

Review

Matrix metalloproteinases (with accent to collagenases)

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Matrix metalloproteinases (MMPs) are a major group of enzymes that regulate cell–matrix composition. The MMPs are zinc-dependent endopeptidases known for their ability to cleave one or several extracellular matrix (ECM) constituents, as well as nonmatrix proteins. This review focuses on structural and functional elements of MMPs, and their roles in physiological and pathological processes, in which it is believed the MMPs play an important, or even indispensable role. According to their structural and functional characteristics, MMP family members have been classified into six different but closely related subgroups with fairly characteristic but often overlapping substrate specificities. MMP synthesis and functions are regulated by transcriptional activation, post-transcriptional processing (release of pro-domain, cell surface shedding), and control of activity by a family of endogenous inhibitors collectively known as tissue inhibitors of metalloproteinases (TIMPs). The balance of MMPs to TIMPs therefore determines matrix turnover, where either an excess of MMPs or a deficit of TIMPs may result in excess ECM degradation. Moreover, the recent development of selective and non-selective inhibitors of MMPs would also provide new insights in the knowledge of the relationship between activation of inflammatory cells and tissue remodeling and propose new therapeutic possibilities to the treatment of inflammatory disease.

Key words: Extracellular matrix, matrix metalloproteinases, inflammation.

INTRODUCTION

Modulation of cell–matrix interactions occurs through the action of unique proteolytic systems responsible for hydrolysis of a variety of extracellular matrix (ECM) components. By regulating the integrity and composition of the ECM structure, these enzyme systems play a pivotal role in the control of signals elicited by matrix molecules, which regulate cell proliferation, differentiation, and cell death. The turnover and remodeling of ECM must be highly regulated, since uncontrolled proteolysis contributes to abnormal development and to the generation of many pathological conditions characterized by either excessive degradation or a lack of degradation of ECM components. Matrix metalloproteinases (MMPs) are a major group of enzymes that regulate cell–matrix composition. The MMPs are zinc-dependent endopeptidases known for their ability to cleave one or several ECM constituents, as well as nonmatrix proteins. They

comprise a large family of proteases that share common structural and functional elements and are products of different genes. The first report of an enzyme from vertebrate (as opposed to bacterial) sources that was capable of attacking the triple helix of native type I collagen was published by Gross and Lapiere (1962). In this report, they demonstrated that the enzyme activity, secreted by cultured tissue fragments of tail fin skin from resorbing tadpole tails in metamorphosis, was true collagenase acting on collagen at 27°C at neutral pH (Vu et al., 2000).

The reason for this interest on MMPs is pointed out most clearly by Table 1, which shows some of the points of involvement of the MMPs in normal biologic and pathologic processes. Embryonic growth and tissue morphogenesis are fundamental events that require disruption of ECM barriers to allow cell migration and micro-environmental matrix remodeling. The ability of MMPs to degrade structural components of ECM and BM has supported their direct roles in these processes (Vu et al., 2000). Although the main function of MMPs is removal of ECM during tissue resorption and progression of many

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Table 1. Involvement of the MMPs in normal and pathological processes (Parks and Mecham, 1998).

Normal and pathological processes in which MMPs are implicated					
Normal			Pathological		
Development	Reproduction	Maintenance	Tissue destruction	Fibrotic diseases	Weakening of matrix
Blastocyst implantation	Endometrial cycling	Remodeling of bone	Rheumatoid arthritis	Liver cirrhosis	Dilated cardiomyopathy
Embryonic development	Graafian follicle rupture	Hair follicle cycle	Osteoarthritis	Fibrotic lung disease	Epidermolysis bullosa
Nerve growth	Luteolysis	Wound healing	Cancer invasion	Otosclerosis	Aortic aneurysm
Growth plate cartilage removal	Cervical dilatation	Angiogenesis	Cancer metastasis	Atherosclerosis	
Skeletal, bone growth	Postpartum uterine involution	Apoptosis	Decubitus ulcer	Multiple sclerosis	
Nerve outgrowth	Mammary gland morphogenesis	Nerve regeneration	Gastric ulcer		
Enamel maturation	Mammary gland involution	Macrophage function	Corneal ulceration		
Primary tooth resorption	Rupture of fetal membranes	Neutrophil function	Periodontal disease		

diseases, it is notable that MMPs also alter biological functions of ECM macromolecules by specific proteolysis. Most MMP genes are highly expressed in a number of reproductive processes, including menstrual cycle, ovulation and uterine, breast and prostate involution (Curry and Osteen, 2003).

