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Progress in matrix metalloproteinase research

Gillian Murphy[a,*] and **Hideaki Nagase**[b]

[a] Department of Oncology, University of Cambridge, Cancer Research UK Cambridge Research Institute, Cambridge, UK

[b] Department of Matrix Biology, The Kennedy Institute of Rheumatology Division, Imperial College London, London W6 8LH, UK

Abstract

Matrix metalloproteinases (MMPs) are now acknowledged as key players in the regulation of both cell–cell and cell–extracellular matrix interactions. They are involved in modifying matrix structure, growth factor availability and the function of cell surface signalling systems, with consequent effects on cellular differentiation, proliferation and apoptosis. They play central roles in morphogenesis, wound healing, tissue repair and remodelling in response to injury and in the progression of diseases such as arthritis, cancer and cardiovascular disease. Because of their wide spectrum of activities and expression sites, the elucidation of their potential as drug targets in disease or as important features of the repair process will be dependent upon careful analysis of their role in different cellular locations and at different disease stages. Novel approaches to the specific regulation of individual MMPs in different contexts are also being developed.

Keywords

Matrixin; Tissue inhibitors of metalloproteinases; Cancer; Cardiovascular disease; Arthritis

1. Introduction

Normal physiological remodelling processes such as development, morphogenesis and tissue repair involve precisely regulated degradation of the extracellular matrix (ECM). Remodelling also occurs during the course of diseases such as arthritis, cancer and cardiovascular disease, in part in an attempt at repair, but is frequently dysregulated and destructive (Milner and Cawston, 2005; Newby, 2005; Sternlicht and Werb, 2001; Burrage and Brinckerhoff, 2007). Various types of proteinases have been implicated in ECM degradation, but a major enzyme group involved is that of the matrix metalloproteinases (MMPs), also called the matrixins. Most recent studies have shown that MMPs have other roles in the regulation of the cellular environment and modulating many bioactive molecules at the cell surface and can act in concert to influence cell behavior (Egeblad and Werb, 2002). In order to ascertain the potential of MMP inhibitors as therapeutics, one of the current major research challenges is to determine the specific function of individual enzymes in different cellular and temporal contexts in both normal and pathological processes.

2. The matrixin family members

In 1962 Gross and Lapiere first reported vertebrate collagenoytic activity in tadpole tissues (tailfin, skin, intestine and gill) undergoing metamorphosis (Gross and Lapiere, 1962). These

*Corresponding author. Tel.: +44 1223 404470; fax: +44 1223 762323, gm290@cam.ac.uk (G. Murphy).

tissues are rapidly remodelling the ECM, hence triggering apoptosis, cell differentiation and growth (Shi et al., 1998). This discovery stimulated many researchers to look in human tissues, since degradation of collagen is important in wound healing, tissue regeneration and diseases such as arthritis, cancer, atherosclerosis, aneurysm and tissue ulcerations. The first human collagenase to be purified was from rheumatoid synovium (Woolley et al., 1975) which has similar properties to tadpole collagenase, cleaving triple helical type I collagen at a single site about 3/4 away from the N-terminus (Woolley et al., 1975). It took another 11 years to deduce the primary structure of human collagenase from fibroblasts (MMP-1) by cDNA cloning (Goldberg et al., 1986). This revealed a homology with a transcript reported in the previous year in rat fibroblasts induced by growth factor, oncogene and cellular transformation (Matrisian et al., 1985) which was later identified as rat MMP-3 (stromelysin 1). The human genome has 24 matrixin genes which include a duplicated *Mmp23* gene. Thus, there are 23 different MMPs. Matrixins are also found in fruit fly (*Drosophila melanogaster*), nematode (*Caenorhabditis elegans*), sea urchin (*Paracentrotus lividus*), hydra (*Hydra vulgaris*) and plants (*Arabidopsis thaliana*).

The matrixins are multidomain zinc metalloproteinases, but sequence homology with the catalytic domain of fibroblast collagenase (collagenase 1) or MMP-1 is a criterion for membership of the family. The MEROPS database (http://www.merops.sanger.ac.uk) classifies the MMPs/matrixin subfamily of metzincins as M10. The catalytic domain contains the Zn^{2+} binding motif HEXXHXXGXXH and a conserved methionine, forming a 'Met-turn' eight residues downstream, which supports the active site cleft structure around the catalytic Zn^{2+}. Thus, they are typical metzincin members (Bode et al., 1993), but what distinguishes MMPs from other metzincins are the primary structures of the catalytic domains. The matrixins are synthesized as pre-proenzymes and the signal peptide is removed during translation to generate proMMPs. The propeptides have the 'cysteine switch' motif PRCGXPD in which the cysteine residue coordinates with the catalytic Zn^{2+} in the catalytic domain, keeping the proMMPs inactive (Van Wart and Birkedal-Hansen, 1990).

Table 1 lists the vertebrate MMPs. They are grouped into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs and others and this grouping is based on domain organization and substrate preference, but is otherwise more or less historical; more recently discovered 'non-conformist' enzymes being grouped in the 'other' category. The use of bioinformatic methods to compare the primary sequences of the MMPs suggests six evolutionary sub-groups (A–F) of the MMPs: sub-group A, MMP-19, -26 and -28 ; sub-group B, MMPs -11, -21 and -23; sub-group C, MMP-17 and -25; sub-group D, MMP-1, -3, -8, -10, -12, -13 and -27; sub-group E, MMP-14, -15, -16 and -24; sub-group F, MMP-2, -7, -9 and -20 (Huxley-Jones et al., 2007).

3. Mammalian matrixins and their domain constituents

In this review we use the conventional grouping of the matrixins and only briefly discuss the main biochemical properties as many reviews have given detailed accounts of their substrate specificities (Overall, 2002; Nagase et al., 2006; Cauwe et al., 2007). Table 1 describes the domain composition of human matrixin members and their chromosome location, and Fig. 1 shows their domain composition and arrangements. Typically MMPs consist of a propeptide of about 80 amino acids, a catalytic metalloproteinase domain of about 170 amino acids, a linker peptide of variable lengths (also called the 'hinge region') and a hemopexin domain of about 200 amino acids. MMP-7 (matrilysin 1), MMP-26 (matrilysin 2) and MMP-23 are exceptions as they lack the linker peptide and the hemopexin domain. MMP-23 has a unique C-terminal cysteine-rich domain and an immunoglobulin-like domain immediately after the C-terminus of the catalytic domain.

3.1. Collagenases

There are three collagenases; collagenase 1(MMP-1), collagenase 2, also called neutrophil collagenase (MMP-8) and collagenase 3 (MMP-13). They consist of propeptide, catalytic and hemopexin domains. They play an important role in cleaving fibrillar collagen types I, II and III into characteristic 3/4 and 1/4 fragments, but they also have activity against other ECM molecules and soluble proteins. The catalytic domains of collagenases can cleave non-collagenous substrates, but they are unable to cleave native fibrillar collagens in the absence of their hemopexin domains. The cooperation between the two domains is considered to be important for the expression of their collagenolytic activity (Chung et al., 2004).

3.2. Gelatinases

Gelatinase A (MMP-2) and gelatinase B (MMP-9) belong to this sub-group. Both enzymes have three repeats of a fibronectin type II motif inserted into the catalytic domain (Figs. 1 and 2). They share similar proteolytic activity and degrade denatured collagens, gelatins and a number of ECM molecules including native type IV, V and XI collagens, laminin and aggrecan core protein. MMP-2 digests native collagen types I, II and III in a similar manner to the collagenases (Aimes and Quigley, 1995;Patterson et al., 2001), but MMP-9 does not. However, the collagenolytic activity of MMP-2 is much weaker than MMP-1 or other collagenases in solution. Nevertheless, because proMMP-2 is recruited to the cell surface and activated by the membrane-bound MT-MMPs, it may accumulate pericellularly and express reasonable local collagenolytic activity. It may also act as a ‘collaborator’ activity, digesting collagenase-clipped collagen to smaller fragments because those fragments denature at body temperature 37 °C (Murphy et al., 1985).

3.3. Stromelysins

MMP-3, MMP-10 and MMP-11 are called stromelysins 1, 2 and 3, respectively. They have the same domain arrangement as the collagenases, but they do not cleave interstitial collagens. MMP-3 and MMP-10 are similar in structure and substrate specificity, but MMP-11 (stromelysin 3) is distantly related. MMP-3 and MMP-10 digest a number of ECM molecules and participate in proMMP activation, but MMP-11has very weak activity toward ECM molecules (Murphy et al., 1993). Another difference is that both MMP-3 and MMP-10 are secreted from the cells as inactive proMMPs, but MMP-11 is activated intracellularly by furin and secreted from the cell as an active enzyme (Pei and Weiss, 1995). The MMP-11 gene is located on chromosome 22, whereas MMP-3 and MMP-10 are on chromosome 11, along with MMP-1, -7, -8, -12, -20, -26 and -27 (Table 1). An alternatively spliced transcript of MMP-11 lacking the signal peptide and the propeptide domain encode an active intracellular form, but its function is not known (Luo et al., 2002).

