Chapter 13
Metalloproteinases in Cartilage Matrix Breakdown: The Roles in Rheumatoid Arthritis and Osteoarthritis

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13.1 Introduction

The cartilage of articular joints consists of a relatively small number of cells and an abundant extracellular matrix (ECM). The major components of its ECM are collagen fibrils and aggregated proteogylcans, aggrecans, which account for approximately 90 % of the total ECM of the cartilage with each roughly in a similar amount. Collagen fibrils, mainly consisting of type II collagen, together with minor types IX and XI collagens, form a meshwork and provide tensile strength to the tissue. Aggrecans, present as large aggregated complexes interacting with hyaluronan and link proteins, form a hydrated gel within the collagen meshwork and give cartilage its ability to withstand mechanical compression. Cartilage also contains less abundant ECM macromolecules such as fibromodulin, decorin, biglycan, cartilage oligomeric matrix protein, types VI collagen, tenascin C, matrilins and cell surface proteoglycans (Heinegård and Saxne 2011). In normal cartilage the turnover of these molecules is at equilibrium, but in arthritic cartilage the loss of ECM components exceeds their synthesis and cause impairment of joint function.

Rheumatoid arthritis (RA) and osteoarthritis (OA) are the two most common joint disorders, which cause pain and disability. The etiology of the two joint diseases is different, but a shared feature is the destruction of articular cartilage. Many possible causes of cartilage destruction are recognized (e.g., injury, inflammation, mechanical load, oxidative stress, aging, apoptosis and extracellular matrix

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K. Brix and W. Stöcker (eds.), *Proteases: Structure and Function*,
DOI 10.1007/978-3-7091-0885-7_13,

disassembly), but the primary cause is elevated levels of active proteinases that degrade the ECM of cartilage. RA is an inflammation driven autoimmune disease associated with hyperplasia of synovial cells and rheumatoid synovia contain infiltrated T and B lymphocytes, macrophages, mast cells, endothelial cells and fibroblastic synovial lining cells. Neutrophils are often found in the cartilage-pannus junction in early erosive lesions and multinucleated osteoclasts at the site of subchondral bone erosion. The main cellular source of ECM-degrading enzyme are macrophages, synovial lining cells and their production is regulated by inflammatory mediators, cytokines and growth factors generated by activated lymphocytes, macrophages and mast cells (Fig. 13.1, left). In OA, on the other hand, destructive proteinases are derived primarily from the cartilage and inductive stimuli include inflammatory cytokines, mechanical load, tissue injury, reactive oxygen species, degraded matrix and aging (Fig 13.1, right). There are a number of proteases, including serine and cysteine proteases that are found in the joint tissues, but the major proteinases that degrade cartilage matrices are the matrix metalloproteinases (MMPs) and the metalloproteinases with disintegrin and thrombospondin domains (ADAMTSs). Aggrecans are degraded by both MMPs and the so-called "aggrecanases" which belong to the ADAMTS family, whereas collagen fibrils are cleaved by collagenases which are members of the MMP family. In addition, the metalloproeineases with disintegrin domain (ADAMs) that shed cell surface cytokines, growth a factors and their receptors also play key roles in inflammation and tissue damage.

Other proteinases such as plasminogen activator-plasminogen system, matriptase, high temperature requirement A (HtrA), activated protein C, cathespins, calpains and caspases are also involved directly and indirectly in cartilage degradation (Troeberg and Nagase 2012). This chapter focuses on the role of metalloproteinases in RA and OA and their structures.

13.2 MMPs and Metzincins

There are 24 MMP genes in the human genome, but there are 23 expressed proteinases because of the duplication of the *MMP23* gene. The MMPs are also called "matrixins" and they are classified as matrixin subfamily of metalloproteinase family M10 in the MEROPS database (http://www.merops.sanger.ac.uk). All matrixins have a signal peptide, a propeptide domain and a catalytic metalloproteinase (M) domain and most have a C-terminal hemopexin (Hpx) domain, with the exception of MMP-7, MMP-23 and MMP-26. MMP-2 and MMP-9 called gelatinase A and gelatinase B, respectively, have three repeats of fibronectin type II (FN) motifs inserted in the catalytic domain. These fibronectin domains interact with collagen and other ECM molecules and influence substrate specificity. The catalytic M domain of MMPs has the zinc-binding motif HEXXHXXGXXH where three histidines bind to the catalytic Zn^{2+} ion. The Hpx domain in MMPs is important for some members to express biological activities,

