physiological and pathological situations. The degradation of ECM is critical under both these conditions. Although the degradation of ECM by MMPs is modulated at many levels including transcriptional and postranslational levels, each enzyme possesses unique characteristics and functions. However, much more is to be learned about the signaling mechanisms which are transduced through receptors, integrins, and the not-as-yet well-characterized feedback mechanisms. A better understanding of the regulation of these enzymes will aid in developing specific therapeutic interventions to pathological situations such as cancer.

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