3.4. Matrilysins

MMP-7 and MMP-26 belong to this category. The structural feature of these MMPs is that they lack the hemopexin domain. MMP-7 is synthesized by epithelial cells and secreted apically. In the intestine it acts intracellularly to process procryptidins to bactericidal forms. It degrades ECM components, but it also cleaves cell surface molecules such as Fas–ligand, pro-tumor necrosis factor α, syndecan 1 and E-cadherin to generate soluble forms, hence acting as a form of ‘sheddase’ (Parks et al., 2004). MMP-26 is expressed in normal cells such as those of the endometrium and in some carcinomas and digests several ECM molecules (Marchenko et al., 2004).

3.5. Membrane-bound MMPs (MT-MMPs)

There are two types of MT-MMPs which include four type I transmembrane proteins (MMP-14, -15, -16 and -24) and two glycosylphosphatidylinositol-anchored proteins

(MMP-17 and -25). They all have a furin-like proprotein convertase recognition sequence RX [R/K]R at the C-terminus of the propeptide. They are therefore activated intracellularly and active enzymes are likely to be expressed on the cell surface. All MT-MMPs, except MT4-MMP (MMP-17) (English et al., 2001) can activate proMMP-2. MT1-MMP (MMP-14) can activate proMMP-13 on the cell surface (Knäuper et al., 1996). However, MT1-MMP itself has collagenolytic activity against type I, II and III collagens (Ohuchi et al., 1997). MT1-MMP null mice exhibit skeletal abnormalities during post-natal development, which is attributed to the lack of collagenolytic activity (Holmbeck et al., 1999).

3.6. Other MMPs

There are seven matrixins which are not catalogued in the above sub-groups. Among them MMP-12, MMP-20 and MMP-27 do have a similar domain arrangement and chromosome location as stromelysins. Therefore, it may be more appropriate to sub-group them into the stromelysins.

Metalloelastase (MMP-12) was originally discovered in macrophages, but is also found in hypertrophic chondrocytes (Kerkelä et al., 2001) and osteoclasts (Hou et al., 2004). It degrades elastin and a number of other ECM molecules and is essential in macrophage migration (Shipley et al., 1996b).

MMP-19 is a potent basement membrane-degrading enzyme, but it also digests other ECM molecules (Stracke et al., 2000). It is widely expressed in human tissues (Pendas et al., 1997) and is considered to play a role in tissue remodelling, wound healing and epithelial cell migration by cleaving the laminin 5 γ2 chain (Sadowski et al., 2003, 2005). MMP-19 deficient mice developed diet induced obesity and reduced skin cancer susceptibility (Pendas et al., 2004).

Enamelysin (MMP-20) is a tooth specific MMP expressed in newly formed tooth enamel and digests amelogenin (Ryu et al., 1999). MMP-20-null mice have a deteriorating enamel organ morphology due to the lack of amelogenin processing (Bartlett et al., 2004). Amelogenin imperfecta, a genetic disorder with defective enamel formation, is due to mutation at MMP-20 cleavage sites (Li et al., 2001).

MMP-21 is expressed in various fetal and adult tissues. It is also found in basal and squamous cell carcinomas (Ahokas et al., 2003) and macrophages of granulomatous skin lesions and in fibroblasts in dermatofibromas (Skoog et al., 2006). Information on its action on ECM components is not available, although it does have gelatinolytic activity.

MMP-23 is unique among the matrixin members as it lacks the cysteine switch motif in the propeptide and the hemopexin domain. The latter is substituted by cysteine-rich immunoglobulin-like domains (Velasco et al., 1999). It is a type II membrane protein having a transmembrane domain at the N-terminal of the propeptide, such that the enzyme is solubilized as the membrane anchored propeptide is cleaved by a proprotein convertase (Pei et al., 2000). It is expressed predominantly in ovary, testis and prostate, suggesting a specialized role in the reproductive process (Velasco et al., 1999).

MMP-27 was first cloned from chicken embryo fibroblast cDNA library (Yang and Kurkinen, 1998). The chicken enzyme digests gelatin and casein and causes autolysis of the enzyme, but little information is available on the activity of mammalian enzyme. MMP-27 is expressed in B-lymphocytes and its levels increase when treated with anti-(IgG/IgM) in culture (Bar-Or et al., 2003).

Epilysin (MMP-28) was first cloned from the human keratinocyte and testis cDNA libraries, and is expressed in many tissues such as lung, placenta, heart, gastrointestinal tract and testis (Lohi et al., 2001). The enzyme expressed in basal keratinocytes in skin is considered to function in wound repair (Saarialho-Kere et al., 2002). It is also elevated in cartilage from patients with osteoarthritis and rheumatoid arthritis (Kevorkian et al., 2004; Momohara et al., 2004). Overexpression of recombinant MMP-28 in lung adenocarcinoma cells induces irreversible epithelial mesenchymal transition, accompanied by cell surface loss of E-cadherin, processing of latent TGFβ complex and increased levels of TGFβ, along with up-regulation of MT1-MMP and MMP-9 and collagen invasive activity (Illman et al., 2006).

4. Three-dimensional (3D) structures

Numerous 3D structures of MMPs have been determined both by X-ray crystallography and NMR spectroscopy (Maskos, 2005) including full-length proMMP-1 (Jozic et al., 2005), proMMP-2 (Morgunova et al., 1999) and the proMMP-2-TIMP-2 complex (Morgunova et al., 2002). Fig. 2 shows the structure of proMMP-2-TIMP-2 complex and individual domain structures. The overall polypeptide folds of propeptide domains, catalytic domains and hemopexin domains from other MMPs are essentially superimposable.

Structures have been solved for the propeptide domain of proMMP-1 (Jozic et al., 2005), proMMP-2 (Morgunova et al., 1999), proMMP-3 without the linker region and the hemopexin domain (Becker et al., 1995) and proMMP-9 without the linker region and the hemopexin domain (Elkins et al., 2002). The propeptide consists of three α chains and connecting loops (Fig. 2). The proteinase susceptible ‘bait region’ is located between helix 1 and helix 2, but the structure of this region has not been resolved in the case of proMMP-1, proMMP-3 and proMMP-9 due to its flexible nature. This region of proMMP-2 is however stabilized by a disulphide bond. The ‘cysteine switch’ motif PRCGXPD in the propeptide is a signature of matrixins as all MMPs, except MMP-23, harbour this motif. The cysteine switch sequence lies in the substrate binding site, but the orientation of this segment is opposite from that of a peptide substrate. The SH group of the cysteine interacts with the catalytic zinc ion. Upon activation the interaction of *Cys*-Zn^{2+} is disrupted, which allows a water molecule to bind to the zinc atom. The propeptide domains of MMPs may also play an important role in folding the proenzymes during synthesis.

The catalytic domain consists of a 5-stranded β-pleated sheet, three α-helices and a connective loops (Fig. 2). It contains two zinc ions; one catalytic and one structural. Usually three calcium ions are found which are thought to stabilize the structure. MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16) and MT5-MMP (MMP-24) have a so-called MT loop, an additional 8 residues between β-strand II and III. This loop is critical for activation of proMMP-2 in the case of MT1-MMP (English et al., 2001). Binding of a peptide substrate to the enzyme is primarily dictated by the structure of the substrate binding site of the catalytic domain, including a pocket called the “S1 pocket” located to the right of the zinc atom. This pocket is hydrophobic in nature, but variable in depth depending on the MMP. It is therefore one of the determining factors of substrate specificity of MMPs. For protein substrate recognition, on the other hand, interaction with the non-catalytic domains is often required (see below). Binding of a substrate to the enzyme is thought to displace the water molecule from the zinc, and peptide bond hydrolysis is facilitated by the carboxylate group of the glutamate in the active site which draws a proton from the displaced water and allows a nucleophilic attack of the polarized water on the carbonyl carbon of the peptide bond.

Fibronectin type II (Fn II) domains are inserted in the loop between the fifth β-strand and the second helix of the catalytic domains of gelatinases (Fig. 2). The Fn II domains in MMP-2 and MMP-9 have a similar conformation. They consist of two antiparallel β-sheets connected with

a short α-helix and stabilized by two disulfide bonds. However, the placements of the Fn II domains in the two gelatinases are significantly different. After superimposing the catalytic domains of proMMP-2 and proMMP-9, Fn II domain 1 and domain 3 fall roughly in the same places, but the position of domain 2 differs. Domain 2 of proMMP-2 has an area that interacts with the catalytic domain, but the corresponding domain of proMMP-9 is rotated and twisted away from the catalytic domain without making contacts (Elkins et al., 2002). Domain 3 in both progelatinases make contact with the propeptide and with the catalytic domain (Morgunova et al., 1999;Elkins et al., 2002).

