

Review

Matrix metalloproteinases: old dogs with new tricks

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Abstract

The matrix metalloproteinase family in humans comprises 23 enzymes, which are involved in many biological processes and diseases. It was previously thought that these enzymes acted only to degrade components of the extracellular matrix, but this view has changed with the discovery that non-extracellular-matrix molecules are also substrates.

Four decades ago a collagenase was discovered that was responsible for involution of the tadpole tail in amphibian morphogenesis [1]. Today, the matrix metalloproteinase (MMP) family, to which this collagenase belongs, has expanded to include 23 gene products in humans (Table 1), which encode zinc-dependent and calcium-dependent proteases that cleave within a polypeptide (endopeptidases). There are also two other large families that have major roles in extracellular proteolysis, the ADAM family (A disintegrin and metalloprotease domain, with about 33 members in humans) and the ADAMTS family (A disintegrin-like and metalloprotease domain (reprolysin type) with thrombospondin type I repeats, with 19 members). Traditionally, the MMPs have always been thought to cleave components of the extracellular matrix (ECM). As the ECM was regarded for a long time as nothing more than a passive structure used for cell attachment, mechanical support and force transmission, extracellular proteases such as MMPs were thought to simply remodel the ECM for its homeostasis or to facilitate cell migration. This view has changed, however [2]: the ECM is now known to contain growth factors, their binding proteins, and other bioactive molecules, as well as binding sites for cell-surface molecules, some of which are revealed only after proteolysis. The proteases responsible for turnover of the ECM therefore contribute significantly to its dynamic interactions with cells. More recently, considerable evidence has emerged showing that the role of MMPs goes far beyond that of digesting ECM molecules

alone; they are now known to process a number of cell-surface and soluble regulators of cell behavior (summarized in Table 1). Mouse genetic models and human diseases have revealed unexpected biological functions of MMPs (summarized in Table 2); many of the phenotypes of these animals are currently unexplained and suggest that there may be as yet unknown substrates for these enzymes that are not ECM components.

One consequence of the previously held view of MMPs as solely ECM-excavating enzymes was the belief that they were uniformly harmful in cancer and should therefore be targeted in cancer therapy. This was not entirely without basis, given that many MMPs are highly expressed in various cancers, both by tumor cells and in surrounding stromal cells such as macrophages. As recent reviews [3-6] have pointed out, however, the unexpectedly complex role of these enzymes in cancer is leading to their re-evaluation as drug targets, or at the very least, a pause while some of the evidence is analyzed in more detail. Although this is disappointing from the clinical perspective, the study of these enzymes has never been more exciting than it is today, because the human genome project has led to identification of all MMPs, and genetic alterations in individual MMP genes have provided new insights into their functions. In this article, we summarize some of the essential concepts in the MMP field at present and the new insights that are pouring in from biological studies.

Table 1**Human matrix metalloproteases and their substrates**

Protein name*	Alternative names	Collagenous substrates	Non-collagenous ECM substrates	Non-structural ECM component substrates
MMP-1	Collagenase-1	Collagen types I, II, III, VII, VIII, X, and gelatin	Aggrecan, casein, nidogen, serpins, versican, perlecan, proteoglycan link protein, and tenascin-C	α_1 -antichymotrypsin, α_1 -antitrypsin/ α_1 -proteinase inhibitor, IGFBP-3, IGFBP-5, IL-1 β , L-selectin, ovostatin, recombinant TNF- α peptide, and SDF-1
MMP-2	Gelatinase-A	Collagen types I, IV, V, VII, X, XI, XIV, and gelatin	Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, and versican	Active MMP-9, active MMP-13, FGF R1, IGF-BP3, IGF-BP5, IL-1 β , recombinant TNF- α peptide, and TGF- β
MMP-3	Stromelysin-1	Collagen types II, IV, IX, X, and gelatin	Aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, and versican	α_1 -antichymotrypsin, α_1 -proteinase inhibitor, antithrombin III, E-cadherin, fibrinogen, IGF-BP3, L-selectin, ovostatin, pro-HB-EGF, pro-IL- β , pro-MMP-1, pro-MMP8, pro-MMP-9, pro-TNF α , and SDF-1
MMP-7	Matrilysin-1, neutrophil collagenase	Collagen types I, II, III, V, IV, and X	Aggrecan, casein, elastin, enactin, laminin, and proteoglycan link protein	β_4 integrin, decorin, defensin, E-cadherin, Fas-L, plasminogen, pro-MMP-2, pro-MMP-7, pro-TNF α , transferrin, and syndecan
MMP-8	Collagenase-2	Collagen types I, II, III, V, VII, VIII, X, and gelatin	Aggrecan, laminin, and nidogen	α_2 -antiplasmin and pro-MMP-8
MMP-9	Gelatinase-B	Collagen types IV, V, VII, X, and XIV	Fibronectin, laminin, nidogen, proteoglycan link protein, and versican	CXCL5, IL-1 β , IL2-R, plasminogen, pro-TNF α , SDF-1, and TGF- β
MMP-10	Stromelysin-2	Collagen types III, IV, V, and gelatin	Fibronectin, laminin, and nidogen	Pro-MMP-1, pro-MMP-8, and pro-MMP-10
MMP-11	Stromelysin-3		Laminin	α_1 -antitrypsin, α_1 -proteinase inhibitor, and IGFBP-1
MMP-12	Macrophage metalloelastase		Elastin	Plasminogen
MMP-13	Collagenase-3	Collagen types I, II, III, IV, V, IX, X, XI, and gelatin	Aggrecan, fibronectin, laminin, perlecan, and tenascin	Plasminogen activator 2, pro-MMP-9, pro-MMP-13, and SDF-1
MMP-14	MT1-MMP	Collagen types I, II, III, and gelatin	Aggrecan, dermatan sulphate proteoglycan, fibrin, fibronectin, laminin, nidogen, perlecan, tenascin, and vitronectin	$\alpha_v\beta_3$ integrin, CD44, gC1qR, pro-MMP2, pro-MMP-13, pro-TNF α , SDF-1, and tissue transglutaminase
MMP-15	MT2-MMP	Collagen types I, II, III, and gelatin	Aggrecan, fibronectin, laminin, nidogen, perlecan, tenascin, and vitronectin	Pro-MMP-2, pro-MMP-13, and tissue transglutaminase
MMP-16	MT3-MMP	Collagen types I, III, and gelatin	Aggrecan, casein, fibronectin, laminin, perlecan, and vitronectin	Pro-MMP-2 and pro-MMP-13
MMP-17	MT4-MMP	Gelatin	Fibrin and fibronectin	
MMP-19	RASI-1	Collagens types I, IV, and gelatin	Aggrecan, casein, fibronectin, laminin, nidogen, and tenascin	
MMP-20	Enamelysin		Aggrecan, amelogenin, and cartilage oligomeric protein	
MMP-21				α_1 -antitrypsin
MMP-23	CA-MMP	Gelatin		
MMP-24	MT5-MMP	Gelatin	Chondroitin sulfate, dermatin sulfate, and fibronectin	Pro-MMP2 and pro-MMP-13
MMP-25	Leukolysin, MT6-MMP		Collagen type IV and gelatin	Fibrin and fibronectin Pro-MMP-2
MMP-26	Matrilysin-2, endometase	Collagen type IV and gelatin	Casein, fibrinogen, and fibronectin	β_1 -proteinase inhibitor
MMP-28	Epilysin		Casein	

*Although there are 23 human MMPs, 29 numbers have been used in the literature. The symbols MMP-4, MMP-5, MMP-6 and MMP-29 are redundant in humans and are no longer in use; MMP-18 corresponds to a *Xenopus laevis* collagenase, for which no human ortholog is known, and a human protein published as MMP-18 is now called MMP-19. Two nearly identical human genes found in a segment of chromosome 1 that is duplicated were called *MMP21* and *MMP22* but are now referred to as *MMP23A* and *MMP23B*.