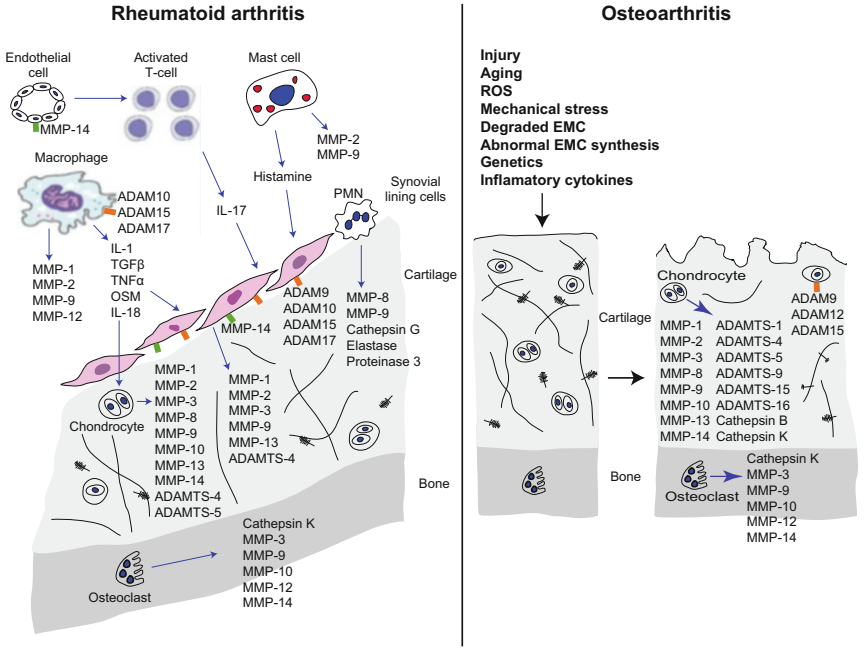
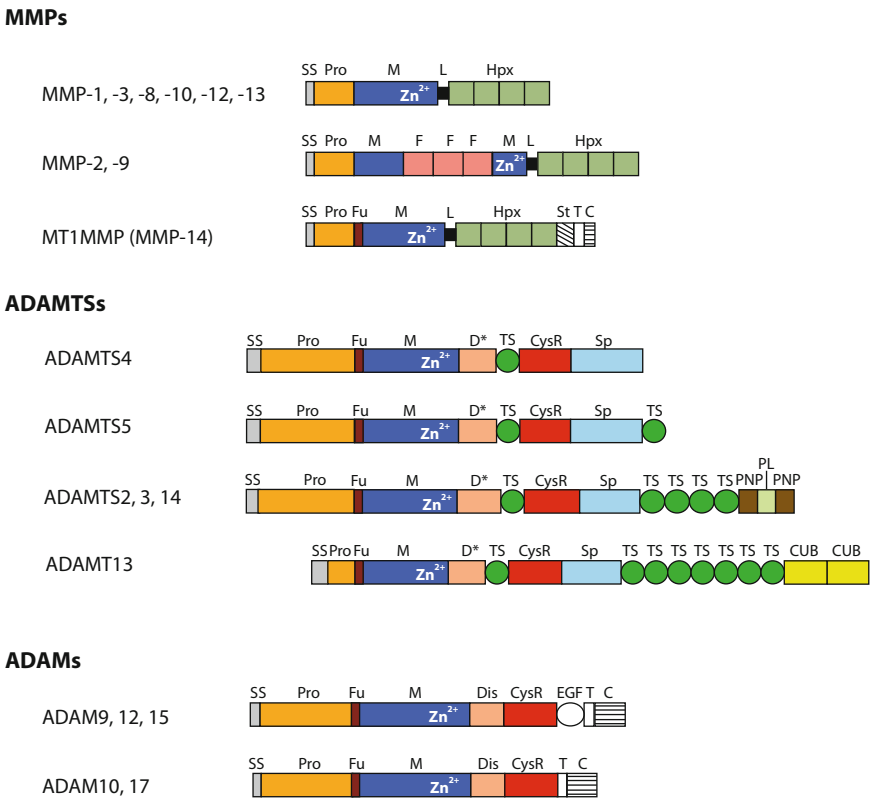