The linker region connects the catalytic domain and the hemopexin domain. This region is considered to be flexible, as shown for MMP-9 (Rosenblum et al., 2007), but a number of prolines are found (Iyer et al., 2006; Jozic et al., 2005; Li et al., 1995; Morgunova et al., 1999). This suggests that this region may have some structural constraint suited for specific functions. Mutation of this region in MMP-1 (Tsukada and Pourmotabbed, 2002) and MMP-8 (Knäuper et al., 1997) reduced the collagenolytic activity, supporting the notion that the correct movement and arrangement between the catalytic domain and the hemopexin domain is important for this activity. Studies of MMP-9 and MMP-14 have found that the linker region can be extensively and heterogeneously N- and O-glycosylated and that these sugar chains have significant effects on the cellular biochemistry of these MMPs (Van den Steen et al., 2006; Wu et al., 2004).

The hemopexin domain has a 4-bladed β-propeller structure with a single disulfide bond between the first and the fourth blades (Fig. 2) (Gomis-Rüth et al., 1996;Li et al., 1995;Libson et al., 1995). Each blade is made up of four antiparallel βstrands and the four β-sheets have similar scaffolds. The centre of the propeller generally contains one calcium ion and a chloride. The hemopexin domains are essential for collagenases to express their triple helical collagen degrading activity and for MT1-MMP to dimerize on the cell surface. The latter event is essential for MT1-MMP to activate proMMP-2 (Itoh et al., 2001) and to cleave collagen (Itoh et al., 2006).

5. Substrate specificity

The substrate specificity profiles of the MMPs have been defined either by identifying the cleavage sites of such protein substrates or by a series of synthetic peptide substrates (Nagase, 2001). More recent approaches include the use of phage display systems and proteomic screening (Overall and Blobel, 2007). In general, MMPs cleave a peptide bond before a residue (referred as the P1′ residue) with a hydrophobic side chain, such as Leu, Ile, Met, Phe, or Tyr. The hydrophobic residues fit into the S1′ specificity pocket of the enzyme, whose size and shape differ considerably among MMPs (Bode et al., 1999). In addition to the S1′ pocket, other substrate contact sites (subsites) also participate in the substrate specificity of the enzyme (Nagase, 2001); particularly Pro in the P3 site (the third residue before the cleavage site) is generally favored by most MMPs.

5.1. The role of the extra-catalytic and non-catalytic domains of MMPs in substrate specificity

Ancillary non-catalytic domains of the matrixins play key roles for some MMPs to recognize their natural ECM substrates. The fibronectin type II domain of gelatinases are important for their effective cleavage of type IV collagen, elastin and gelatins, but they do not affect hydrolysis of small peptides (Murphy et al., 1994; Shipley et al., 1996a). Their interaction with specific ECM molecules allows the catalytic site to be oriented to the scissile peptide bond. For collagenases to cleave triple helical collagens their hemopexin domains are essential. Studies by Chung et al. (2004) have demonstrated that collagenases locally unwind the triple helical collagen chains before they cleave the peptide bonds. It is postulated that the collagen binding groove consists of the catalytic domain and the hemopexin domain. In the case of

proMMP-1, the prodomain interacts with both the catalytic and hemopexin domains (Jozic et al., 2005), rendering it in a 'closed' configuration of proMMP-1 and the pro-domain partially blocks the putative collagen binding site. The removal of the pro-domain upon activation opens the groove ('open' form) and allows collagenases to bind to collagen (Iyer et al., 2006; Li et al., 1995). This explains the earlier reports that proMMP-1 does not bind to collagen I, but the activated collagenase does (Allan et al., 1991; Welgus et al., 1985). Nevertheless, it is not yet known how collagenases unwind the triple helical strands. Effective unwinding may require cooperative molecular interactions between collagenase and the triple helical substrate.

ProMMP-2 forms a complex with TIMP-2. This is due to a tight binding between the hemopexin domain of the proenzyme and the non-inhibitory C-terminal domain of the inhibitor. This complex formation is essential for proMMP-2 activation by MT1-MMP on the cell surface, which requires a quaternary molecular complex (Itoh and Seiki, 2005). Because the proMMP-2-TIMP-2 complex can inhibit active MMPs, the complex binds to an active MT1-MMP on the cell surface through the free N-terminal MMP inhibitory domain of TIMP-2. This presumably orients the propeptide of proMMP-2 to an adjacent active MT1-MMP, and the specific reaction of two MT1-MMP molecules is driven by the hemopexin domains. TIMP-3 and TIMP-4 form a complex with proMMP-2 in a similar manner to TIMP-2 and the proMMP-2-TIMP-4 complex interacts with MT1-MMP, but it is non-productive in terms of MMP-2 activation (Bigg et al., 2001). The biological significance of the proMMP- 2-TIMP-3 complex is not known.

The hemopexin domain of proMMP-9 can also bind to TIMP-1 and TIMP-3 through their C-terminal domains. ProMMP-9 in neutrophils partially binds to neutrophil gelatinase associated lipocalin-like molecule (NGAL) through an intermolecular disulfide bond (Kjeldsen et al., 1993). However, the biological significance of these complexes is not clear, except that proMMP- 9-TIMP complexes inhibit metalloproteinases and activation of proMMP-9 of the complex by MMPs is restricted.

6. Metalloproteinase inhibitors

6.1. Endogenous inhibitors of MMPs

α_2-Macroglobulin and tissue inhibitors of metalloproteinases (TIMPs) are two major inhibitors of MMPs. Human α_2-macroglobin is a glycoprotein of 725 kDa consisting of four identical subunits of 180 kDa. It acts as a general proteinase inhibitor and is found in the blood and in tissue fluids. Most endopepidases, regardless of their class, are inhibited by entrapping the enzyme within the macroglobulin (Barrett and Starkey, 1973). The complex is then rapidly cleared by the low density lipoprotein receptor-related protein-1-mediated endocytosis (Strickland et al., 1990). MMP activities in the fluid phase are mainly regulated by α_2-macroglobulin and related proteins.

TIMPs were originally found as collagenase inhibitors in serum and in the conditioned medium from fibroblast cultures (Vater et al., 1979; Welgus et al., 1979). There are four TIMPs in humans (TIMP-1 to -4) of 22–29 kDs. TIMP-1 and TIMP-3 are glycoproteins and TIMP-2 and TIMP-4 do not contain carbohydrates. They inhibit all MMPs tested so far, but TIMP-1 is a poor inhibitor of MT1-MMP, MT3-MMP, MT5-MMP and MMP-19 (Baker et al., 2002). Recent studies have shown that TIMPs can inhibit a broader spectrum of metalloproteinases. For example, TIMP-1 inhibits ADAM-10 (Amour et al., 2000), whilst TIMP-2 inhibits ADAM12 (Jacobsen et al., 2008). TIMP-3 has a much broader inhibition profile including ADAM-10 (Amour et al., 2000), -12 (Jacobsen et al., 2008) and -17 (Amour et al., 1999) and ADAMTSs (ADAMTS-1, -2, -4 and -5) (Kashiwagi et al., 2001; Rodriguez-Manzaneque et al., 2002; Wang et al., 2006). Because of this broad inhibitory spectrum, TIMP-3 ablation in mice causes lung emphysema-like alveolar damage (Leco et al., 2001) and faster apoptosis of

mammary epithelial cells after weaning (Fata et al., 2001), whereas TIMP-1-null mice and TIMP-2-null mice do not exhibit obvious abnormalities unless those animals are challenged. This indicates that TIMP-3 is a major regulator of metalloproteinase activities *in vivo*.

Several other proteins have been reported to inhibit selected members of MMPs: a secreted form of β-amyloid precursor protein inhibits MMP-2 (Higashi and Miyazaki, 2003); A C-terminal fragment of procollagen C-proteinase enhancer protein inhibits MMP-2 (Mott et al., 2000); and RECK, a GPI-anchored glycoprotein that suppresses angiogenesis inhibits MMP-2, MMP-9 and MMP-14 (Oh et al., 2001). However, the mechanisms of action of these proteins are not known. Tissue factor pathway inhibitor-2, a serine proteinase inhibitor, was reported to inhibit MMP-1 and MMP-2 (Herman et al., 2001), but this effect could not be confirmed (Du et al., 2003).