MMPs are also involved in wound healing, a tissue-remodeling process which involves the migration of keratinocytes at the edge of the wound to re-epithelialize the damaged surface. The role of MMPs in angiogenesis is also wide and complex. Many MMPs are produced by endothelial cells and have been described to be important for the formation of new blood vessels in both physiological and pathological conditions. MMPs are considered to participate in dissemination of cancer cells by breaking down ECM, but they are also important in creating an environment that supports the initiation and maintenance of growth of primary and metastatic tumors (Chambers and Matrisian, 1997). MMPs have recently been found also in pulpal tissue and odontoblasts, where they play a role in dentin matrix formation and modulation during caries progression and secondary dentin formation

(Tjäderhane et al., 1998).

DOMAIN STRUCTURE AND FUNCTION

If one examines the MMPs starting from the N terminus, the following features are seen: 1) signal peptide, 2) propeptide, 3) furin-cleavage site insert, 4) catalytic domain, 5) fibronectin-like repeats, 6) hinge region, 7) hemopexin domain, and 8) membrane insertion extension (Figure 1) (Massova et al., 1998).

Signal peptide: It is typically a stretch of 17 to 20 residues, rich in hydrophobic amino acids, that serves as a signal for secretion into the endoplasmic reticulum for eventual export from the cell.

Propeptide domain: It consists of approximately 80 to 90 amino acids containing a cysteine residue, which interacts with the catalytic zinc atom via its side chain thiol group. A highly conserved sequence (...PRCGXPD...) is present in the propeptide. Removal of the propeptide by proteolysis results in proenzyme activation, as all members of the MMPs family are produced

in a latent form. The Cys within this sequence (the "cysteine switch") ligates the catalytic zinc to maintain the latency of pro-MMPs (Borden and Heller, 1997). This cysteine is found in all MMPs, including those that have the furin-cleavage site.

Furin-cleavage site insert: This stretch of about nine residues includes the consensus sequence of RXKR/RRKR that leads to intracellular cleavage by furin. MMP-11, -14, -15, -16, and -17 possess this sequence. In the remaining enzymes, a cleavage by external proteases occurs in the middle of the propeptide, partially exposing the zinc and leading to autolytic cleavage of the remainder of the propeptide. The exact site of final cleavage may vary within a given enzyme leading to different degrees of activation, particularly in the case of MMP-1.

The catalytic domain (about 170 amino acids): It contains two zinc ions and at least one calcium ion coordinated to various residues. One of the two zinc ions is present in the active site and is involved in the catalytic processes of the MMPs. The catalytic zinc ion is essential for the proteolytic activity of MMPs; the three histidine