Table 2**Phenotypes of knockout mice lacking MMP genes**

Genotype	Phenotype
<i>MMP2</i> ^{-/-}	Suppression of experimentally induced pancreatic carcinogenesis [85]; suppression of angiogenesis and tumor growth [86]; delayed mammary gland differentiation; mild growth retardation.
<i>MMP3</i> ^{-/-}	Accelerated mammary gland adipogenesis [87]; delayed incisional wound healing [88]; resistance to contact dermatitis [89]; impaired ex vivo herniated disc resorption [90].
<i>MMP7</i> ^{-/-}	Suppression of experimental adenoma growth [91]; defective innate intestinal immunity [70]; impaired tracheal wound re-epithelization [92]; defective prostate involution after castration [93]; impaired ex vivo herniated disc resorption [94]; impaired transepithelial migration of neutrophils [32]; reduced syndecan-1 shedding [32].
<i>MMP9</i> ^{-/-}	Suppression of experimentally induced skin carcinogenesis [95]; suppression of experimentally induced pancreatic carcinogenesis [85]; decreased experimental metastasis [86]; delayed growth plate vascularization [96]; abnormal endochondral ossification [96]; defective in osteoclast recruitment [97]; resistance to bullous pemphigoid (skin blistering) [98]; resistance to experimentally induced aortic aneurysms [99]; prolonged contact dermatitis [89]; protection from ventricular enlargement after infarction [100]; protection from cardiac rupture after infarction [101]; diminished peripheral mononucleocyte infiltrate in glomerular nephritis [102]; impaired smooth muscle cell migration and geometrical arterial remodeling [103,104]; impaired cellular infiltration and bronchial hyper-responsiveness during allergen-induced airway inflammation [105]; impaired recruitment of stem and progenitor cells from the bone marrow following irradiation [106]; myocardial protection from ischemia and/or reperfusion [107].
<i>MMP11</i> ^{-/-}	Suppression of experimentally induced mammary carcinogenesis [108]; decreased tumor cell survival and growth [109]; accelerated neointima formation after vessel injury [110].
<i>MMP12</i> ^{-/-}	Resistance to cigarette-smoke-induced emphysema [111].
<i>MMP14</i> ^{-/-}	Severe abnormalities in bone and connective tissue [76,77]; reduced collagen turnover [76]; impaired endochondral ossification [76,77]; defective angiogenesis [76,77].

Data in the table are modified from [5,77,83,112].

Classification and evolution

There are 23 human MMP genes (Table 1). Before this genetic diversity was known, it was customary to divide the MMPs into collagenases, gelatinases, stromelysins, elastase and others, on the basis of the known substrates, but as some MMPs have overlapping substrate specificities, the boundary between the previously used enzyme classes is now blurred. Nevertheless, the trivial names are often useful, particularly if they reflect a function or a distinct structural feature or location, and they have therefore been retained (and are indicated in Table 1). MMP genes are structurally similar to each other, indicating that they evolved by duplication of a common ancestral gene followed by divergent evolution. A number of MMP genes (*MMP1*, *MMP3*, *MMP7*, *MMP8*, *MMP10*, *MMP12*, *MMP13*, *MMP20*, and *MMP26*) are found in a cluster on human chromosome 11 (11q21-23) [7,8]. The human and mouse genomes appear not to contain an identical complement of MMPs, which may complicate the use of transgenic mice for insights into functions of the human genes. For example, human *MMP1* does not have a clear-cut ortholog in the mouse but instead is most similar to two genes, *mColA* and *mColB*, whose functions are at present unknown [9].

MMP genes have been identified in a number of non-vertebrate species, and the number will undoubtedly increase as more genome projects are completed. The nematode *Caenorhabditis elegans* has a number of MMPs, including

C31, H19 and Y19 [10]; there is a *Drosophila* MMP [11]; sea urchins contain an MMP essential for hatching, known as envlysin [12]; and the hydra has an MMP that is required for foot-process development [13]. Interestingly, there is an MMP in green algae [14] as well as in plants such as the soybean [15] and *Arabidopsis thaliana* [16].

Domain organization and protein structure

The close evolutionary relationship between MMPs is further reflected in the conservation of their domain structure (Figure 1) and of their mechanisms of catalysis and regulation. Most MMPs are secreted, although some are anchored to the cell surface by a transmembrane segment (MMP-14, MMP-15, MMP-16 and MMP-24, referred to as membrane-type MMPs or MT-MMPs) [17] or via a glycosylphosphoinositol (GPI) anchor (MMP-17 and MMP-25, sometimes also included in the MT-MMPs) [18]. Most of the MT-MMPs are type I transmembrane proteins - oriented with the amino terminus outside the cell - although one, MMP-23, is unusual in apparently having the amino terminus positioned intracellularly (a type II orientation) [19]. The MT-MMPs have a single transmembrane domain followed by a short, highly conserved cytosolic segment of 20 amino acids. 'Soluble' MMPs may also be located at the cell surface via interaction with membrane-bound molecules or complexes such as integrin $\alpha_v\beta_3$ [20], the extracellular matrix metalloproteinase inducer EMMPRIN/CD147 [21], the hyaluronan receptor CD44 [22],