6.2. Inhibition mechanism of TIMPs and TIMP variants

The primary structure of TIMP-1 was deduced from cDNA sequencing (Docherty et al., 1985). There are four TIMPs which are homologous to each other and consisting of 184–194 amino acids with 12 conserved cysteines. The determination of the six disulfide bond arrangement of TIMP-1 indicated that it consists of an N-terminal domain and a C-terminal domain, each containing three disulfide bonds(Williamson et al., 1990). The N-terminal domain alone is fully functional for MMP inhibition (Murphy et al., 1991). The first 3D structure was solved for the N-terminal domain of TIMP-2 by NMR spectroscopy and revealed of a β-barrel structure with oligosaccharide–oligonucleotide binding (OB)-fold and two small α-helices at the C-terminal end (Williamson et al., 1994), but it did not show how TIMP-2 interacts with MMPs. A possible MMP binding site of TIMP-1 was first proposed to be around Val69-Cys70, based on the observation that the Val69-Cys70 bond was cleaved by neutrophil elastase which inactivated TIMP-1, but this cleavage was protected in the TIMP-1-MMP-3 complex (Nagase et al., 1997). The NMR structure of N-TIMP-2 showed that Val69 is a part of exposed ridge-like structure formed with the N-terminal segment of the inhibitor where Cys1 and Cys70 are linked by disulfide bond. Mutagenesis around the ridge region altered the reactivity of TIMP-1 with MMPs, which further supported that this region might be the MMP reactive site in the TIMPs (Huang et al., 1997). The exact mechanism by which TIMP inhibit MMPs had to wait for the crystal structures of the TIMP-1-MMP-3 catalytic domain complexes (Gomis-Rüth et al., 1997), which showed that the N-terminal four residues Cys1-Thr-Cys-Val4 and the residues Glu67-Ser-Val-Cys70 that are linked by a disulfide bond slots into the active site of the MMPs (Fig. 3A). The catalytic zinc atom of MMP-3 is bidentately chelated by the N-terminal amino group and the carbonyl group of Cys1, which expels the water molecule bound to the zinc atom (Fig. 3B). This region occupies about 75% of the protein–protein interaction in case of the complex of the catalytic domain of MMP-3 and TIMP-1 and the side chain of Thr2 enter into the S1′ pocket of the enzyme. The crystal structure of TIMP-2-MMP-14 catalytic domain showed a similar complex formation as TIMP-1 and MMP-3, but TIMP-2 has a longer AB loop (see Fig. 4) which make additional contact with MMP-14 (Fernandez-Catalan et al., 1998).

The structural information on TIMP-MMP complexes led to further mutagenesis studies to address the nature of the specificity of TIMPs. The residues in TIMP-1 and TIMP-2 which showed significant changes in inhibition specificity upon mutagenesis are highlighted in Fig. 4. Mutation of the position 2 (Thr in TIMP-1) greatly affected the affinity of TIMPs for MMPs, but there was a very poor correlation in residues that affect inhibition potency and substrate specificity for different MMPs (Meng et al., 1999). Substitution to glycine essentially inactivated TIMP-1 to inhibit MMPs (Meng et al., 1999). Additional mutations at position Val4 and Ser68 and their combination generated inhibitors discriminatory between MMP-1, -2 and -3 (Wei et al., 2003), indicating that MMP reactive ridge may be modified to make TIMPs

selective. Ile35, Tyr36 and Asn38, located in the longer AB loop of TIMP-2, fit into a special cavity on the surface of the MMP-14 molecule and mutation of Tyr36 indicated that it is a key residues for this interaction (Williamson et al., 2001).

There are several studies which represent gain of function mutagenesis. TIMP-1 is a poor inhibitor of MT1-MMP, MMP-19 and tumor necrosis factor α converting enzyme (TACE/ADAM17), but it gains reactivity for all three metalloproteinases by replacing Thr98 with Leu, which is found in both TIMP-2 and TIMP-3 (Lee et al., 2003, 2004a), and further improved with replacement of the AB loop (Lee et al., 2004a). TIMP-2 does not inhibit TACE, but it was also converted to a functional inhibitor by replacing key residues of the AB loop with those of TIMP-3, in combination with S2T/A70S/V71L mutation (Lee et al., 2004b). Full-length TIMP-4 is a weak inhibitor of TACE, but truncation of the C-terminal domain increased the reactivity and replacement with the TIMP-3 AB loop further improved the inhibitory activity with a sub-nanomolar K_i (Lee et al., 2005).

The importance of chelation of the N-terminal amino group of TIMPs to the catalytic zinc ion of the MMPs, was confirmed by carbamylation of the N-terminus (Higashi and Miyazaki, 1999), or addition of an extra Ala at the N-terminus (Wingfield et al., 1999), both of which inactivated the TIMPs with respect to MMP inhibition. However, Wei et al. (2005) have reported that addition of Ala to the N-terminus of TIMP-3 did not significantly alter the ability to inhibit ADAM-17, although it impaired the inhibition of MMPs. Mutation of Thr2 of TIMP-3 to Gly also inactivated TIMP-3 to inhibit MMPs, but retained the inhibitory activity for ADAM17. Those studies suggest that ADAM inhibition mechanism by TIMP-3 may be different from that for MMPs. The 3D structure of the complex formed between TIMP-3 with an extra Ala at its N-terminus and ADAM17 would provide new insights into this interaction.

6.3. Other biological activities of TIMPs

When TIMP-1 was cloned its identity with the protein having erythroid potentiating activity (EPA) became apparent (Docherty et al., 1985; Gasson et al., 1985). Later, TIMP-2 was also shown to have EPA (Stetler-Stevenson et al., 1992). Hayakawa et al. (1992, 1994) reported that TIMP-1 and TIMP-2 have cell growth promoting activity for many types of cells, which may explain the EPA properties of TIMP-1 and TIMP-2. Both TIMPs also protect cells from apoptosis (Valente et al., 1998; Guedez et al., 1998). On the other hand, TIMP-3 causes apoptosis of tumor cells and smooth muscle cells (Ahonen et al., 2003; Baker et al., 1998; Smith et al., 1997). This activity is thought to be due to the inhibition of TACE which prevents the shedding of cell death receptors such as Fas, tumor necrosis factor receptor-1 and TNF-related apoptosis inducing ligand receptor-1 (Ahonen et al., 2003). TIMP-3 can also bind to vascular endothelial growth factor (VEGF) receptor 2 and inhibits downstream signalling of VEGF and angiogenesis (Qi et al., 2003). The phenotypes of *TIMP-3* null mice have largely been ascribed to the loss of metalloproteinase function but there is clearly a need to be aware of other non-inhibitory functions of all the TIMPs.

6.4. Synthetic inhibitors of matrix MMPs

The development of synthetic inhibitors of MMPs initially relied on the use of a peptide sequence recognized by the targeted protease, to which a chelating moiety is introduced to interact with the zinc ion of the active site. This strategy generated several potent non-peptidic inhibitors of metalloproteinases, but one drawback of this approach has been the poor selectivity displayed by this class of inhibitors. Despite knowledge of the three-dimensional structure of numerous catalytic domains of metalloproteinases, the development of highly specific synthetic active-site-directed inhibitors of metalloproteinases that discriminate members of the matrixinin family remains a challenge. Due to the nature of MMP active sites, the development of specific MMP inhibitors will need to combine sophisticated theoretical and

experimental approaches to identify the specific structural features of each individual metalloproteinase that can be exploited to obtain the desired selectivity. Site specific delivery is also a worthwhile goal and may permit the use of inhibitors with lower in vitro potency.

The majority of the clinical trials using synthetic metalloproteinase inhibitors were conducted with cancer patients and proved unsuccessful, principally due to the lack of efficacy and untoward side effects including musculoskeletal pain and tendonitis in some cases (Milner and Cawston, 2005). In animal models metalloproteinase inhibitors have been relatively effective in preventing development and progression of early disease, but have had little effect on advanced disease. Hence their poor performance is unsurprising given the design of the trials that have been undertaken to date (Coussens et al., 2002), many of which involved the use of MMP inhibitors as single agent therapies for patients with advanced disease. It is clear that a more detailed knowledge of the roles of the metalloproteinases in each disease and their spatiotemporal expression are needed to guide the development and use of inhibitors of clinical value. This would need to be coupled with the development of robust markers to detect efficacy.

New generation variants of the hydroxamate based inhibitors which are relatively more specific are now being evaluated (Fisher and Mobashery, 2006). Newer trends in inhibitor design are also in evidence, due to concerns about the metabolism of hydroxamates and the ability to chelate other metalloproteins. Thiol, hydroxypyrone and barbiturate based inhibitors have been described, as well as phosphinates which act as transition state analogues. Tetracycline based inhibitors appear to have some efficacy which is hard to explain on the basis of their weak MMP binding and may be due to effects on MMP synthesis. It is clear that MMP inhibitors could be extremely efficacious as therapeutics in a number of diseases but the design criteria are very demanding. Good selectivity for the target MMP, correct spatial and temporal administration and appropriate dosage regimes all need to be fulfilled.