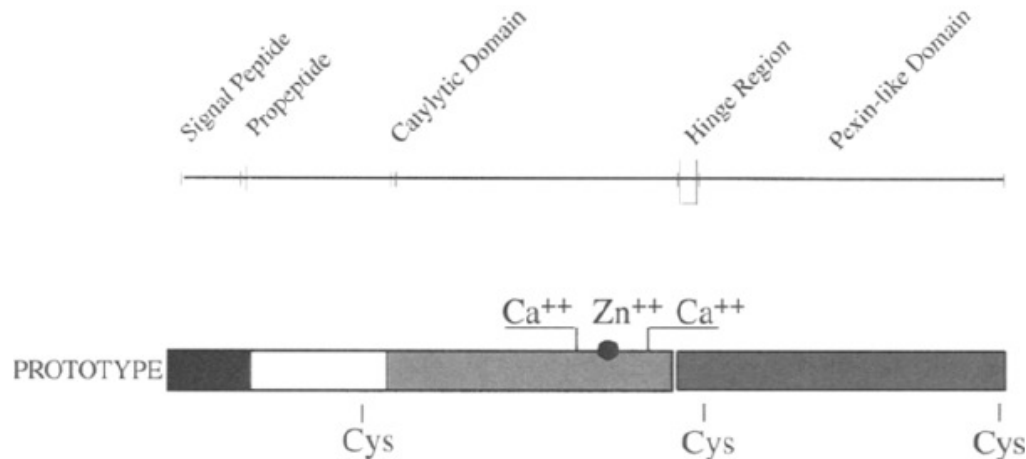


Figure 1. Representation of a prototype matrix metalloproteinase. All the interstitial collagenases contain variations of the domains indicated in this representation (Birkedal-Hansen et al., 1993).

residues that coordinate with the catalytic zinc are conserved among all the MMPs. The 50 to 54 residues at the C-terminal end of the catalytic domain include the site of binding of the catalytic zinc. Little is known about the roles of the second zinc ion and the calcium ion within the catalytic domain, but the MMPs are shown to possess high affinities for structural zinc and calcium ions (Bode et al., 1994).

Fibronectin-like repeats: These specialized structures aid the binding of enzyme to gelatin and collagen substrates (Steffensen et al., 1995).

Hinge region: The catalytic domain is connected to the following hemopexin domain by a linker region usually referred to as the hinge region. It ranges in length from 0 to 75 residues. The longest hinge is found in MMP-9 and shows considerable homology to type V collagen in that it is rich in proline. A typical hinge contains about 16 residues including a number of proline residues (Pendas et al., 1997).

The hemopexin-like domain (about 210 amino acid) of MMPs: It is highly conserved and shows sequence similarity to the plasma protein, hemopexin. The hemopexin-like domain has been shown to play a functional role in substrate binding and/or in interactions with the TIMPs, a family of specific MMP protein inhibitors (Gomis-Rüth et al., 1997). The hemopexin domain is an absolute requirement for collagenases to cleave triple helical interstitial collagens (Bode, 1995), although the catalytic domains alone retain proteolytic activity toward other substrates.

Membrane insertion extension: Although most MMPs have their C terminus at the end of the hemopexin domain, the four MT-MMPs have a further extension that governs insertion of these proteases into the cell

membrane. Its length ranges from about 80 to 110 residues.

Classification of MMPs

There are now at least 18 distinct vertebrate MMPs (Barrett et al., 1998). They were first described in vertebrates (Gross and Lapiere, 1962) including humans, but have since been found in invertebrates and plants. They are distinguished from other endopeptidases by their dependence on metal ions as cofactors, their ability to degrade extracellular matrix, and their specific evolutionary DNA sequence. Table 2 lists the currently known MMPs and related forms from lower organisms. The MMPs share high protein sequence homology and have defined domain structures and thus, according to their structural properties, the MMPs are classified as either secreted MMPs or membrane anchored MMPs, which are further divided into eight discrete subgroups: five are secreted and three are membrane-type MMPs (MT-MMPs) (Heikkilä, 2005).

According to their structural and functional characteristics, MMPs family members have been classified into different but closely related subgroups with fairly characteristic but often overlapping substrate specificities. This classification considers collagenases (MMP-1, 8, 13 and 18), gelatinases (MMP-2 and 9), stromelysins (MMP-3, 10, 11), elastases (MMP-7 and 12), and membrane type MMPs (MT-MMPs, MMP-14, 15, 16 and 17) and a group of unnamed members (Massova et al., 1998). In addition to a remarkably common 3-D structure (Vu et al., 2000), MMPs share a similar gene arrangement, suggesting that they arose by duplications of an ancestor gene. At least eight of the known human MMPs genes (MMP-1, -3, -7, -8, -10, -12, -13 and -20) are clustered on chromosome 11 at 11q21-23. Other MMP genes are scattered among chromosomes 1, 8, 12,

Table 2. MMP (Clark, 2001).