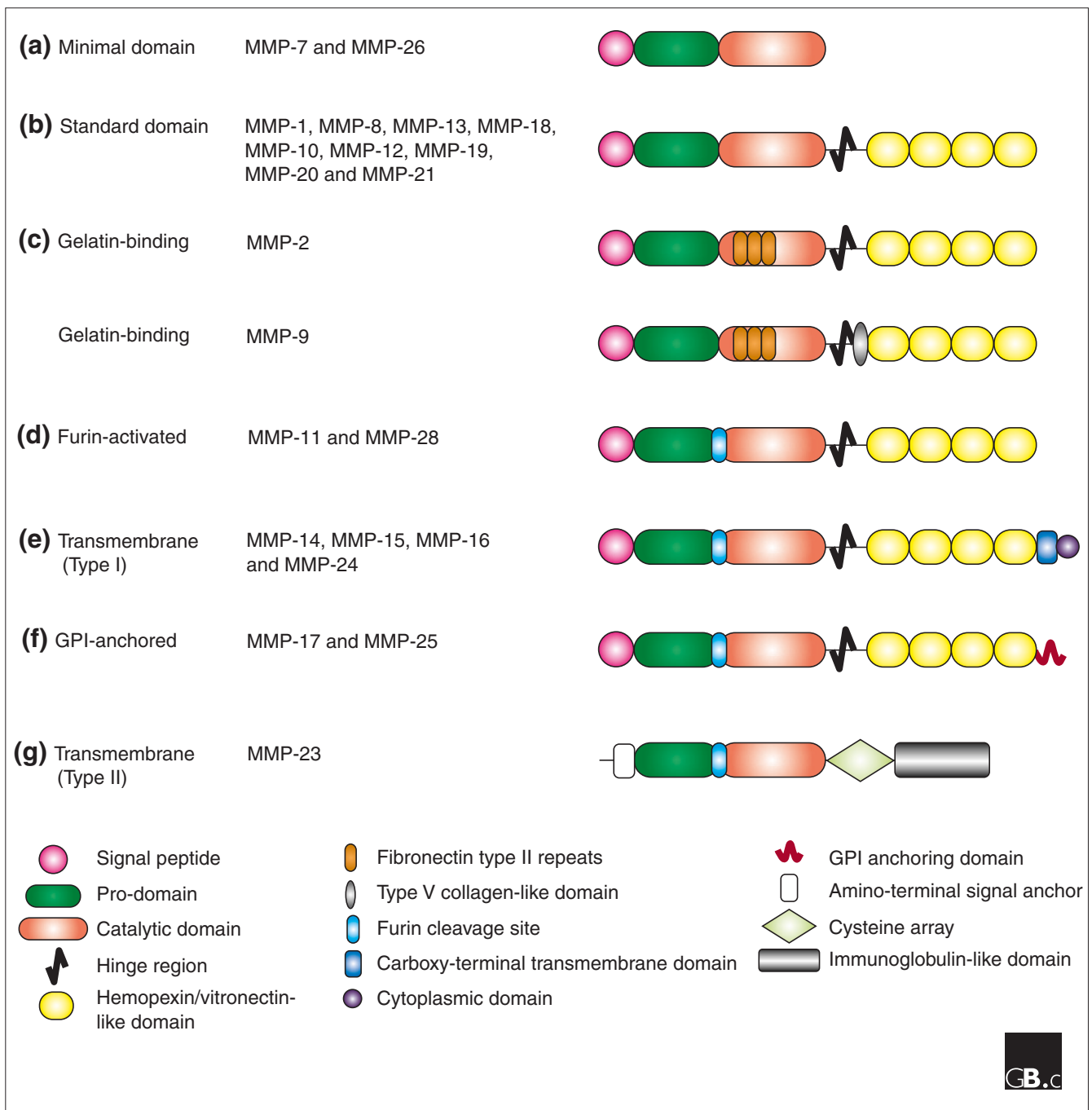


Figure 1
The domain composition and important structural features of the various subtypes of MMPs.

cell-surface heparan-sulfate proteoglycans such as syndecans [23], or transmembrane MMPs [17].

All MMPs except MMP-7 (matrilysin-1) and MMP-26 (endometase or matrilysin-2) consist of two domains connected by a flexible proline-rich hinge peptide. The domains comprise a protease domain and an ancillary (helper)

domain - most commonly a domain similar to sequences in the heme-binding protein hemopexin and the soluble fibronectin-related protein vitronectin [24,25] (see Figure 1). The protease domain contains a signal peptide, the pro-domain, and the catalytic module, in that order. MMP-2 and MMP-9, which are the main gelatinases, differ from other MMPs in that they have three tandem fibronectin type II

repeats within the amino terminus of the catalytic module that mediate gelatin binding (Figure 1c) [26-28]. MMP-9 has an additional insert within its hinge region similar to collagen V; its function is unknown. MMP-7 and MMP-26 [29] lack the hemopexin/vitronectin-like domain (Figure 1a), whereas MMP-23 has a unique cysteine- and proline-rich sequence and a domain similar to the immunoglobulin-like domains of the interleukin-1 receptor instead of the hemopexin/vitronectin-like domain (Figure 1g) [30].

X-ray crystal structures are now available for a number of MMPs, including full-length activated enzymes and zymogen forms that include the pro-domain [31]. Crystallography has revealed how a cleft in the active site contributes to enzyme specificity [32]. The catalytic domain of all MMPs has a unique signature of amino-acid residues (His-Glu-X-Gly-His-X-X-Gly-X-X-His-Ser, where X is any amino acid) within the active site [25] that coordinates the catalytic zinc atom. The hemopexin/vitronectin-like domain consists of four parts arranged symmetrically around a central axis, forming a four-bladed propeller-like structure that helps to confer substrate-binding specificity on the enzyme.

Regulation of MMPs

In keeping with their potential for tissue destruction, MMPs are stringently regulated at multiple levels, including transcription, activation of the zymogen forms, extracellular inhibitors, location inside or outside the cell and internalization by endocytosis. The pro-domain keeps the enzyme latent using the thiol group of a highly conserved, unpaired cysteine at its carboxyl terminus. This conserved cysteine acts as a fourth inactivating ligand for the catalytic zinc atom in the active site, resulting in the exclusion of water and rendering the enzyme inactive (Figure 2). For the enzyme to be activated, this cysteine-zinc pairing needs to be disrupted by a conformational change or by proteolysis (such as by the protease plasmin or by other MMPs). Once the thiol group is replaced by water, the enzyme is able to hydrolyze the propeptide to complete the activation process and can then cleave the peptide bonds of its substrates. This system of regulation is referred to as the 'cysteine-switch' mechanism [33]. Most MMPs are not activated until they are outside the cell, but the MT-MMPs and MMP-11, MMP-23 and MMP-28 are activated by a proprotein convertase (such as furin) within the secretory pathway [34-37].

Once activated, there are multiple mechanisms that can inactivate the MMPs [38]. Four classes of metalloproteinase inhibitors are found in extracellular spaces and body fluids that have broad inhibitory activity against many MMPs. One class is the tissue inhibitors of metalloproteinases (TIMPs), which are disulfide-bonded proteins of 20-30 kDa that directly interact with the MMP active site through a small number of their amino acids. An unrelated small inhibitor derived by proteolysis of the procollagen C-proteinase

enhancer has structural similarity to TIMPs and may inhibit MMPs through a similar mechanism [39]. Recently, a membrane-anchored molecule, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), has been discovered that appears to regulate MMP-2, MMP-9 and MMP-14 post-transcriptionally by affecting secretion and activation as well as by inhibition of the active site [40]. In the circulation, the protease inhibitor α 2-macroglobulin inactivates active MMPs by a 'bait and trap' mechanism [38,41]: when protease-sensitive sites within the inhibitor are cleaved, it springs closed around the proteinase and isolates it from potential substrates.

MMP zymogens can also be activated by MMPs themselves; for example, MMP-2 is activated by MT-MMPs such as MMP-14 [17]. Paradoxically, the inhibitor TIMP-2 is absolutely required for this process, which requires it to bind to the active site of MMP-14 and also, through its carboxy-terminal domain, to the hemopexin/vitronectin-like domain of MMP-2. The essential role of TIMP-2 is emphasized by the lack of pro-MMP-2 activation in *Timp2*-null mice [42] as well as by the exquisite co-regulation of the *Mmp14* and *Timp2* genes (but not other TIMP genes) during mouse development [43]. (MMP-14 also activates MMP-13 at the cell surface, but this does not seem to require TIMP-2 [44].)

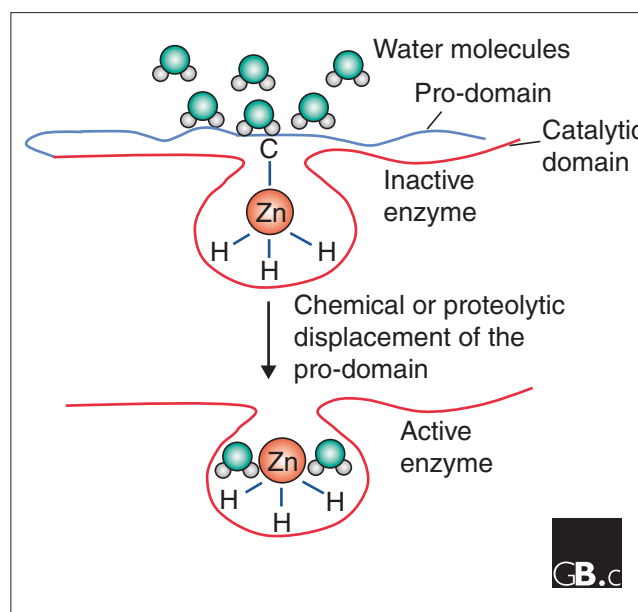


Figure 2
The 'cysteine-switch' mechanism regulating the MMP zymogen. The thiol group of a conserved cysteine (C) at the carboxyl terminus of the pro-domain acts as a fourth inactivating ligand for the catalytic zinc atom in the active site; this results in the exclusion of water and keeps the enzyme latent. Displacement of the pro-domain by conformational change or proteolysis disrupts this cysteine-zinc pairing and the thiol group is replaced by water. The enzyme can then cleave the peptide bonds of its substrates.