Fig. 13.1 Sources of cartilage matrix-degrading metalloproteinases in RA and OA. In RA (*left*), inflammation or tissue damage drives the recruitment of lymphocytes, macrophages and mast cells and polymorphonuclear leukocytes (PMN). Those cells produce metalloproteinases and/or cytokines that stimulate fibroblastic synovial lining cells and chondrocytes in cartilage to produce metalloproteinases. MMP-14 is expressed on the surface of the synovial lining cells when cells contact to collagen of the cartilage. In OA (*right*), the primary cellular source of matrix-degrading enzymes are the chondrocytes, which respond to tissue injury, aging, mechanical stimuli, inflammatory cytokines, reactive oxygen species and other factors. Bone erosion is due to osteoclasts that migrate from blood vessels into the bony region

e.g, for collagenases to cleave triple helical collagen (Visse and Nagase 2003), and for membrane-type 1 MMP (MT1-MMP or MMP-14) to assume a dimeric form on the cell surface which is crucial for its biological activities, including the activation of proMMP-2, cell migration and collagenolysis (Tochowicz et al. 2011). Most MMPs are secreted from the cell as inactive pro-enzymes, but six are plasma membrane-anchored membrane-type MMPs (MT-MMPs), of which four are type I transmembrane-type harbouring a cytosolic domain and two are glycosylphosphatidyl inositol-anchored. Pro-enzymes secreted from the cell secreted into the ECM are activated by tissue or plasma proteinases or by reactive oxygen species (Hadler-Olsen et al. 2011). Six pro-MT-MMPs and proMMP-11, -21, -23 and -28 are, on the other hand, most likely to be activated intracellularly by furin or a related pro-proteinases (Nagase et al. 2006).

MMPs have been characterized for their abilities to cleave ECM components, and the domain arrangements of MMPs that are found in cartilage and symonvium are shown in Fig. 13.2. They cleave aggrecan core protein at several sites, but the



C	Cytoplasmic domain	F	Fibronectin type II domain	Pro	Pro-domain
CUB	Complement C1r/C1s - Urchin epidermal growth factor - Bone morphogenetic protein1 domain	Fu	Furin recognition motif	Sp	Spacer domain
		Hpx	Hemopexin-like domain	SS	Signal sequence
		L	Linker region	St	Stalk region
CysR	Cysteine-rich domain	M	Metalloproteinase domain	T	Transmembrane domain
D*	Disintegrin domain with a Ch fold	Mu	Mucin-like	TS	Thrombospondin type 1 repeat
Dis	Disintegrin domain	PL	Proteinase and lacunin domain		
EGF	Epidermal growth factor domain	PNP	Procollagen N-proteinase specific		

Fig. 13.2 Domain arrangement of MMPs, ADAMTSs and ADAMs that are involved in cartilage matrix degradation. ADAMTS2, 3 and 14 are procollagen N-proteinases. ADAMTS13 is shown as the structure of its non-catalytic domains was used in model ADAMTS4

cleavage at the Asn^{341}–Phe^{342} bond located in the interglobular domain between the N-terminal globular domain (G1) and the second globular domain (G2) has been characterized as a signature of the MMP activity (see Fig. 13.3). ADAMTSs, on the other hand, cleave the the Glu^{373}–Ala^{374} bond, which is the signature of "aggrecanase" activity. Collagenolytic MMPs are so-called collagenases (MMP-1, MMP-8 and MMP-13) and MMP-2 (gelatinase A) and MMP-14 (membrane type-1 MMP). It should also be noted that many MMPs cleave non-ECM