MMP-No.	Name	E.C. number	Other names/notes
MMP-1	Collagenase 1	3.4.24.7	Interstitial collagenase
MMP-2	Gelatinase A	3.4.24.24	72 kDa gel'ase, type IV collagenase
MMP-3	Stromelysin 1	3.4.24.17	Transin, proteoglycanase, CAP
(MMP-4)	Procollagen peptidase	—	Discontinued = MMP-3)
(MMP-5)	3/4-Collagenase	—	Discontinued = MMP-2)
(MMP-6)	Acid metalloprotease	—	Discontinued = MMP-3)
MMP-7	Matrilysin	3.4.24.23	Pump-1
MMP-8	Collagenase 2	3.4.24.34	Neutrophil collagenase
MMP-9	Gelatinase B	3.4.24.35	92 kDa gel'ase, type V collagenase
MMP-10	Stromelysin 2	3.4.24.22	Transin 2
MMP-11	Stromelysin 3		Furin motif
MMP-12	Macrophage elastase	3.4.24.65	Metalloelastase
MMP-13	Collagenase 3		Rodent intersititial collagenase
MMP-14	Membrane type matrix metalloproteinase 1		MT1-MMP, furin motif
MMP-15	Membrane type matrix metalloproteinase 2		MT2-MMP, furin motif
MMP-16	Membrane type matrix metalloproteinase 3		MT3-MMP, furin motif
MMP-17	Membrane type matrix metalloproteinase 4		MT4-MMP, furin motif
MMP-18	Collagenase 4		<i>Xenopus</i>
MMP-19	No trivial name		RASI-1
MMP-20	Enamelysin		
XMMP	No trivial name		<i>Xenopus</i> , furin motif
MMP-C31	(also H19, Y19)		<i>Caenorhabditis elegans</i>
—	Envelysin	3.4.24.12	Sea urchin
—	Soybean MMP		<i>Glycine max</i> , no cDNA
—	Fragilysin	3.4.24.74	<i>Bacteroides fragilis</i>

14, 16, 18, 20, and 22 (Shapiro, 1998).

A BRIEF OVERVIEW OF THE INDIVIDUAL INTERSTITIAL COLLAGENASES

The role of all the factors participating in the spread of inflammation and necrosis in pulp tissue is not completely understood. Since MMPs, the host enzymes responsible for the extracellular matrix degradation, have been suggested to be important in other inflammatory conditions such as periodontitis, it is possible that they also participate in the pathogenesis of pulp and periapical inflammation. The area of interstitial collagenase chemistry and biology, in parallel with that of the matrix metalloproteinases in general, has undergone explosive growth. These enzymes are known to be effective in various inflammatory and other tissue destructive diseases. Collagenases are actively taking part in the tissue destruction and granulation tissue formation in chronic apical periodontitis (Wahlgren, 2003). Collagenase-1 (MMP-1), collagenase-2 (MMP-8) and collagenase-3 (MMP-13) comprise the collagenase subfamily capable of initiating degradation of native fibrillar collagen types I, II, III, V and IX (Knäuper et al., 1996).

Collagenases can also digest a number of other ECM and non-ECM molecules.

The collagenases differ in their substrate specificities and functional roles. MMP-1 preferably degrades collagen III, MMP-8 prefers type I collagen, and MMP-13 prefers collagen II (Massova et al., 1998; Knäuper et al., 1996). MMP-1 is the most often associated collagenase with normal tissue remodeling. MMP-1 is expressed by fibroblasts, endothelial cells, macrophages, hepatocytes, chondrocytes, osteoblasts, odontoclasts, tumors cells and migrating epidermal keratinocytes and its expression can be induced in certain inflammatory diseases and cancers (Giambenardi et al., 1998).

The MMP-1 gene is localized on chromosome 11q22 (Brinckerhoff et al., 2000). It is constitutively expressed at low levels under normal physiological conditions; however, its expression may increase markedly in pathological conditions. MMP-1 is present in jaw cyst wall extracts and cyst fluids (Teronen et al., 1995). MMP-8 was first cloned from messenger RNA (mRNA) extracted from the peripheral leucocytes of a patient with chronic granulocytic leukemia (Hasty et al., 1990). MMP-8 is synthesized in polymorphonuclear leukocytes during their maturation in bone marrow and stored in specific intracellular granules, out of which it is secreted to the

Table 3. Tissue inhibitors of metalloproteinases (TIMPs) (Clark, 2001).