The further activation of MMP-2 bound to TIMP2 probably involves complex but highly regulated repositioning of membrane-bound MMPs through their cytoplasmic tails or transmembrane domains or through interaction with cellular receptors. Lehti *et al.* [45,46] have suggested that oligomerization may occur between the cytoplasmic domains of the two MMP-14 molecules, an observation that ties in with a report that a conserved cysteine in the MMP-14 cytoplasmic tail is essential for dimerization [47].

In addition to a possible role in oligomerization, the cytoplasmic domain of MT-MMPs is essential for regulating the activity of these enzymes; it acts by altering their spatial distribution in the cell in response to intracellular signaling events. The cytoplasmic sequence targets the MT-MMPs to specific domains within the cell membrane, including invadopodia (surface protrusions of invasive cells) [48] and caveolae (specialized membrane invaginations) [49]. Recently, a role for the cytoplasmic domain has been identified in the cycling of MT-MMPs between an intracellular pool and the cell surface via clathrin-coated pits and vesicles [50,51]; this may be one of several cellular mechanisms by which the amount of enzyme at the cell surface is regulated. Studies of *thrombospondin-2* null mice have indicated that thrombospondin-2 has a role in targeting active MMP-2 to the scavenger-receptor pathway [52], and it has been shown that MMP-13 binds to a specific cell-surface receptor before it is internalized via the low-density lipoprotein receptor [53].

Functions of MMPs

The substrates of MMPs are given in Table 1, and the functions of MT-MMPs in particular are illustrated in Figure 3.

Enzyme-substrate co-localization and interactions

The collagenases MMP-1, MMP-8, MMP-13, and MMP-14 are the only MMPs that can efficiently degrade the fibrillar collagens (types I, II and III) in their triple-helical domains [54]. Cleavage by these enzymes renders the collagen molecules thermally unstable, so that they unwind to form gelatin, after which they can be degraded by other members of the MMP family such as the major gelatinases MMP-2 and MMP-9. The collagenase active site is unable to accommodate the entire cross-section of the collagen triple helix, an observation that has generated much interest in how this substrate is actually cleaved.

The two major gelatinases, MMP-2 and MMP-9, have several distinctive features. They can be distinguished by the fact that MMP-2 binds preferentially to TIMP-2, which is required for its activation, whereas MMP-9 is preferentially inhibited by TIMP-1 [38]. MMP-2 becomes located at the cell surface by binding of its carboxyl terminus to the integrin $\alpha_v\beta_3$ [29] or the MMP-14-TIMP-2 complex; when bound, the catalytic site of MMP-2 is exposed and can be cleaved and activated. The α_2 chains of collagen IV bind

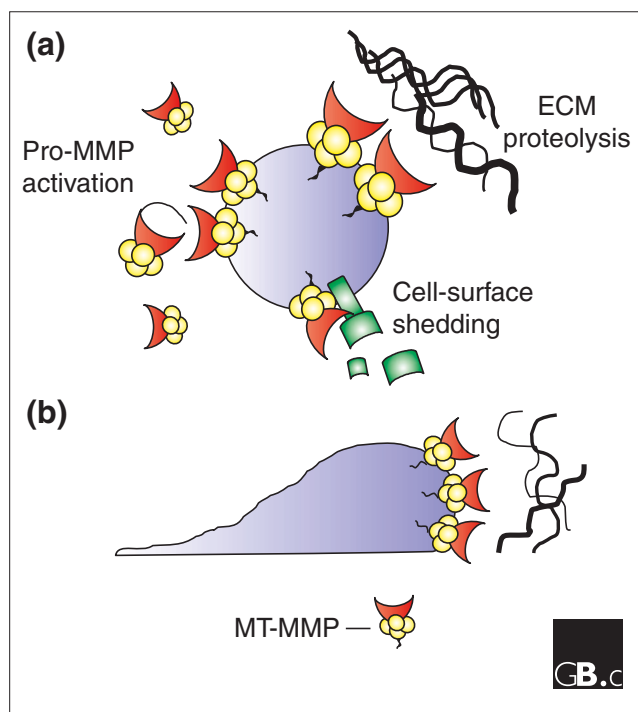


Figure 3

The locations of MT-MMPs. **(a)** The location of MT-MMPs lends them critical biological roles at the cell surface: they cleave components of the ECM, other MMPs and receptors for growth factors (which leads to shedding of the receptors from the cells). **(b)** Mobilization of MT-MMPs to the leading edge of cancer cells, where they remodel the ECM, facilitates cell migration and tumor invasion.

MMP-9 with a high affinity even when MMP-9 is inactive [55]; this juxtaposition of enzyme and substrate means that a pool of the enzyme is rapidly available upon activation for any remodeling events.

Non-matrix substrates and consequences of MMP activity

The extracellular matrix contains sites that can bind growth factors, either directly and via growth-factor-binding proteins. When bound, growth factors - such as transforming growth factor β (TGF- β), fibroblast growth factor 1 (FGF-1) and insulin-like growth factor 1 (IGF-1), tumor necrosis factor α , and heparin-binding epidermal growth factor-like growth factor (HB-EGF) [56] - are unable to bind their receptors and signal to the nucleus [57-61]. Several MMPs are able to release growth factors by cleaving either the growth-factor-binding protein or the matrix molecule to which these proteins attach. In addition, MMP-3 and MMP-7 can cleave the adherens-junction protein E-cadherin, thus promoting cell invasion by disrupting cell aggregation [62,63]. MMP-3 can release a soluble form of the adhesion molecule L-selectin [64] from leukocytes. It also sheds membrane-bound HB-EGF, so that it can exert signaling functions [56]. MMP-7 releases soluble Fas ligand; this occurs during involution of

the prostate after castration and induces apoptosis [65]. In contrast to the activation of growth factors by proteolytic release, many growth factors are proteolytically inactivated by MMPs, including the chemokine connective tissue activating peptide III (CTAP-III), monocyte chemoattractant protein and stromal cell-derived factor 1 (SDF-1) [66,67]. A second mechanism by which growth factors and cytokines are negatively regulated is when MMPs cause the shedding of their receptors from the cell membranes, as in the case of surface FGF receptor 1 [68].

The immune system is also influenced by MMPs. Firstly, the defensins are a family of polar antimicrobial peptides that make up part of the innate immune system of some animals. Defensins are synthesized in an inactive form and activated by the proteolytic removal of the pro-domain by MMP-7, which allows them to insert into the bacterial membrane and disrupt its integrity [69,70]. Secondly, MMP-3 (stromelysin-1) and MMP-7 (matrilysin-1) can cleave all immunoglobulin G proteins; this cleavage is important as it prevents the initiation of the complement cascade and is potentially beneficial in the removal of the immunoglobulin G from damaged or inflamed tissue [71]. And finally, the receptor of the complement component C1q, gC1qR, exists in both a membrane-bound form and a soluble form that may inhibit the hemolytic activity of C1q. By releasing gC1qR, MT1-MMP may help tumor cells to avoid targeted destruction by the complement system and may thus facilitate tumor-cell survival [72-74].