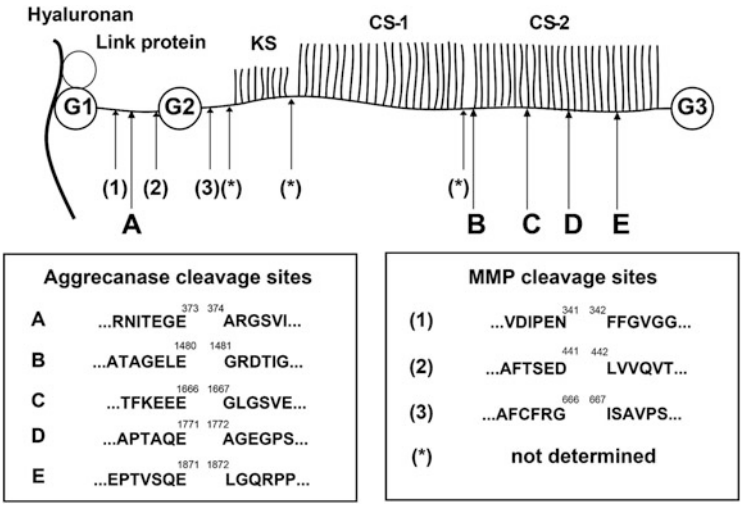


Fig. 13.3 Sites cleaved in aggrecan core protein by aggrecanase and MMPs

molecules such as cytokines, chemokines, growth factors, growth factor binding proteins, proteinase inhibitors, cell surface adhesion molecules and receptors to regulate their biological activities (Overall 2002). MMP activities are inhibited by endogenous inhibitors called TIMPs (tissue inhibitors of metalloproteinases), but only limited members of the ADAMTSs and the ADAMs are inhibited by TIMPs (see below).

Crystal structures of MMPs, a crayfish metalloproteinase astacin, snake venom metalloproteinase adamalysin, and a bacterial metalloproteinase serralysin, all revealed similar polypeptide folds, although their amino acid sequences are not related each other, except the catalytic zinc ion-binding motif HEXXHXXGXXH and the 'Met-turn' located after the zinc-binding motif. This methionine forms a base to create the active site for these metalloproteinases. Those are collectively called 'metzincins' (Bode et al. 1993; Gomis-Rüth 2009). The astacin family includes bone morphogenetic protein 1, tolloid-like metalloproteinases, meprins and ovastacin; the adamalysins include the ADAMTSs and the ADAMs; and the pappalysins including human pregnancy-associated plasma protein A (Boldt et al. 2004) and archeae metalloproteinases ulilysin (Tallant et al. 2006). The serralysins are related bacterial metalloproteinase. Fragilysin 3 from *Bacteroides fragilis* was recently classified as a metzincin metalloproteinase, which shows structural similarity to mammalian ADAMs with little sequence identity (Goulas et al. 2011). Since similar proteins have not been found in species other than *B. fragilis*, it is considered to result from horizontal gene transfer from a eukaryotic cell to a bacterial cell (Goulas et al. 2011).

13.2.1 Three-Dimensional (3D) Structures of MMPs

Numerous 3D structures of the catalytic domains of MMPs, including those of all collagenolytic MMPs (MMP-1, MMP-2, MMP-8, MMP-13 and MMP-14) have been solved by X-ray crystallography and some by NMR spectroscopy [see Maskos (2005) for review]. Full-length proMMP-1 (Jozic et al. 2005), proMMP-2 (Morgunova et al. 1999) and proMMP-2-TIMP-2 complex (Morgunova et al. 2002), activated from of full-length MMP-1 (Iyer et al. 2006; Li et al. 1995) and the full-length MMP-1-triple helical collagen peptide complex (Manka et al. 2012) were also solved. Those studies have helped us understand the mechanisms of peptide hydrolysis by MMPs and design their inhibitors. The overall polypeptide folds of prodomains, catalytic domains and Hpx domains are very similar among MMPs. Figure 13.4 shows the crystal structure of the proMMP-2-TIMP-2 complex to indicate examples of individual domains found in MMPs.