6.5. Novel approaches to metalloproteinase inhibition

Alternative strategies for the development of metalloproteinase inhibitors are being considered (Overall and Lopez-Otin, 2002). The production of specific antibody fragments developed from phage library screens could be promising. Such proteins could target the active site in a more specific way than chemical inhibitors. They may also be used to define exo sites on the metalloproteinases that determine their substrate specificity or extracellular location. For instance, the hemopexin domain of the collagenase, MMP-1, is essential for the specificity of the catalytic domain cleavage of collagen. MMP-2 is localized to specific extracellular collagenous sites by its fibronectin domains and MT1-MMP (MMP-14) requires the hemopexin domain for cell surface clustering as part of its collagenolytic capacity and ability to mediate proMMP 2 activation. The hemopexin domain also determines its binding to CD44 (Suenaga et al., 2005). Further understanding of the nature of these interactions will allow the development of either specific chemical antagonists of binding or of fragment antibodies that target these interactions. Several other strategies may potentially downregulate metalloproteinases. Both the intracellular signalling pathways and the downstream transcription factors which induce gene expression are being intensively studied. Blockade of mitogen activated protein kinase (MAPK) pathways, nuclear factor (NF)-κB or activator protein(AP)-1 have all been shown to have some efficacy in vitro or in animal models of arthritis (Mix et al., 2004). The use of biological reagents to block inflammatory cytokines also reduces metalloproteinase expression in many cases. The tetracyclines, which are rather weak inhibitors of MMP catalytic activity, also influence on their synthesis and have been successfully trialed in rheumatoid arthritis (Voils et al., 2005). Gene therapy, either overexpressing TIMPs or using cytokines/growth factors, has been successful in animal models and may have future uses once the problems of the safe and efficient delivery of genes into target cells and tissues have been overcome (Van der Laan et al., 2003).

7. MMP pathophysiology

The expression of MMPs has been documented during many stages of mammalian development, from embryonic implantation (Harvey et al., 1995) to the morphogenesis of specific tissues, including lung, bone and mammary gland (reviewed by Vu and Werb, 2000; Page-McCaw et al., 2007). Other physiological processes, including growth and wound healing (Ravanti and Kähäri, 2000), also involve the expression of abundant MMPs in specific spatial and temporal locations. Similarly, many disease processes show specific patterns of MMP expression, such as cancer (reviewed by (Sternlicht and Werb, 2001; Egeblad and Werb, 2002), cardiovascular disease (Newby, 2005) and arthritis (Milner and Cawston, 2005; Burrage and Brinckerhoff, 2007; Murphy and Nagase, 2008). Many animal models of disease have been developed and studied with respect to the relevance of MMPs in disease progression. Such studies have reiterated the observation that the relevance of MMP expression profiles varies with the nature of the cells, the stage of the disease and the genetic make up of the animal (Hu et al., 2007; Lemaitre and D'Armiento, 2006; Martin and Matrisian, 2007).

Inactivation of specific genes in mice has presented a rather different picture. With very few exceptions, embryogenesis and subsequent growth have been relatively normal, indicating the potential for both compensatory effects of MMP function and adaptive development. However, pathological challenges to the system have proved fruitful in promoting the understanding of the roles of MMPs. Table 2 gives examples of the roles of MMPs in the mouse models of cancer, cardiovascular disease and arthritis. For example, deficiency of MMP-7 decreased the development of tumors in a mouse model of human cancer (Wilson et al., 1997). Over-expression of MMPs in mice has also been useful; excess MMP-1 in the heart caused functional defects due to the reduction in collagen (Kim et al., 2000) and overexpression of MMP-3 in the mammary gland leads to spontaneous pre-malignant lesions and the development of cancers (Sternlicht et al., 1999).

7.1. MMPs and cancer

A number of cancer studies have correlated MMP expression and disease outcome (comprehensively documented by Egeblad and Werb, 2002). MMPs can contribute to tumor growth not only by degradation of the ECM but by the release of sequestered growth factors or the generation of bioactive fragments (Noel et al., 2008). For instance, MMP-9 mobilizes VEGF from the ECM (Bergers et al., 2000) and cleaves type IV collagen to generate tumstatin, an angiogenesis inhibitor (Hamano et al., 2003). MMPs are also important in tumor progression, promoting invasion, immune escape and many other events. However, particularly in carcinomas, MMPs are associated with the supporting 'host' cells rather than the tumor cells (Noel et al., 2008) and this finding has emphasized the emerging theme of the importance of the microenvironment in tumorigenesis. The task of determining the specific role of individual enzymes in each case is hence somewhat daunting. However, the widespread use of both gene and tissue microarrays is generating informative data. For example, Minn et al. (2007) identified a set of genes, including MMP-1 and MMP-2 that mediate breast cancer metastasis to lungs in human patients using careful transcriptomic analyses coupled with functional verification and clinical validation. Acuff et al. (2006) used a customized Affymetrix protease microarray (Hu/Mu ProtIn chip) designed to distinguish human and mouse genes to analyze the expression of proteases and protease inhibitors in lung cancer. Using an orthotopic lung cancer model, they showed that murine MMP-12, MMP-13 and cathepsin K were up-regulated in tumor tissue compared with normal mouse lung. To determine the relevance of stromal proteases detected using this model system, they compared the results to an analysis of human lung adenocarcinoma specimens using Affymetrix microarray. MMP-12, MMP-13 and cathepsin K showed an increase in expression in human tumors compared with normal lung similar to that seen in the orthotopic model. Immunohistochemical analysis also confirmed

MMP-12 expression in the stroma of human lung tumor samples. MMP-14 (MT1-MMP) deficient mice have been crossed with the MMTV-polyoma virus middle T antigen mice (PyMT) to evaluate its significance in this tumorigenesis model. MMP-14 deficient tumors grew to the experimental endpoint more quickly, but demonstrated a reduced ability to metastasize to the lungs (Itoh et al., 2001).

The ablation of individual MMP and TIMP genes in mice and the study of the subsequent effects on tumorigenesis has become a standard approach to the analysis of their functions; some examples are listed in Table 2. It is notable that animal studies have shown that MMPs sometimes have 'protective' roles in cancer development. *Mmp8*-null male or ovariectomized female mice develop significantly larger numbers of skin papillomas after a chemical carcinogen treatment (Balbin et al., 2003) and higher grade and more aggressive skin tumors developed in *Mmp9*-null mice (Coussens et al., 2000). In a human study of MMP-12 in squamous cell carcinoma it was found that high expression in tumor cells was associated with more aggressive tumors and high expression in tumor macrophages was correlated with lower grade tumors (Kerkelä et al., 2002). It is possible that obtaining a detailed understanding of the potential role of MMPs in the different cell types associated with tumors may be vital for the design of therapeutic anti-MMP strategies.

7.2. MMPs and vascular disease

It is well accepted that MMPs are key players in most vascular diseases and a similar elucidation of the spectrum of expression and specific roles has to be effected before a therapeutic opportunity can be clearly identified (Galis and Khatri, 2002; Newby, 2005; Newby and Johnson, 2005). MMPs have been implicated in intimal thickening, a repair response to damage of the walls of large arteries in human atherosclerotic pathologies, as well as in the subsequent plaque rupture. Hence, the concept of 'good' and 'bad' MMPs can be invoked in cardiovascular disease as in cancer, but the existing data from animal model studies are not clear cut. Studies using MMP gene knockout mice have indicated that MMP-2 and MMP-9 play key roles in cardiac rupture after myocardial infarction (Hayashidani et al., 2003; Heymans et al., 1999; Matsumura et al., 2005a,b; Romanic et al., 2002a; Romanic et al., 2002b; Romanic et al., 2002c). A recent study showed that MT1-MMP (MMP-14) is increased after ischemia-reperfusion (Deschamps et al., 2005). TIMP-3 deficiency in mice disrupted matrix homeostasis and caused spontaneous left ventricular dilation, cardiomyocyte hypertrophy and contractile dysfunction (Fedak et al., 2004). A critical role of MMP-2 and MMP-9 has been also shown for the development of abdominal aortic aneurysm using MMP gene deletion mice (Longo et al., 2002). Recent studies on atherosclerotic plaque stability using a series of apoE/MMP double knockout mice have indicated that MMP-3 and MMP-9 have protective roles by limiting plaque growth and enhancing plaque stability, but MMP-12 promotes lesion expansion and destabilization (Johnson et al., 2005). Chase and Newby (Chase and Newby, 2003) have proposed that multiple steps of MMP gene induction occur in vascular pathologies due to the progressive recruitment of different cell types and inductive factors during different phases of the disease. A widening spectrum of MMPs, starting with MMP-2 and MMP-9, leading to MMP-1, MMP-12 and MMP-14, followed by MMP-3, MMP-11 and MMP-13 ultimately leads to a transition from matrix turnover to matrix destruction.