Genetic alterations in human and mouse MMPs

An extensive body of literature indicates an association of MMPs with cancer, arthritis, numerous other inflammatory or autoimmune disorders, cardiovascular and cerebrovascular diseases, and fibrotic diseases. Despite this, there are relatively few instances in which MMPs have proved to be the primary cause of disease. One of these is a rare inherited disorder of bone (nodulosis arthropathy osteolysis syndrome), one of a set of 'vanishing bone syndromes', in which there is severe resorption and destruction of bones, primarily those of the hands and feet [75]. The affected individuals also have chronic arthritis and subcutaneous nodules in the hands and feet. The disease is inherited in an autosomally recessive manner, and loss-of-heterozygosity analysis mapped the responsible gene to 16q12-q22, an interval containing the *MMP2* gene. Analysis of the serum and fibroblasts of affected individuals showed a complete absence of *MMP2*. The family was found to have two homoallelic *MMP2* mutations, resulting in the ablation of *MMP2* activity. The presence of collagenous nodules fits with the role of MMPs in removing excess ECM; the paradox in these patients, however, is that excessive destruction of bones and arthritis are caused by an absence of a destructive enzyme, not an excess as might have been expected. There are a number of mechanisms by which this may occur: there may be compensatory overproduction of another MMP or protease, MMP-2 may be essential for processing of an inductive factor required for the activity of

bone-forming cells (osteoblasts), or it may be required to regulate the activity of bone-degrading cells (osteoclasts) in tissues. Interestingly, the *Mmp2* knockout mouse is not known to recapitulate the human disease, which resembles more closely the phenotype of *Mmp14* knockout mice [76,77]. Given that MMP-14 and TIMP-2 are required for MMP-2 activation, it is possible that mutations in the genes encoding these enzymes may result in a similar human disease.

The chromosome 1p36.3 region that contains the two closely related *MMP23* genes (see Table 1) is altered or frequently deleted in neuroblastomas in which the oncogene *MYCN* has been amplified, in a subset of malignant melanomas and in a 1p35 deletion disorder [78], but the role of the two MMP genes in these disorders has not been investigated.

A polymorphism in the *MMP1* promoter can result in one allele having a sequence of either one or two guanines in a particular position. The presence of two guanines results in the *de novo* appearance of a binding site for the ETS-1 transcription factor that cooperates with an adjacent site for the AP-1 transcription factor to enhance the expression of *MMP1* [79-81]. It is interesting to note that cancer patients, on average, have a higher incidence of the two-guanine allele than have people in the unaffected population. A regulatory polymorphism is also present in the *MMP3* promoter, leading to the presence of either five or six adenosines [82]. The allele with six (6A) has a reduced transcriptional activity compared to the allele with five. A study on patients with coronary artery atherosclerosis revealed that patients homozygous for the 6A allele showed a more rapid progression of both global and focal atherosclerotic lesions.

The creation of transgenic mice that are null for specific genes has been useful in attributing functions to a number of MMP family members (Table 2). In general, most MMP gene knockouts are viable, with subtle phenotypic differences from their wild-type counterparts [83]. This could reflect either a lack of function in vital developmental processes or a significant amount of redundancy amongst these enzymes for substrates, with the consequence that other family members can be upregulated to compensate for the loss of an individual enzyme. Although the lack of a dramatic phenotype in many of the knockout mice may initially have seemed a disappointing outcome, it has in fact been very valuable for dissecting the role of MMPs in cancer and inflammation. The exception to the rule is the *Mmp14*-knockout mouse [76,77], which has severe postnatal growth-retardation and skeletal anomalies and dies before the onset of sexual maturity (Figure 4). The severity of the *Mmp14* knockout, in contrast to the other MMP knockout animals, may reflect the position of this enzyme as an initiator of a number of proteolytic cascades, as well as its obligate location at the cell surface. (Note that none of the other MT-MMPs has yet been inactivated in mice.) The defects seen in these animals are probably attributable to both deficiencies in the turnover of direct MMP-14 substrates



Figure 4

The phenotype of *Mmp14*-null mice, which is the most dramatic developmental phenotype of all MMP null transgenes. A rare example of an *Mmp14*-null mouse that has survived to 10 weeks of age is shown (front, alongside a normal littermate); note the severe dwarfism and craniofacial anomalies.

and abnormalities in the indirect substrates that are mediated by the activation of intermediary enzymes such as MMP-2 and MMP-13.

The importance of MMPs in physiological processes and human disease is now undisputed. What is not known, however, is the full extent to which they are involved in every process in mammals. The failure of broad-spectrum and even relatively specific MMP inhibitors in cancer therapy is not surprising, because the complete effects for a given inhibitor depend on the functions of its targets, and these are mostly unidentified and unknown. The fact that there are two other large families of related proteases with important physiological functions, the ADAM and ADAMTS families, suggests that additional caution should be taken when using MMP inhibitors to treat disease states. More subtle approaches are going to be required to modulate the functions of these enzymes in a more selective fashion [84]. By understanding the precise mechanisms by which MMPs are regulated and their interactions with various binding partners, it may be possible to block the deleterious functions of these enzymes without the concomitant loss of beneficial functions. This underscores the need for a greater investment than ever before in MMP biology and biochemistry. The next five years are expected to bring many exciting insights into the role of MMPs in development and human disease and may lead to even broader interest in what was once thought to be a highly specialized and restricted field.

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References

- Gross J, Lapiere CM: **Collagenolytic activity in amphibian tissues: a tissue culture assay.** *Proc Natl Acad Sci USA* 1962, **48**:1014-1022.
- Brinckerhoff CE, Matrisian LM: **Matrix metalloproteinases: a tail of a frog that became a prince.** *Nat Rev Mol Cell Biol* 2002, **3**:207-214.
- Coussens LM, Fingleton B, Matrisian LM: **Matrix metalloproteinase inhibitors and cancer: trials and tribulations.** *Science* 2002, **295**:2387-2392.
- Vihinen P, Kahari VM: **Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets.** *Int J Cancer* 2002, **99**:157-166.
- Egeblad M, Werb Z: **New functions for the matrix metalloproteinases in cancer progression.** *Nat Rev Cancer* 2002, **2**:161-174.
- Itoh Y, Nagase H: **Matrix metalloproteinases in cancer.** *Essays Biochem* 2002, **38**:21-36.
- Pendas AM, Balbin M, Llano E, Jimenez MG, Lopez-Otin C: **Structural analysis and promoter characterization of the human collagenase-3 gene (MMP13).** *Genomics* 1997, **40**:222-233.
- Shapiro SD: **Matrix metalloproteinase degradation of extracellular matrix: biological consequences.** *Curr Opin Cell Biol* 1998, **10**:602-608.
- Balbin M, Fueyo A, Knauper V, Lopez JM, Alvarez J, Sanchez LM, Quesada V, Bordallo J, Murphy G, Lopez-Otin C: **Identification and enzymatic characterization of two diverging murine counterparts of human interstitial collagenase (MMP-1) expressed at sites of embryo implantation.** *J Biol Chem* 2001, **276**:10253-10262.
- Wada K, Sato H, Kinoh H, Kajita M, Yamamoto H, Seiki M: **Cloning of three *Caenorhabditis elegans* genes potentially encoding novel matrix metalloproteinases.** *Gene* 1998, **211**:57-62.
- Llano E, Pendas AM, Aza-Blanc P, Kornberg TB, Lopez-Otin C: **Dm1-MMP, a matrix metalloproteinase from *Drosophila* with a potential role in extracellular matrix remodeling during neural development.** *J Biol Chem* 2000, **275**:35978-35985.
- Lepage T, Gache C: **Early expression of a collagenase-like hatching enzyme gene in the sea urchin embryo.** *EMBO J* 1990, **9**:3003-3012.
- Leontovich AA, Zhang J, Shimokawa K, Nagase H, Sarras MP Jr: **A novel hydra matrix metalloproteinase (HMMP) functions in extracellular matrix degradation, morphogenesis and the maintenance of differentiated cells in the foot process.** *Development* 2000, **127**:907-920.
- Kinoshita T, Fukuzawa H, Shimada T, Saito T, Matsuda Y: **Primary structure and expression of a gamete lytic enzyme in *Chlamydomonas reinhardtii*: similarity of functional domains to matrix metalloproteases.** *Proc Natl Acad Sci USA* 1992, **89**:4693-4687.
- McGeehan G, Burkhart W, Anderegg R, Becherer JD, Gillikin JW, Graham JS: **Sequencing and characterization of the soybean leaf metalloproteinase: structural and functional similarity to the matrix metalloproteinase family.** *Plant Physiol* 1992, **99**:1179-1193.
- Maidment JM, Moore D, Murphy GP, Clark IM: **Matrix metalloproteinase homologues from *Arabidopsis thaliana*. Expression and activity.** *J Biol Chem* 1999, **274**:34706-34712.
- Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M: **A matrix metalloproteinase expressed on the surface of invasive tumour cells.** *Nature* 1994, **370**:61-65.
- Itoh Y, Kajita M, Kinoh H, Mori H, Okada A, Seiki M: **Membrane type 4 matrix metalloproteinase (MT4-MMP, MMP-17) is a glycosylphosphatidylinositol-anchored proteinase.** *J Biol Chem* 1999, **274**:34260-34266.
- Pei D: **CA-MMP: a matrix metalloproteinase with a novel cysteine array, but without the classic cysteine switch.** *FEBS Lett* 1999, **457**:262-270.
- Brooks PC, Stromblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP, Chersesh DA: **Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3.** *Cell* 1996, **85**:683-693.
- Guo H, Li R, Zucker S, Toole BP: **EMMPRIN (CD147), an inducer of matrix metalloproteinase synthesis, also binds interstitial collagenase to the tumor cell surface.** *Cancer Res* 2000, **60**:888-891.
- Yu WH, Woessner JF Jr, McNeish JD, Stamenkovic I: **CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling.** *Genes Dev* 2002, **16**:307-323.