Family member	Reported functions
TIMP-1	Inhibition of all known MMP family members. Associates with proMMP-9. Inhibits angiogenesis. Erythroid-potentiating activity.
TIMP-2	Inhibitions all known MMP family members. Associates with MT1-MMP and MMP-2 at cell surface and regulates MMP-2 activation.
TIMP-3	Inhibition of all known MMP family members. Extracellular matrix-associated. Mutation associated with Sorsby's fundus dystrophy.
TIMP-4	Inhibition of all known MMP family members. Restricted expression suggests tissue specific TIMP function.

cell environment in response to external triggering stimuli and the enzyme thus plays a key role in tissue destruction during inflammatory diseases (Ding et al., 1997). The presence of MMP-8 in healthy odontoblasts and pulp tissue suggests that MMP-8, an efficient inducer of type I collagenolysis, may contribute to the modulation of dentin and secondary dentin formation (Wahlgren, 2003). In pulpitis, the MMP-8 was expressed in various inflammatory cells, such as PMNs, macrophages, plasma cells and in endothelial cells. In pulpitis, MMP-8 was considered to originate from both PMN and non-PMN lineage cells. The main MMP-8 positivity was accumulated around the pulp abscess suggesting that it also contributes to the abscess formation (Wahlgren, 2003).

Monocytes/macrophages express MMP-8 and MMP-13, and these MMPs may work not only extracellularly, taking part in tissue destruction, but also intracellularly, in the phagocytic process (Knäuper et al., 1996). MMP-8 can also be detected in mucosal fibroblasts, chondrocytes, odontoblasts, monocyte/macrophages, melanoma cells, leukaemia cells, malignant plasma cells and human endothelial cells (Kiili et al., 2002; Moilanen et al., 2003; Wahlgren et al., 2001). The major collagenase species detected in inflamed human periodontium is MMP-8 (Romanelli et al., 1999). MMP-8 is expressed *in vivo* by bronchial epithelial cells and macrophages involved in bronchiectasis, chondrocytes in rheumatoid arthritis and osteoarthritic lesions, rheumatoid synovial fibroblasts, in human gingival sulcular epithelial cells, in cells of human atheroma, in plasma cells associated with oral keratocysts and by cells in proliferating and migratory wound epithelia, in dermal fibroblasts and inflammatory cells (Tervahartiala et al., 2000).

In vitro, MMP-8 is released out of leucocytes during the chemotactic stimulation and, *in vivo*, response to local inflammatory conditions (Tschesche, 1995). MMP-13 was originally cloned from human breast tumor cDNA library (Hasty et al., 1990). It has the widest substrate selection among the interstitial collagenases and, in addition to collagens, it is able to cleave various BM components. MMP-13 cleaves type II collagen more efficiently than types I and III, and among interstitial collagenases, it is

most effective in cleaving gelatin (Ding et al., 1997). The physiological expression of MMP-13 seems to be limited only to developing bone (Kiili et al., 2002), wound healing and teeth (Moilanen et al., 2003). It is widely expressed in pathological conditions including rheumatoid arthritis, osteoarthritis, periodontitis, chronic ulcerations, hypertrophic chondrocytes, osteoblasts as well as plasma cells and many carcinoma and melanoma cells (Wahlgren et al., 2001; Knäuper et al., 1996; Romanelli et al., 1999). MMP-13 is predicted to have an important role in tumors invasion and metastasis due to its wide substrate-specificity together with catalytic efficiency and its upregulated expression in cancer cells (Tervahartiala et al., 2000).

Cleavage of type I collagen by MMP-1 and by MMP-13 initiates keratinocyte migration during reepithelialization and osteoclast activation, respectively (Tschesche, 1995). In the study of Andonovska et al. (2008), results from quantitative enzyme method demonstrated that the concentrations of collagenases (MMP-1, -8, -13) in chronic pulp inflammation were significantly higher than those of the control group (normal pulp tissue). In addition, concentrations of collagenases in patients with diagnosed periapical lesions were significantly higher than those of the control group and were higher from the patients with chronic pulp inflammation.