7.3. MMPs and arthritis

The expression of matrix metalloproteinases in synovial membranes, cartilage, tendon and bone of synovial joints in both rheumatoid arthritis (RA) and osteoarthritis (OA) is well documented and often correlates with tissue destruction (Smeets et al., 2001; Murphy et al., 2002; Murphy and Nagase, 2008). As for other diseases, the spectrum of these enzymes orchestrating not only connective tissue turnover but other aspects of tissue biology, is much larger than anticipated. In both diseases, inflammatory mediators stimulate the production of

MMPs that degrade most components of the extracellular matrix. The collagenases, MMP-1 and MMP-13 are produced by synovial fibroblasts and macrophages and chondrocytes and have important roles in RA and OA because they are rate limiting in the process of collagen degradation. Antibodies that recognize terminal amino acid sequences generated by proteolysis at specific sites in type II collagen have been invaluable for identifying the proteinases responsible for cartilage breakdown both in vitro and in vivo (Hollander et al., 1994; Wu et al., 2002). Preliminary results with quantitative assays of type II collagen neoepitopes suggest that they may be useful markers of joint disease in humans (Fosang et al., 2003). MMP-8 and MMP-14 are collagenases and are also expressed by cells within synovial joints, but their precise contribution to collagen degradation has not yet been established. MMP-13 also degrades the proteoglycan molecule, aggrecan, giving it a dual role in matrix destruction. MMPs such as MMP-2, MMP-3, MMP-9, MMP-10 and MMP-19 are also elevated in arthritis and, along with MMP-13 these enzymes degrade non-collagen matrix components of the joints (Cawston and Wilson, 2006). Many MMPs have been associated with bone matrix turnover, involving virtually all the cell types present. Collagenolytic MMPs have a role in the actions of the bone forming cells, the osteoblasts, but the major collagenolytic proteinase in osteoclastic bone resorption appears to be the cysteine proteinase, cathepsin K which can function in the acidic phagolysosomal resorption zone of the osteoclast. However, MMPs do play a role in osteoclastic resorption in pathological conditions, including MMP-1, MMP-2, MMP-3, MMP-9, MMP-12, MMP -3 and MMP-14 (Andersen et al., 2004). They degrade a number of the non-collagenous proteins of the bone matrix and clearly play roles beyond ECM cleavage.

7.4. Genetic disorders of MMPs and TIMPs

Only a few human genetic disorders of the MMPs and TIMPs have been described in relation to human disease. An autosomal recessive disorder, multicentric osteolysis or ‘vanishing’ bone syndrome resulting from inactivating mutations in the *Mmp2* gene has been described (Martignetti et al., 2001). Mosig et al. (2007) have also shown that the loss of MMP-2 in mice disrupts skeletal and craniofacial development due to defects in osteblast and osteoclast growth. A missense mutation in the *MMP13* gene, spondyloepimetaphyseal dysplasia, Missouri type, causes an autosomal dominant disorder characterized by defective growth and remodelling of long bones (Kennedy et al., 2005). In comparison, *Mmp13*-null mice have defects in growth plate cartilage with increased hypertrophic domains and delayed endochondral ossification during development (Inada et al., 2004; Stickens et al., 2004). An *MMP20* gene mutation in the splice acceptor site of intron 6 was shown to link to autosomal recessive pigment hypomaturation amelogenesis imperfecta (Kim et al., 2005). This mutation is postulated to cause either a loss of the functional hemopexin domain or reduction of MMP-20 cased by degradation of its mRNA.

Sorsby’s fundus dystrophy (SFD) is a rare autosomal dominant disorder that results in degeneration of the macular region of the retina, with onset usually in the fourth to fifth decade of life. It leads to the rapid loss of central vision, often followed by further loss of peripheral vision. SFD has been shown to be a single-gene disorder, linked to mutations in exon 5 of the tissue inhibitor of metalloproteinases 3 (TIMP3) gene on chromosome 22q12-q13 (Apte et al., 1994).

7.5. MMP and TIMP regulation in relation to disease

Studies on translational and post-translational regulation of theMMPfamily have focussed largely on the understanding of tissue specific expression. From promoter studies through to the role of specific growth factors, signalling pathways and transcription factors, some idea of the intricacies of regulation are emerging (Yan and Boyd, 2007). Functional polymorphisms derived from nucleotide insertions, substitutions or micro-satellite instability have been reported in several MMP promoters and have been shown to contribute to inter-individual

susceptibility and outcome in several cardiovascular diseases including coronary artery disease, stenosis, myocardial infarction, coronary aneurysm, stroke, large artery stiffness (Ye, 2000; Newby and Johnson, 2005; Abilleira et al., 2006). The clinical impact of such polymorphisms in gastric cancer (Kubben et al., 2006) and in breast cancer progression (Hughes et al., 2007) are examples from cancer studies. Variation in *MMP3*, *MMP7*, *MMP12* and *MMP13* genes has been found to be associated with functional status in rheumatoid arthritis (Ye et al., 2007) and specific haplotypes of the *MMP1* and *MMP3* genes associate with radiographic joint destruction (Dörr et al., 2004).

Both DNA methylation and altered chromatin structure contribute to the epigenetic regulation of MMP and TIMP gene expression. Hypo- or hypermethylation of *MMP2*, *MMP3*, *MMP9* and *MMP10* have been described in different tumor types (Yan and Boyd, 2007). Nakamura et al. (2005) found that frequent hypermethylation of a locus at 22q12.3 correlated with loss of TIMP-3 expression in secondary glioblastoma. This epigenetic change was significantly correlated to poor survival in patients with grade II diffuse astrocytoma.

8. Conclusions and perspectives

The potential involvement of any of the 23 MMPs in both normal tissue maintenance and the destructive or reparative aspects of many diseases has produced many challenges when considering the potential application of MMP inhibitors as therapeutic agents. A detailed understanding of MMP expression patterns, in terms of both cellular origins and timing is a necessity. Furthermore, some knowledge of the precise role of an individual MMP within each context is important. This needs to be coupled with the ability to generate specific inhibitors, either targeting the active site or other interaction sites. Like all molecularly targeted cytostatic agents, there is a need for good clinical evaluation of efficacy in the form of appropriate biomarkers and therapeutic endpoints. The recent developments in techniques for imaging active MMPs will prove useful in this regard. Imaging of the cleavage of near infra red fluorescent substrates allows the visualization of the expression and activity of MMPs, as well as their influence on the tumor via imaging based biomarkers (Weissleder, 2006). Novel MMP-7 substrates have also recently been described for MRI imaging. These are based on the concept of a solubility switch on proteolysis to act as a product trap (Lepage et al., 2007). Cleavage of MMP substrates can also be utilized in photodynamic therapies where a non-toxic agent becomes both fluorescent and releases toxic singlet oxygen upon proteolysis (Choi et al., 2006).

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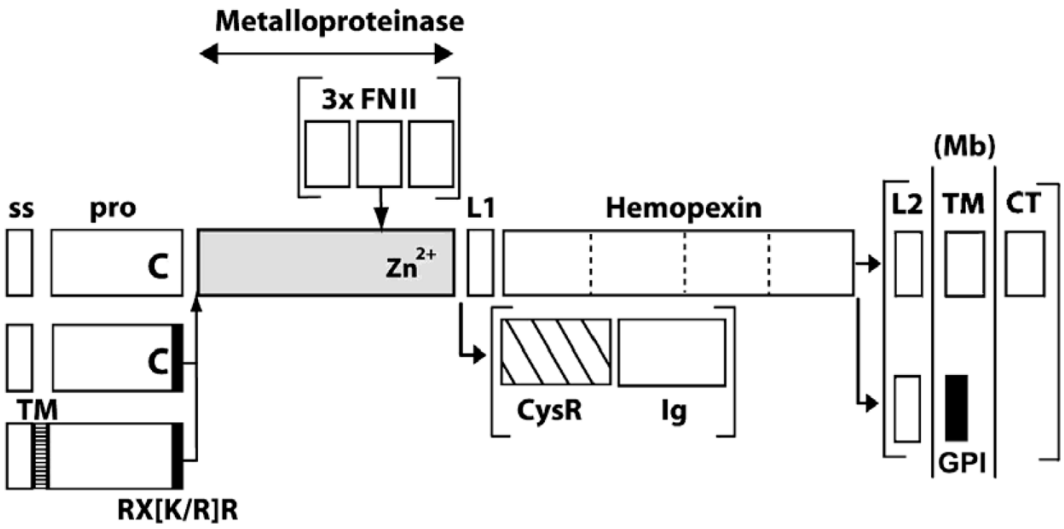

Fig. 1.

Domain structures of the matrixin family. See Table 1 for the domain arrangement of each MMP. ss, signal sequence; pro, pro-domain, FNII, fibronectin type II motif; L1, linker 1; L2, linker 2; Mb, plasma membrane; TM, transmembrane domain; CT, cytoplamic tail; CysR, cysteine rich; Ig, immunoglobulin domain; GPI, glycosylphosphatidylinositol anchor; C, cysteine.