23. Li Q, Park PW, Wilson CL, Parks WC: **Matrilysin shedding of syndecan-1 regulates chemokine mobilization and trans-epithelial efflux of neutrophils in acute lung injury.** *Cell* 2002, **111**:635-646.
24. Bode W, Grams F, Reinemer P, Gomis-Ruth FX, Baumann U, McKay DB, Stocker W: **The metzincin-superfamily of zinc-peptidases.** *Adv Exp Med Biol* 1996, **389**:1-11.
25. Stocker W, Bode W: **Structural features of a superfamily of zinc-endopeptidases: the metzincins.** *Curr Opin Struct Biol* 1995, **5**:383-390.
26. Steffensen B, Wallon UM, Overall CM: **Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen.** *J Biol Chem* 1995, **270**:11555-11566.
27. Shipley JM, Doyle GA, Fliszar CJ, Ye QZ, Johnson LL, Shapiro SD, Welgus HG, Senior RM: **The structural basis for the elastolytic activity of the 92-kDa and 72-kDa gelatinases. Role of the fibronectin type II-like repeats.** *J Biol Chem* 1996, **271**:4335-4341.
28. Murphy G, Nguyen Q, Cockett MI, Atkinson SJ, Allan JA, Knight CG, Willenbrock F, Docherty AJ: **Assessment of the role of the fibronectin-like domain of gelatinase A by analysis of a deletion mutant.** *J Biol Chem* 1994, **269**:6632-6636.
29. Park HI, Ni J, Gerkema FE, Liu D, Belozero V, Sang QX: **Identification and characterization of human endometase (matrix metalloproteinase-26) from endometrial tumor.** *J Biol Chem* 2000, **275**:20540-20544.
30. Velasco G, Pendas AM, Fueyo A, Knauper V, Murphy G, Lopez-Otin C: **Cloning and characterization of human MMP-23, a new matrix metalloproteinase predominantly expressed in reproductive tissues and lacking conserved domains in other family members.** *J Biol Chem* 1999, **274**:4570-4576.
31. Morgunova E, Tuuttila A, Bergmann U, Isupov M, Lindqvist Y, Schneider G, Tryggvason K: **Structure of human pro-matrix metalloproteinase-2: activation mechanism revealed.** *Science* 1999, **284**:1667-1670.
32. Nagase H, Meng Q, Malinovsky V, Huang W, Chung L, Bode W, Maskos K, Brew K: **Engineering of selective TIMPs.** *Ann NY Acad Sci* 1999, **878**:1-11.
33. Van Wart HE, Birkedal-Hansen H: **The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family.** *Proc Natl Acad Sci USA* 1990, **87**:5578-5582.
34. Sato H, Kinoshita T, Takino T, Nakayama K, Seiki M: **Activation of a recombinant membrane type I-matrix metalloproteinase (MTI-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2.** *FEBS Lett* 1996, **393**:101-104.
35. Wang X, Pei D: **Shedding of membrane type matrix metalloproteinase 5 by a furin-type convertase: a potential mechanism for down-regulation.** *J Biol Chem* 2001, **276**:35953-35960.
36. Kang T, Nagase H, Pei D: **Activation of membrane-type matrix metalloproteinase 3 zymogen by the proprotein convertase furin in the trans-Golgi network.** *Cancer Res* 2002, **62**:675-681.
37. Pei D, Weiss SJ: **Furin-dependent intracellular activation of the human stromelysin-3 zymogen.** *Nature* 1995, **375**:244-247.
38. Baker AH, Edwards DR, Murphy G: **Metalloproteinase inhibitors: biological actions and therapeutic opportunities.** *J Cell Sci* 2002, **115**:3719-3727.
39. Mott JD, Thomas CL, Rosenbach MT, Takahara K, Greenspan DS, Banda MJ: **Post-translational proteolytic processing of procollagen C-terminal proteinase enhancer releases a metalloproteinase inhibitor.** *J Biol Chem* 2000, **275**:1384-1390.
40. Oh J, Takahashi R, Kondo S, Mizoguchi A, Adachi E, Sasahara RM, Nishimura S, Imamura Y, Kitayama H, Alexander DB, et al.: **The membrane-anchored MMP inhibitor RECK is a key regulator of extracellular matrix integrity and angiogenesis.** *Cell* 2001, **107**:789-800.
41. Takahashi C, Sheng Z, Horan TP, Kitayama H, Maki M, Hitomi K, Kitaura Y, Takai S, Sasahara RM, Horimoto A, et al.: **Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK.** *Proc Natl Acad Sci USA* 1998, **95**:13221-13226.
42. Wang Z, Juttermann R, Soloway PD: **TIMP-2 is required for efficient activation of proMMP-2 in vivo.** *J Biol Chem* 2000, **275**:26411-26415.
43. Apte SS, Fukai N, Beier DR, Olsen BR: **The matrix metalloproteinase-14 (MMP-14) gene is structurally distinct from other MMP genes and is co-expressed with the TIMP-2 gene during mouse embryogenesis.** *J Biol Chem* 1997, **272**:25511-25517.
44. Knauper V, Will H, Lopez-Otin C, Smith B, Atkinson SJ, Stanton H, Hembry RM, Murphy G: **Cellular mechanisms for human pro-collagenase-3 (MMP-13) activation. Evidence that MTI-MMP (MMP-14) and gelatinase a (MMP-2) are able to generate active enzyme.** *J Biol Chem* 1996, **271**:17124-17131.
45. Lehti K, Valtanen H, Wickstrom S, Lohi J, Keski-Oja J: **Regulation of membrane-type-I matrix metalloproteinase activity by its cytoplasmic domain.** *J Biol Chem* 2000, **275**:15006-15013.
46. Lehti K, Lohi J, Juntunen MM, Pei D, Keski-Oja J: **Oligomerization through hemopexin and cytoplasmic domains regulates the activity and turnover of membrane-type I matrix metalloproteinase.** *J Biol Chem* 2002, **277**:8440-8448.
47. Rozanov DV, Deryugina EI, Ratnikov BI, Monosov EZ, Marchenko GN, Quigley JP, Strongin AY: **Mutation analysis of membrane type-I matrix metalloproteinase (MTI-MMP). The role of the cytoplasmic tail Cys(574), the active site Glu(240), and furin cleavage motifs in oligomerization, processing, and self-proteolysis of MTI-MMP expressed in breast carcinoma cells.** *J Biol Chem* 2001, **276**:25705-25714.
48. Nakahara H, Howard L, Thompson EW, Sato H, Seiki M, Yeh Y, Chen WT: **Transmembrane/cytoplasmic domain-mediated membrane type I-matrix metalloproteinase docking to invadopodia is required for cell invasion.** *Proc Natl Acad Sci USA* 1997, **94**:7959-7964.
49. Annabi B, Lachambre M, Bousquet-Gagnon N, Page M, Gingras D, Beliveau R: **Localization of membrane-type I matrix metalloproteinase in caveolae membrane domains.** *Biochem J* 2001, **353**:547-553.
50. Jiang A, Lehti K, Wang X, Weiss SJ, Keski-Oja J, Pei D: **Regulation of membrane-type matrix metalloproteinase I activity by dynamin-mediated endocytosis.** *Proc Natl Acad Sci USA* 2001, **98**:13693-13698.
51. Uekita T, Itoh Y, Yana I, Ohno H, Seiki M: **Cytoplasmic tail-dependent internalization of membrane-type I matrix metalloproteinase is important for its invasion-promoting activity.** *J Cell Biol* 2001, **155**:1345-1356.
52. Yang Z, Strickland DK, Bornstein P: **Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2.** *J Biol Chem* 2001, **276**:8403-8408.
53. Barmina OY, Walling HW, Fiocco GJ, Freije JM, Lopez-Otin C, Jeffrey JJ, Partridge NC: **Collagenase-3 binds to a specific receptor and requires the low density lipoprotein receptor-related protein for internalization.** *J Biol Chem* 1999, **274**:30087-30093.
54. Lovejoy B, Welch AR, Carr S, Luong C, Broka C, Hendricks RT, Campbell JA, Walker KA, Martin R, Van Wart H, Browner MF: **Crystal structures of MMP-1 and -13 reveal the structural basis for selectivity of collagenase inhibitors.** *Nat Struct Biol* 1999, **6**:217-221.
55. Olson MW, Toth M, Gervasi DC, Sado Y, Ninomiya Y, Fridman R: **High affinity binding of latent matrix metalloproteinase-9 to the alpha2(IV) chain of collagen IV.** *J Biol Chem* 1998, **273**:10672-10681.
56. Suzuki M, Raab G, Moses MA, Fernandez CA, Klagsbrun M: **Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site.** *J Biol Chem* 1997, **272**:31730-31737.
57. Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y: **Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release.** *Biochem J* 1997, **322**:809-814.
58. Whitelock JM, Murdoch AD, Iozzo RV, Underwood PA: **The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases.** *J Biol Chem* 1996, **271**:10079-10086.
59. Manes S, Mira E, Barbacid MM, Cipres A, Fernandez-Resa P, Buesa JM, Merida I, Aracil M, Marquez G, Martinez AC: **Identification of insulin-like growth factor-binding protein-1 as a potential physiological substrate for human stromelysin-3.** *J Biol Chem* 1997, **272**:25706-25712.
60. Fowlkes JL, Thrailkill KM, Serra DM, Suzuki K, Nagase H: **Matrix metalloproteinases as insulin-like growth factor binding protein-degrading proteinases.** *Prog Growth Factor Res* 1995, **6**:255-263.