13.2.1.1 Pro-domains

Pro-domains of the secreted proMMPs consist of about 80 amino acids, and they are folded into three α-helices and connecting loops (Becker et al. 1995; Elkins et al. 2002; Jozic et al. 2005; Morgunova et al. 1999). The pro-domain functions as a chaperone as well as keeping the enzyme in an inactive zymogen form. The sequence PRCGXPD called "cysteine switch" is conserved in proMMPs and it lies in the substrate binding groove of the catalytic domain. It forms hydrogen bonds in a similar manner as in those of a peptide substrate deduced from the interactions of peptide inhibitors (Grams et al. 1995). However, the direction of the cysteine switch sequence is opposite from that of a peptide substrate. The SH group of the cysteine interacts with the catalytic Zn^{2+}, which keep the enzyme inactive. Upon activation of a proMMP, the Cys-Zn^{2+} interaction is disrupted, which enables Zn^{2+} to interact with H_2O necessary for peptide hydrolysis. Disruption of the Cys-Zn^{2+} interaction can be triggered by proteolytic processing of the pro-peptide or by structural perturbation of the pro-domain by chemicals such as chaotropic agents, sodium dodecyl sulphate, SH reactive agents, or oxidation of the SH group (Nagase 1997). The activation of proMMPs by reactive oxygen species such as hypochlorous acid generated by myeloperoxidase, or by NO from phagocytes may be important mechanism of proMMP activation (Fu et al. 2004; Gu et al. 2002; Peppin and Weiss 1986). However, a longer reaction may inactivate the enzyme (Fu et al. 2004).

13.2.1.2 M Domain

The catalytic domain consists of a five-stranded β-sheet and three α helices, two Zn^{2+} ions and one to three Ca^{2+} ions. Calcium ions and one Zn^{2+} ion hold the structure

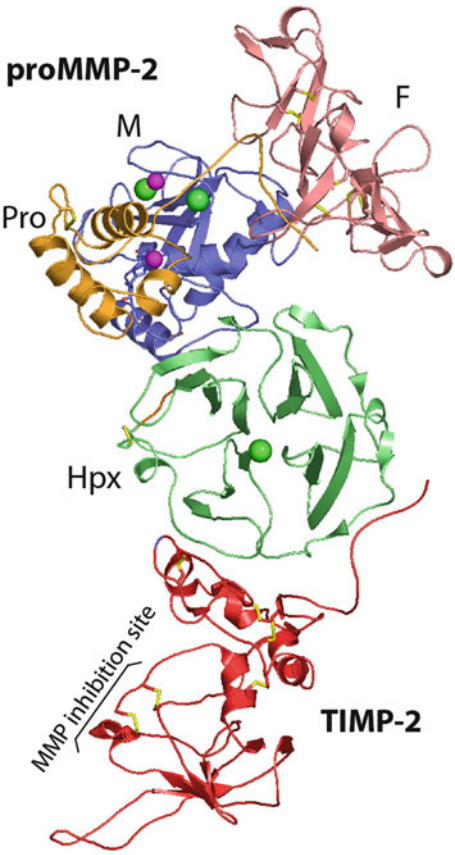


Fig. 13.4 Ribbon structure of the proMMP-2-TIMP-2 complex. The pro-domain is shown in *gold*, catalytic domain in *blue*, fibronectin type II motifs in *salmon*, hemopexin domain in *green*, TIMP-2 in *red*, zinc ions in *purple sphere*, calcium ions in *green sphere*, and disulfide bonds in *yellow*. The image was prepared by Robert Visse based on PDB ID: 1GXY

and one Zn^{2+} in the active site is essential for peptide hydrolysis. The catalytic Zn^{2+} is coordinated with three histidines in the HEXGHXXGXXH sequence conserved in all MMPs. The substrate binding S1′ pocket located adjacent to the catalytic Zn^{2+} is hydrophobic in nature and variable in depth and shape among MMPs. It is therefore one of the determinant factors for substrate specificity of MMPs. However, for MMPs to recognize large extended ECM molecules, extended substrate recognition sites (exosites) are found in both the catalytic and non-catalytic domain (Bertini et al. 2012; Fulcher and Van Doren 2011; Manka et al. 2012; Palmier et al. 2010).