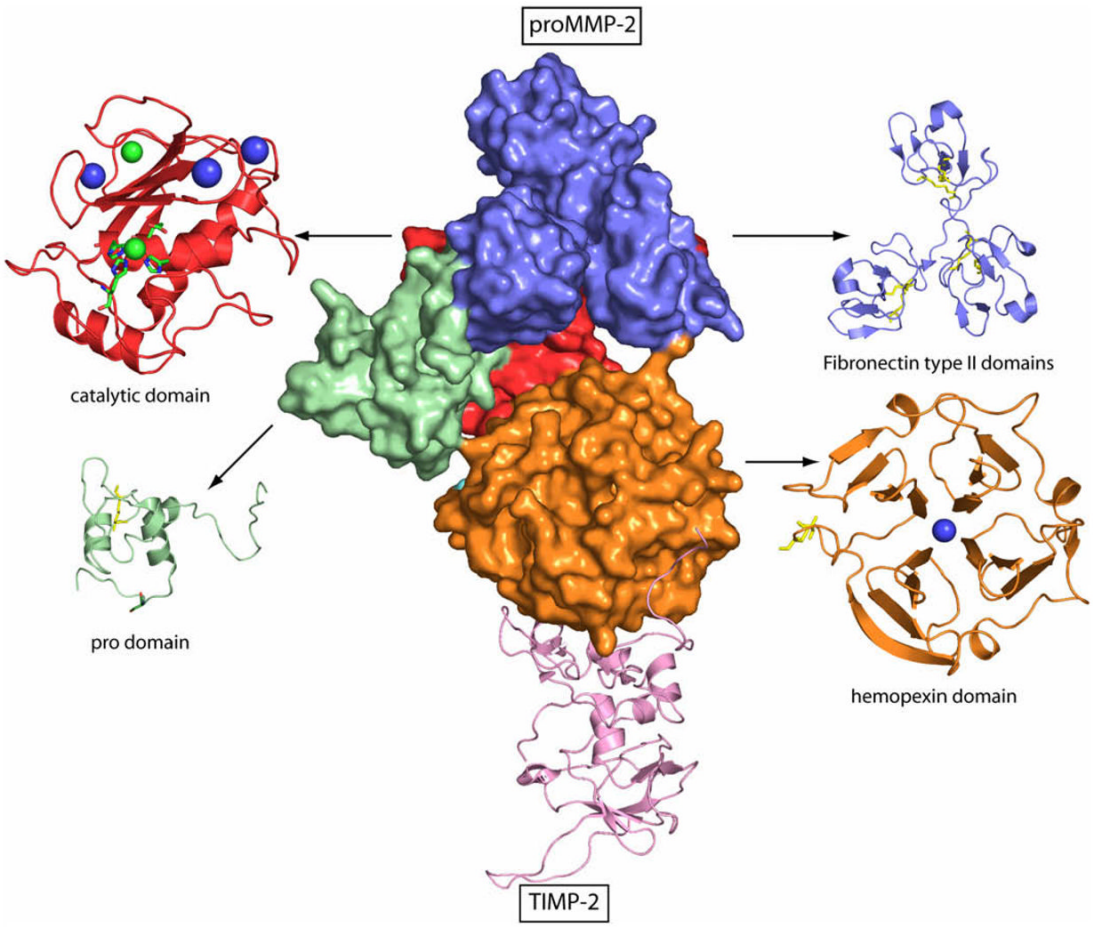

Fig. 2.

The three-dimensional diagram of human proMMP-2 and TIMP-2 complex. The pro-domain is shown in green, catalytic domain in red, fibronectin type II domains in blue, hemopexin domain in orange, and TIMP-2 in pink. Zinc ion is in green sphere, calcium ion in blue sphere and disulfide bonds in yellow. The image was prepared by Rob Visse of Imperial College London based on Brookhaven Protein Data Bank entry 1GXD.[11]

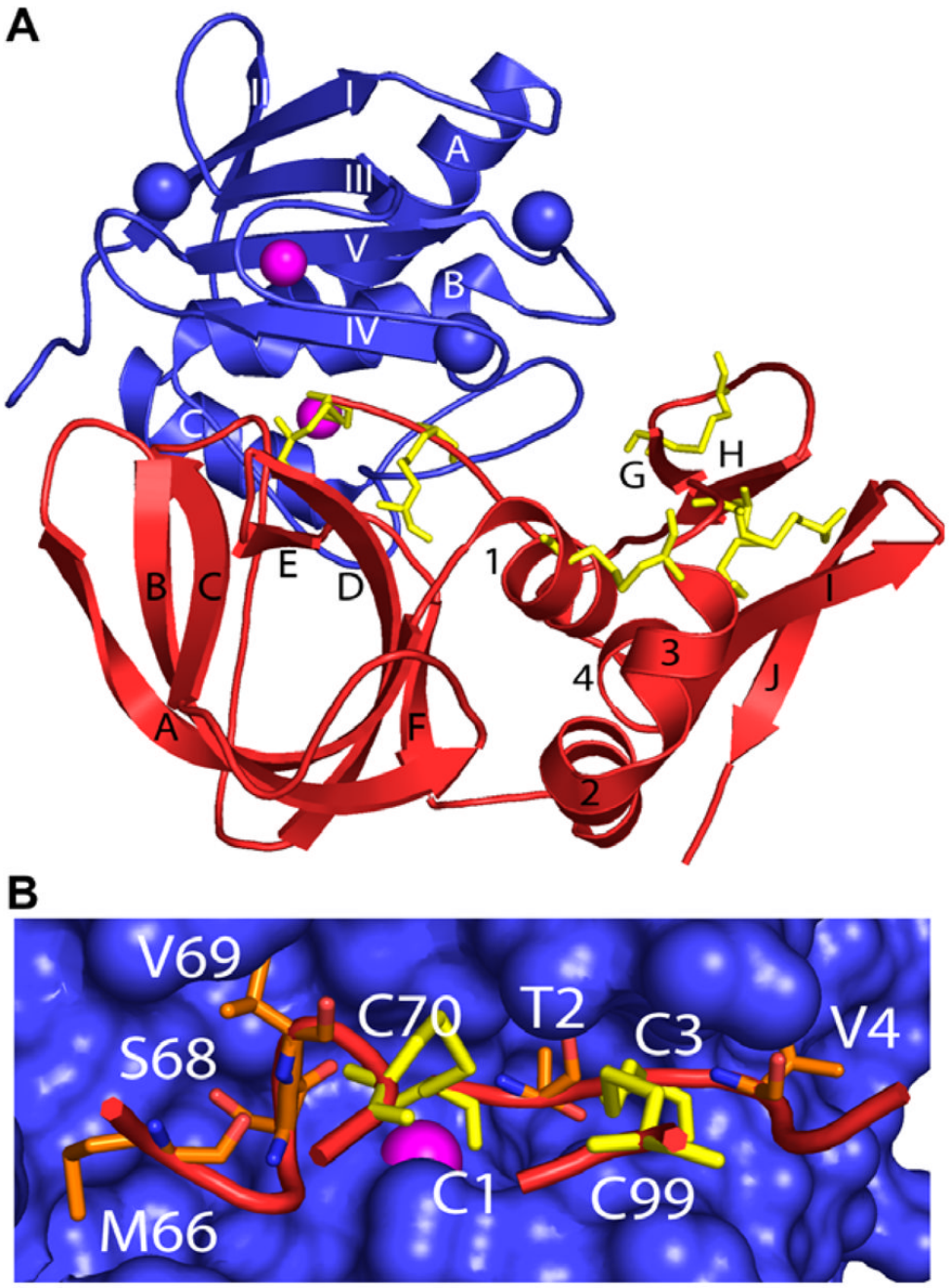

Fig. 3.

The three-dimensional diagram of TIMP-1 bound to the catalytic domain of MMP-3. (A) TIMP-1-MMP-3 complex. (B) The reactive centre residues of TIMP-1 in the active site of MMP-3. TIMP-1 is in red, MMP-3 is in blue. Disulfide bonds are shown in yellow, zinc ions in pink and calcium ions in blue. The image was prepared by Rob Visse of Imperial College London based on Brookhaven Protein Data Bank entry 1UEA.