61. Fowlkes JL, Enghild JJ, Suzuki K, Nagase H: **Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures.** *J Biol Chem* 1994, **269**:25742-25746.
62. Noe V, Fingleton B, Jacobs K, Crawford HC, Vermeulen S, Steelant W, Bruyneel E, Matrisian LM, Mareel M: **Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1.** *J Cell Sci* 2001, **114**:111-118.
63. Steinhilber U, Weiske J, Badock V, Tauber R, Bommert K, Huber O: **Cleavage and shedding of E-cadherin after induction of apoptosis.** *J Biol Chem* 2001, **276**:4972-4980.
64. Preece G, Murphy G, Ager A: **Metalloproteinase-mediated regulation of L-selectin levels on leucocytes.** *J Biol Chem* 1996, **271**:11634-11640.
65. Asamoto M, Hokaiwado N, Cho YM, Takahashi S, Ikeda Y, Imaida K, Shirai T: **Prostate carcinomas developing in transgenic rats with SV40 T antigen expression under probasin promoter control are strictly androgen dependent.** *Cancer Res* 2001, **61**:4693-4700.
66. Fujiwara K, Matsukawa A, Ohkawara S, Takagi K, Yoshinaga M: **Functional distinction between CXC chemokines, interleukin-8 (IL-8), and growth related oncogene (GRO)alpha in neutrophil infiltration.** *Lab Invest* 2002, **82**:15-23.
67. McQuibban GA, Butler GS, Gong JH, Bendall L, Power C, Clark-Lewis I, Overall CM: **Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1.** *J Biol Chem* 2001, **276**:43503-43508.
68. Levi E, Fridman R, Miao HQ, Ma YS, Yayon A, Vlodavsky I: **Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1.** *Proc Natl Acad Sci USA* 1996, **93**:7069-7074.
69. Ganz T: **Defensins and host defense.** *Science* 1999, **286**:420-421.
70. Wilson CL, Ouellette AJ, Satchell DP, Ayabe T, Lopez-Boado YS, Stratman JL, Hultgren SJ, Matrisian LM, Parks WC: **Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense.** *Science* 1999, **286**:113-117.
71. Gearing AJ, Thorpe SJ, Miller K, Mangan M, Varley PG, Dudgeon T, Ward G, Turner C, Thorpe R: **Selective cleavage of human IgG by the matrix metalloproteinases, matrilysin and stromelysin.** *Immunol Lett* 2002, **81**:41-48.
72. Ruiz S, Henschen-Edman AH, Nagase H, Tenner AJ: **Digestion of C1q collagen-like domain with MMPs-1,-2,-3, and -9 further defines the sequence involved in the stimulation of neutrophil superoxide production.** *J Leukoc Biol* 1999, **66**:416-422.
73. Feng X, Tonnesen MG, Peerschke EI, Ghebrehiwet B: **Cooperation of C1q receptors and integrins in C1q-mediated endothelial cell adhesion and spreading.** *J Immunol* 2002, **168**:2441-2448.
74. Rozanov DV, Ghebrehiwet B, Postnova TI, Eichinger A, Deryugina EI, Strongin AY: **The hemopexin-like C-terminal domain of membrane type 1 matrix metalloproteinase regulates proteolysis of a multifunctional protein, gC1qR.** *J Biol Chem* 2002, **277**:9318-9325.
75. Martignetti JA, Aqeel AA, Sewairi WA, Boumah CE, Kambouris M, Mayouf SA, Sheth KV, Eid WA, Dowling O, Harris J, et al.: **Mutation of the matrix metalloproteinase 2 gene (MMP2) causes a multicentric osteolysis and arthritis syndrome.** *Nat Genet* 2001, **28**:261-265.
76. Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, et al.: **MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover.** *Cell* 1999, **99**:81-92.
77. Zhou Z, Apte SS, Soininen R, Cao R, Baaklini GY, Rauser RW, Wang J, Cao Y, Tryggvason K: **Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase 1.** *Proc Natl Acad Sci USA* 2000, **97**:4052-4057.
78. Gururajan R, Lahti JM, Grenet J, Easton J, Gruber I, Ambros PF, Kidd VJ: **Duplication of a genomic region containing the Cdc2L1-2 and MMP21-22 genes on human chromosome 1p36.3 and their linkage to DIZ2.** *Genome Res* 1998, **8**:929-939.
79. Zhu Y, Spitz MR, Lei L, Mills GB, Wu X: **A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility.** *Cancer Res* 2001, **61**:7825-7829.
80. Ghilardi G, Biondi ML, Mangoni J, Leviti S, DeMonti M, Guagnellini E, Scorza R: **Matrix metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness.** *Clin Cancer Res* 2001, **7**:2344-2346.
81. Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, Brinckerhoff CE: **A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription.** *Cancer Res* 1998, **58**:5321-5325.
82. Biondi ML, Turri O, Leviti S, Seminati R, Cecchini F, Bernini M, Ghilardi G, Guagnellini E: **MMP1 and MMP3 polymorphisms in promoter regions and cancer.** *Clin Chem* 2000, **46**:2023-2024.
83. Parks WC, Shapiro SD: **Matrix metalloproteinases in lung biology.** *Respir Res* 2001, **2**:10-19.
84. Lopez-Otin C, Overall CM: **Protease degradomics: a new challenge for proteomics.** *Nat Rev Mol Cell Biol* 2002, **3**:509-519.
85. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D: **Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis.** *Nat Cell Biol* 2000, **2**:737-744.
86. Itoh T, Tanioka M, Matsuda H, Nishimoto H, Yoshioka T, Suzuki R, Uehira M: **Experimental metastasis is suppressed in MMP-9-deficient mice.** *Clin Exp Metastasis* 1999, **17**:177-181.
87. Alexander CM, Selvarajan S, Mudgett J, Werb Z: **Stromelysin-1 regulates adipogenesis during mammary gland involution.** *J Cell Biol* 2001, **152**:693-703.
88. Bullard KM, Lund L, Mudgett JS, Mellin TN, Hunt TK, Murphy B, Ronan J, Werb Z, Banda MJ: **Impaired wound contraction in stromelysin-1-deficient mice.** *Ann Surg* 1999, **230**:260-265.
89. Wang M, Qin X, Mudgett JS, Ferguson TA, Senior RM, Welgus HG: **Matrix metalloproteinase deficiencies affect contact hypersensitivity: stromelysin-1 deficiency prevents the response and gelatinase B deficiency prolongs the response.** *Proc Natl Acad Sci USA* 1999, **96**:6885-6889.
90. Haro H, Crawford HC, Fingleton B, MacDougall JR, Shinomiya K, Spengler DM, Matrisian LM: **Matrix metalloproteinase-3-dependent generation of a macrophage chemoattractant in a model of herniated disc resorption.** *J Clin Invest* 2000, **105**:133-141.
91. Wilson CL, Heppner KJ, Labosky PA, Hogan BL, Matrisian LM: **Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin.** *Proc Natl Acad Sci USA* 1997, **94**:1402-1407.
92. Dunsmore SE, Saarialho-Kere UK, Roby JD, Wilson CL, Matrisian LM, Welgus HG, Parks WC: **Matrilysin expression and function in airway epithelium.** *J Clin Invest* 1998, **102**:1321-1331.
93. Powell WC, Fingleton B, Wilson CL, Boothby M, Matrisian LM: **The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis.** *Curr Biol* 1999, **9**:1441-1447.
94. Haro H, Crawford HC, Fingleton B, Shinomiya K, Spengler DM, Matrisian LM: **Matrix metalloproteinase-7-dependent release of tumor necrosis factor-alpha in a model of herniated disc resorption.** *J Clin Invest* 2000, **105**:143-150.
95. Coussens LM, Tinkle CL, Hanahan D, Werb Z: **MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis.** *Cell* 2000, **103**:481-490.
96. Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z: **MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes.** *Cell* 1998, **93**:411-422.
97. Engsig MT, Chen QJ, Vu TH, Pedersen AC, Therkidsen B, Lund LR, Henriksen K, Lenhard T, Foged NT, Werb Z, Delaisse JM: **Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones.** *J Cell Biol* 2000, **151**:879-889.
98. Liu Z, Shapiro SD, Zhou X, Twining SS, Senior RM, Giudice GJ, Fairley JA, Diaz LA: **A critical role for neutrophil elastase in experimental bullous pemphigoid.** *J Clin Invest* 2000, **105**:113-123.
99. Pyo R, Lee JK, Shipley OJM, Curci JA, Mao D, Ziporin SJ, Ennis TL, Shapiro SD, Senior RM, Thompson RW: **Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms.** *J Clin Invest* 2000, **105**:1641-1649.
100. Ducharme A, Frantz S, Aikawa M, Rakbin E, Lindsey M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P, Lee RT: **Targeted deletion**