13.2.1.3 Fibronectin Type II Domain

MMP-2 and MMP-9 have three repeats of fibronectin type II (Fn II) domain and three domains participate in their specificity for gelatin, collagen IV and laminin. They are inserted into the loop between the fifth β strand and the second helix of the catalytic M domain. The Fn II domains in MMP-2 and MMP-9 have similar

conformations. Each domain consists of two antiparallel β-sheets connected with a short α-helix which are stabilized by two disulfide bonds. However, the placement of the three Fn II domains in the two gelatinases are different in that domain 2 of proMMP-2 has an area that interacts with the M domain, but the corresponding domain of proMMP-9 is rotated and twisted away from the M domain (Elkins et al. 2002). Domain 3 in the two progelatinases makes contact with the propeptide and with the M domain (Elkins et al. 2002; Morgunova et al. 1999).

13.2.1.4 Linker Region

The linker region connects the M domain and the Hpx domain and it is variable in length. As shown for MMP-1, MMP-9 and MMP-12, linker regions are considered to be flexible (Bertini et al. 2008, 2009; Jozic et al. 2005; Rosenblum et al. 2007), but they may have some structural constraint as they contain a number of proline residues and therefore are suited for specific functions. Mutational studies of this region in MMP-1 (Tsukada and Pourmotabbed 2002) and MMP-8 (Knäuper et al. 1997) significantly decreased the collagenolytic activity, supporting the idea that correct movement around the M and the Hpx domain is important for the expression of collagenase activity. The linker region of MMP-9 and MMP-14 are N- and O-glycosylated and these carbohydrates have effects on the cellular biochemistry of these MMPs (Van den Steen et al. 2006; Wu et al. 2004).

13.2.1.5 Hpx Domain

The Hpx domain is found in most MMPs, with the exception of MMP-7, -23 and -26. It is a ellipsoidal disk shape, four-bladed β propeller structure with a single disulphide bond between the first and fourth blades (Gomis-Rüth et al. 1996; Li et al. 1995; Libson et al. 1995). Each blade is made up of four antiparallel β-strands and the four β-sheets have similar scaffolds and are arranged almost symmetrically around a central core axis. The centre of the propeller usually contains one calcium ion and a chloride. The Hpx domains are essential for collagenases to degrade triple helical collagens and MMP-14 to dimerize on the cell surface (Tochowicz et al. 2011).

13.2.2 Mechanisms of Collagenolysis by MMPs

The fibril-forming insterstitial collagens consist of three α chains of approximately 1,000 amino acid residues with repeating Gly-Xaa-Yaa triplets, where Xaa and Yaa are often Pro and Hyp respectively. Due to a high imino acid content and repeated

Gly in every third residue, the α chain adopts a left-handed poly-proline II-like helix and three left-handed chains intertwine to form a right-handed superhelix (Kramer et al. 2001; Ramachandran and Kartha 1955). The triple helical structures make interstitial collagen I, II and III resistant to most proteinases, and in vertebrates, only collagenolytic MMPs (also referred to as "collagenases") and cathepsin K can cleave the helical region of these collagens. While MMP collagenases cleave collagen near neutral pH, cathepsin K, mainly produced by osteoclasts, functions in acidic environments, e.g. in resorbing bones (Garnero et al. 1998). Collagenolytic MMPs are MMP-1 (collagenase 1), MMP-8 (collagenase 2/neutrophil collagenase) and MMP-13 (collagenase 3), MMP-2 (gelatinase A) and MMP-14 (MT1-MMP). They cleave native collagen I at a single site ¾ of the way from the N-terminus and generate characteristic ¾-long N-terminal and ¼-long C-terminal fragments.