Regulation of activity of MMPs

The activity of MMPs is regulated at several different stages: transcriptional, post-transcriptional, controlled at the protein level via their activators, inhibitors, and/or cell surface location. MMPs levels are usually low in normal adult tissues, and with some exceptions, their production and activity are maintained at practically undetectable levels. The MMPs are tightly regulated, as they need to be present in the right cell type and pericellular location at the right time and in the right amount. A loss of activity control may result in diseases. Thus, MMPs are regulated at the transcriptional and post-transcriptional levels and their expression becomes elevated, when there is a challenge to the system, such as wound healing, repair or

remodeling processes, in diseased tissues, and even in several cell types grown in culture (Clark, 2001).

MMPs are also regulated by activation of the precursor zymogens and inhibition by endogenous inhibitors, tissue inhibitors of metalloproteases (TIMPs). The extracellular activation of most MMPs is regulated by a proteolytic cascade and can be initiated by other already activated MMPs or by several serine proteases. The TIMPs are the main natural inhibitors of MMPs and, as such, they tightly control metalloproteases catalytic activity. Thus, the balance between MMPs and TIMPs is critical for the eventual ECM remodeling. Disruption of the balance between MMPs and TIMPs contributes to pathophysiological processes, such as many chronic tissue destructive inflammatory and autoimmune diseases.

Also, MMPs activity can be inhibited by the synthetic inhibitors. Synthetic inhibitors of MMP generally contain a chelating group which binds the catalytic zinc atom at the MMP active site tightly. Common chelating groups include hydroxamates, carboxylates, thiols, and phosphinyls. Hydroxamates are particularly potent inhibitors of MMPs and other zinc-dependent enzymes, due to their bidentate chelation of the zinc atom. Other substituents of the inhibitors are more or less specific for given MMPs (Meng et al., 2008). The transcriptional regulation of MMPs appears to represent the key step in MMPs regulation, since most MMPs genes are expressed only when active physiological or pathological tissue remodeling takes place (Matrisian, 1990).

Overall, it is clear that the transcriptional regulation of MMPs production is a very complicated phenomenon including the regulation of the production and degradation of transcription factors and the regulation of their transactivating activities and via these processes the modulation of MMPs production in cells. Spontaneous sequence variations in the promoters of MMPs could influence critical steps in binding to transcription factors or overall transcriptional efficiency, which results in discrepancy in the expression of MMPs in different individuals. In fact, genetic polymorphisms can alter the binding sites of some functional regulating factors and influence the level of MMPs expression (Arakaki et al., 2009).

Inhibition mechanism of TIMPs

There are different mechanisms to inhibit or down-regulate MMPs. Inhibition may take place by interaction with an active Zn^{2+} -site or by cleaving the active enzyme or binding it to a non-active complex form. To influence the MMP levels, it is also possible to down-regulate MMPs at the transcriptional level (Birkedal-Hansen et al., 1993). The proteolytic activity of the MMPs involved in extracellular matrix degradation must be precisely regulated by their endogenous protein inhibitors, the TIMPs. Disruption of this balance results in serious diseases. TIMPs (21 to 30 kDa) are the major

endogenous regulators of MMPs activities in the tissue, and four homologous TIMPs (TIMPs-1 to -4) have been identified to date (Table 3) (Matrisian, 1990).

The TIMPs are secreted proteins, but may be found at the cell surface in association with membrane-bound proteins. TIMPs have effects on cell growth and survival that cannot always be clearly reconciled with their ability to abrogate MMPs activity. TIMP-1 was first identified as an agent that stimulates growth of some cell lines (Gasson et al., 1985). It was first noted by Bauer et al. (1975) that cultured human fibroblasts produced an inhibitory protein. TIMP-1 inhibits MMP-1, MMP-3 and MMP-9 more effectively than TIMP-2. TIMP-2 also has erythroid potentiating activity and stimulates the growth of lymphoma cells (Stetler-Stevenson et al., 1997) and fibroblasts. TIMP-2 protein has been detected around migrating keratinocytes in human wounds and is believed, at least partially, to be produced by these cells (Vaalamo et al., 1997).