- of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction.** *J Clin Invest* 2000, **106**:55-62.
101. Heymans S, Luttun A, Nuyens D, Theilmeier G, Creemers E, Moons L, Dyspersin GD, Cleutjens JP, Shipley M, Angellilo A, et al.: **Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure.** *Nat Med* 1999, **5**:1135-1142.
 102. Lelongt B, Bengatta S, Delauche M, Lund LR, Werb Z, Ronco PM: **Matrix metalloproteinase 9 protects mice from anti-glomerular basement membrane nephritis through its fibrinolytic activity.** *J Exp Med* 2001, **193**:793-802.
 103. Galis ZS, Johnson C, Godin D, Magid R, Shipley JM, Senior RM, Ivan E: **Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling.** *Circ Res* 2002, **91**:852-859.
 104. Cho A, Reidy MA: **Matrix metalloproteinase-9 is necessary for the regulation of smooth muscle cell replication and migration after arterial injury.** *Circ Res* 2002, **91**:845-851.
 105. Cataldo DD, Tournoy KG, Vermaelen K, Munaut C, Foidart JM, Louis R, Noel A, Pauwels RA: **Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation.** *Am J Pathol* 2002, **161**:491-498.
 106. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii, S: **Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand.** *Cell* 2002, **109**:625-637.
 107. Romanic AM, Harrison SM, Bao W, Burns-Kurtis CL, Pickering S, Gu J, Grau E, Mao J, Sathe GM, Ohlstein EH, Yue TL: **Myocardial protection from ischemia/reperfusion injury by targeted deletion of matrix metalloproteinase-9.** *Cardiovasc Res* 2002, **54**:549-558.
 108. Masson R, Lefebvre O, Noel A, Fahime ME, Chenard MP, Wendling C, Kebers F, LeMeur M, Dierich A, Foidart JM, Basset P, Rio MC: **In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy.** *J Cell Biol* 1998, **140**:1535-1541.
 109. Boulay A, Masson R, Chenard MP, El Fahime M, Cassard L, Bellocq JP, Sautes-Fridman C, Basset P, Rio MC: **High cancer cell death in syngeneic tumors developed in host mice deficient for the stromelysin-3 matrix metalloproteinase.** *Cancer Res* 2001, **61**:2189-2193.
 110. Lijnen HR, Van Hoef B, Vanlinthout I, Verstreken M, Rio MC, Collen D: **Accelerated neointima formation after vascular injury in mice with stromelysin-3 (MMP-11) gene inactivation.** *Arterioscler Thromb Vasc Biol* 1999, **19**:2863-2870.
 111. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD: **Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice.** *Science* 1997, **277**:2002-2004.
 112. Sternlicht MD, Werb Z: **How matrix metalloproteinases regulate cell behavior.** *Annu Rev Cell Dev Biol* 2001, **17**:463-516.