However, interstitial collagens in the tissue form insoluble fibrils and fibrils are arranged as suprafibrillar architectures with the non-helical telopeptide regions cross-linked intra- and inter-molecularly. Cartilage collagen fibrils are comprised mainly of collagen II, but contain collagens IX and XI as minor components (Fig. 13.5a). Collagen IX maintains the structural integrity of cartilage by aligning along the surface of collagen II fibril and other ECM molecules through the basic non-collagenous 4 (NC4) domain which projects out from the surface of fibrils (Heinegård and Saxne 2011). Collagen fibrils are much more resistant to mammalian collagenases than acid-extracted monomeric collagens in solution (Welgus et al. 1980). This is explained by the accessibility of the collagenase cleavage site which is largely blocked by the C-telopeptide of neighbouring collagen molecules (Perumal et al. 2008). Thus, proteinases that cleave non-collagenous telopeptide regions increase the accessibility of collagenolytic MMPs. These enzymes are MMP-3, neutrophil elastase, cathepsin G and lysosomal cysteine proteinases (Fig. 13.5a). Once collagen fibrils are cleaved or depolymerized, the helical structures unfold at the body temperature and become susceptible to gelatinases (MMP-2 and MMP-9) and other tissue proteinases.

Mammalian collagenases have the unique ability to cleave triple helical interstitial collagens, which requires both the catalytic M domain and the Hpx domain. However, when the 3D structure of MMP-1 was reported it became apparent that the active site of the enzyme cannot accommodate the triple helical collagens in the active site for the enzyme, because the entrance of the active site is only 5 Å wide and the diameter of the triple helix is 15 Å (Bode 1995). Simple docking of triple helical peptide indicated that the closest peptide bond is ~7 Å away from the catalytic Zn^{2+}. It has therefore been postulated that either the active site of collagenase undergoes large conformational changes to accommodate triple helix or the collagen needs to be unwound to present a single α chain to the active site to initiate collagenolysis.

The evidence that collagenase unwinds triple helical collagen was presented by Chung et al. (2004). They demonstrated that proteinases that do not cleave native collagen I (e.g., MMP-3, neutrophil elastase) can cleave collagen I in the presence of MMP-1(E200A) mutant which lacks the ability to hydrolyze peptide. Thus, it

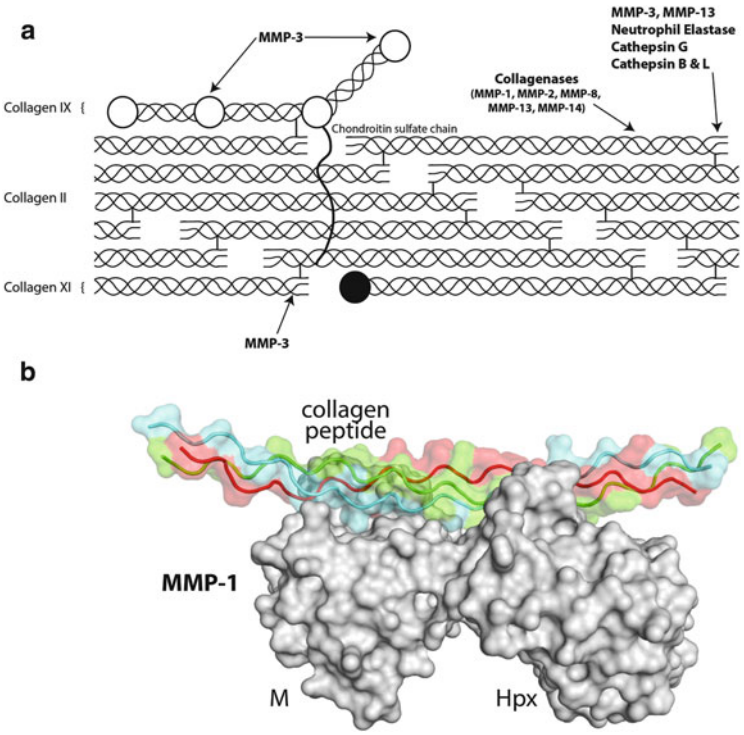


Fig. 13.5 The sites of proteinase action on cartilage collagen fibrils. (**a**) Schematic presentation of cartilage collagen fibrils and the sites of proteinase action. (**b**) MMP-1 and collagen peptide interaction. MMP-1 is represented as a *grey* surface and the three collagen chains are colored in *cyan*, *green* and *red*. Both catalytic M domain and the C-terminal Hpx domain interact with collagen and the collagen peptide is slightly bent through this interaction. The image was prepared by Robert Visse based on PDB ID: 4AUO