TIMP-2 inhibits proMMP-2 over 10-fold more effectively than TIMP-1. However, TIMP-2 has a bi-functional effect on MMP-2, since MT-MMP mediated proMMP-2 activation requires a tiny amount of TIMP-2 to make activation progress, whereas a greater concentration of TIMP-2 inhibits MMP-2 (Brew et al., 2000). High levels of TIMP-3 promote apoptosis in many cell types *in vitro* and *in vivo* (Vaalamo et al., 1997), and this effect is associated with death receptor modulation. TIMP-3 inhibits at least MMP-2 and MMP-9, whereas TIMP-4 is a good inhibitor for all classes of MMPs without remarkable preference for specific MMPs. Active MMP-13 is an example of an MMP, which is inhibited by all types of TIMPs (Palosaari, 2003). TIMP-4 can also instigate apoptosis in transformed cardiac fibroblasts but inhibits apoptosis in human breast cancer cells *in vitro* and mammary tumors *in vivo*; it is thus a tumor promoter when over expressed. TIMP-4 regulates MMP-2 activity both by inhibiting MT1-MMP and by inhibiting activated MMP-2 (Palosaari, 2003).

While TIMPs usually inhibit already active MMP, gelatin-binding MMPs are an exception, since TIMP reversibly binds to the proforms of both MMP-2 and MMP-9. It is widely appreciated that TIMPs inhibit cell invasion *in vitro*, tumor genesis, metastasis *in vivo*, and angiogenesis. TIMPs exhibit additional biological functions. TIMP-1 and TIMP-2 have mitogenic activities on a number of cell types, whereas over expression of these inhibitors reduces tumor cell growth, and TIMP-2, but not TIMP-1, inhibits basic fibroblast growth factor-induced human endothelial cell growth. These biological activities of TIMPs are independent of MMP-inhibitory activities. TIMPs are therefore important regulators not only in matrix turnover but also in cellular activities (Palosaari, 2003).

SUMMARY AND FUTURE PROSPECTS

In recent years, MMPs have gained considerable

attention in many studies on normal tissue events, inflammation, and disease processes, and this enhanced interest is probably due to several factors. For the most part, MMPs are produced or activated when needed, and expression of these enzymes provides a reliable indicator of ongoing tissue remodeling. Thus, for investigators, these enzymes are models of gene products that are accurately regulated and precisely targeted to specific extracellular substrates by a wide variety of cells during numerous normal tissue processes, such as wound healing, bone resorption, and morphogenesis. In contrast, exuberant production of MMPs is a hallmark of many destructive diseases, such as arthritis and chronic ulcerations, and of many disease-related processes, such as inflammation, metastasis, and angiogenesis, and aberrant regulation of MMPs production is thought to be a primary mechanism contributing to disease progression and injury.

As such, understanding how MMPs expression and activation are controlled and identifying which proteinases are produced by which cells under defined conditions will have a great impact on our understanding of normal biology and disease pathogenesis (Sorsa et al., 2004). Several reports in recent years have demonstrated that various MMPs act on non-matrix proteins, such as cytokines, chemokines, receptors and antimicrobial peptides (Sorsa et al., 2004). Thus, MMPs should not be viewed solely as proteinases for matrix catalysis, but rather as extracellular processing enzymes involved in regulating cell-cell and cell-matrix signaling events, typically gain-of-function processing of latent proteins (Page-McCaw et al., 2007).

MMPs continue to be discovered. Highly specialized MMPs such as enamelysin, found only in the embryonic tooth enamel may have corresponding undiscovered counterparts in other tissues or organs. The human genome project will no doubt uncover further examples. A great deal still remains to be learned about the MMPs and their inhibition. We know almost nothing about the natural substrates of the MMPs in vivo, about how the cell senses and regulates the level of enzymes and inhibitors out in the matrix, and about the ability of the cell to substitute one enzyme for another in knockouts. Attention will no doubt focus on ways to regulate the activities through gene manipulation and through specific inhibitors of enzyme expression or activity.

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