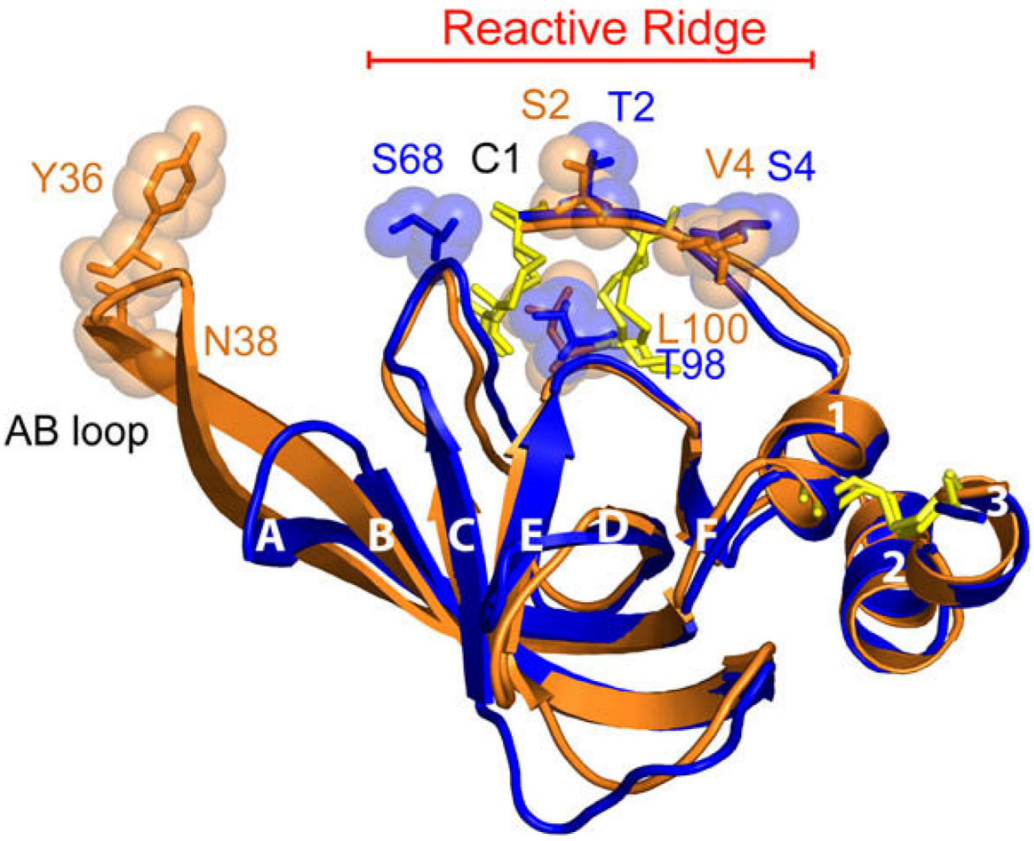

Fig. 4.

Superimposition of the N-terminal domains of TIMP-1 and TIMP-2. TIMP-1 is shown in blue and TIMP-2 in orange. The residues subjected to mutagenesis studies are illustrated and disulfide bonds are in yellow. The image was prepared by Rob Visse of Imperial College London based on Brookhaven Protein Data Bank entries 1UEA for TIMP-1 and IBQQ for TIMP-2.

Table 1

Human matrixins and their domain composition

Enzyme	MMP	Chromosomal location (human)	Domain composition												
			SS	Pro	CS	RX[R/K]R	Cat	FN2	Lk 1	Hpx	Lk 2	TM	GPI	Cyt	CysR-Ig
Collagenases															
Interstitial collagenase; Collagenase 1	MMP-1	11q22–q23	+	+	+	−	+	−	+	+					
Neutrophil collagenase; Collagenase 2	MMP-8	11q21-q22	+	+	+	−	+	−	+	+					
Collagenase 3	MMP-13	11q22.3	+	+	+	−	+	−	+	+					
Gelatinases															
Gelatinase A	MMP-2	16q13	+	+	+	−	+	+	+	+					
Gelatinase B	MMP-9	20q11.2–q13.1	+	+	+	−	+	+	+	+					
Stromelysins															
Stromelysin 1	MMP-3	11q23	+	+	+	−	+	−	+	+					
Stromelysin 2	MMP-10	11q22.3–q23	+	+	+	−	+	−	+	+					
Matrilysins															
Matrilysin 1	MMP-7	11q21–q22	+	+	+	−	+	−	−	−					
Matrilysin 2	MMP-26	11p15	+	+	+	−	+	−	−	−					
Stromelysin 3	MMP-11	22q11.2	(+)	(+)	+	+	+	−	+	+					
Membrane-type MMPs															
(A) Transmembrane-type															
MT1-MMP	MMP-14	14q11–q12	+	+	+	+	+	−	+	+	+	+	−	+	
MT2-MMP	MMP-15	15q13–q21	+	+	+	+	+	−	+	+	+	+	−	+	
MT3-MMP	MMP-16	8q21	+	+	+	+	+	−	+	+	+	+	−	+	
MT5-MMP	MMP-24	20q11.2	+	+	+	+	+	−	+	+	+	+	−	+	
(B) GPI-anchored															
MT4-MMP	MMP-17	12q24.3	+	+	+	+	+	−	+	+	+	−	+	−	
MT6-MMP	MMP-25	16p13.3	+	+	+	+	+	−	+	+	+	−	+	−	
Others															
Macrophage elastase	MMP-12	11q22.2–q22.3	+	+	+	−	+	−	+	+					

Enzyme	MMP	Chromosomal location (human)	Domain composition												
			SS	Pro	CS	RX[R/K]R	Cat	FN2	Lk 1	Hpx	Lk 2	TM	GPI	Cyt	CysR-Ig
–	MMP-19	12q14	+	+	+	–	+	–	+	+					
Enamelysin	MMP-20	11q22.3	+	+	+	–	+	–	+	+					
–	MMP-21	10	+	+	+	+	+	–	+	+					
CA-MMP	MMP-23	1p36.3	+	+	–	+	+	–	–	–	–	–	–	–	+
–	MMP-27	11q24	+	+	+	–	+	–	+	+					
Epilysin	MMP-28	17q21.1	+	+	+	+	+	–	+	+					

Human matrixins are grouped based on trivial names. SS, signal peptide; Pro, pro-domain; CS, cysteine switch motif; RX[R/K]R, proprotein convertase recognition sequence; FN2, fibronectin type II motif; LK, linker; TM, transmembrane domain; GPI, glycosylphophatidylinositol anchoring sequence; Cyt, cytoplasmic domain; CyR-Ig, cysteine rich and Ig domain; ( ) indicates a splice variant lacking SS and Pro.

Table 2

Mmp and *Timp* gene ablation in mice: specific disease phenotypes

Gene	*In vivo* phenotype	Reference
Cancer		
Mmp2	Reduced pancreatic tumorigenesis	Bergers et al. (2000)
Mmp7	Reduced intestinal adenoma formation	Wilson et al. (1997)
Mmp8	Papillomas increased size (males)	Balbin et al. (2003)
Mmp9	Reduced pancreatic tumorigenesis	Bergers et al. (2000)
Mmp11	Delayed mammary tumorigenesis	Masson et al. (1998)
Mmp14	Accelerated mammary tumorigenesis, reduced metastases	Szabova et al. (2007)
Mmp9	Less skin fibrosarcomas, longer latency	Pendas et al. (2004)
Timp1	Suppression or potentiation of lung tumor metastases	Soloway et al. (1996)
	Increased tumor cell sensitivity to chemotherapy	Davidsen et al. (2006)
Timp3	Enhanced metastatic dissemination of melanoma and lymphoma	Cruz-Munoz et al. (2006)
Vascular diseases		
Mmp2	Reduction neointima formation on vascular injury Protection from cardiac rupture post-myocardial infarction	Johnson and Galis (2004) and Matsumura et al. (2005b)
Mmp9	Reduction neointima formation on vascular injury	Johnson and Galis (2004)
	Protection from cardiac rupture post-myocardial infarction	Ducharme et al. (2000)
Mmp11	Accelerated neointima formation on vascular injury	Lijnen et al. (1999b)
Timp1	Accelerated neointima formation on vascular injury	Lijnen et al. (1999a)
	Spontaneous cardiac dilatation, augmented dysfunction post-myocardial infarction	Creemers et al. (2003)
Timp3	Spontaneous dilated cardiomyopathy	Fedak et al. (2004)
Arthritis		
		Reference
Mmp2	Increased immune-complex mediated arthritis	Itoh et al. (2002)
Mmp9	Decreased immune-complex mediated arthritis	Itoh et al. (2002)
Mmp12	Reduced macrophage numbers in ligament injury	Wright et al. (2006)
Mmp13	Spontaneous abnormal growth plate, increased trabecular bone	Stickens et al. (2004)
Mmp14	Defects in skeletal development, arthritis and connective tissue disease	Holmbeck et al. (1999)
Timp3	Enhanced inflammatory response in antigen induced arthritis	Mahmoodi et al. (2005)

Ablation of individual *Mmp* or *Timp* genes in mice has been used to study the change in phenotype of established disease models. Some representative examples of the major changes seen in cancer, vascular diseases and arthritis models are cited.