was concluded this MMP-1 mutant unwinds collagen without cleaving the peptide bonds. This proposal was challenged by Stultz and colleagues who proposed that collagen around the collagenase-cleavage site is less stable than the rest of the collagen molecule and it tends to be partially unfolded, based on their theoretical molecular simulation (Nerenberg et al. 2008). Such an unfolded "vulnerable" state was considered to be sufficient for collagenases to recognize and hydrolyze triple helical collagens. This model, however, does not explain why MMP-3 or neutrophil elastase alone does not cleave collagens (Chung et al. 2004). Recent NMR studies of MMP-1(E200A) mutant and triple helical collagen peptide demonstrated unfolding of the triple helix upon interacting with MMP-1(E200A) (Bertini et al. 2012). The importance of the Hpx domain for collagenolysis was shown by that the M domain and the Hpx domain cooperatively bind to collagen and it is temperature dependent, suggesting a subtle structural changes in triple helix enhance collagenase-collagen interaction and this subtle changes are prone to be the C-terminal side of collagenase cleavage site in collagen (Manka et al. 2012).

The crystal structure of the MMP-1(E200A)-collagen peptide complex indicated that MMP-1 has an extended collagen binding sites in both the M and the Hpx domains and all three chains of collagen interact with the enzyme (Manka et al. 2012) (Fig. 13.5b). It is suggested a flexing of the M and the Hpx domains help unwind collagen (Bertini et al. 2012; Manka et al. 2012).

13.2.3 MMPs in Arthritis

MMP activities are not readily detected in the normal steady state tissues, but the production of many MMPs is transcriptionally regulated by cytokines, growth factors, hormones, cell-cell and cell-matrix interaction, physical and chemical stimuli (Sternlicht and Werb 2001). MMP activities are also functionally regulated through their activation from their precursors, inhibition by endogenous inhibitors and cellular uptake by endocytic pathways. They are functional according to physiological demands and pathological situations.

The main cellular sources of MMPs in RA are synovial lining cells and macrophages, and their production is greatly influenced by inflammatory mediators, cytokines and growth factors (e.g. IL-1, TNF, IL-17, IL-18, oncostatin M, transforming growth factor-β, histamine) derived primarily from activated lymphocytes, macrophages and mast cells (see Fig. 13.1).The production of some of these MMPs is suppressed by IL-4, IL-10 and IL-13. These MMPs are considered to act mainly on ECM components, but they clearly have a number of other functions that orchestrate cell migration and infiltration of the tissue, and the release of growth factors (Overall 2002). For example, MMP-8-null mice have exacerbated adjuvant-induced arthritis due to suppression of the transcripts of the apoptosis initiator caspase 11 (the murine homologue of human caspases 4 and 5), resulting in delayed neutrophil apoposis, although the mechanism of the reduced transcripts is not known (Cox et al. 2010). MT1-MMP (MMP-14) plays a key role in migration of many cell types. Little MMP-14 is expressed in normal synovial cells, but it is highly expressed in the pannus-cartilage junction in RA joints, where synovial cells are in contact with cartilage collagen, and allows the cells to invade into cartilage (Miller et al. 2009).

In OA the ECM-degrading proteinases are derived primarily from the cartilage. Although inflammation is less prevalent, synovial membrane inflammation may be observed particularly at an early stage (Benito et al. 2005). Thus, inflammatory cytokines may participate in enhancing the production of MMPs. Cartilage explant studies suggest that cytokines such as IL-1, IL-17, TNFα and oncostatin M (Milner and Cawston 2005), proteolytic fragments of fibronectin (Homandberg 1999), endothelin 1 (Roy-Beaudry et al. 2003), mechanical compression (Blain et al. 2001) and mechanical injury (Lee et al. 2005a) are potential stimulatory factors. Instability of the joint, ageing and oxidative stress also contribute to cartilage matrix catabolism (Aigner et al. 2007).

MMPs are able to cleave a number of ECM components of cartilage. MMPs cleave aggrecan at the Glu^{341}–Phe^{342} bond in the interglobular domain of the core protein and MMP-generated G1-VDIPEN^{341} fragments are found in RA and OA cartilage (Lark et al. 1997; Struglics et al. 2006). However, in vitro studies indicate that aggrecanolytic activities of MMPs are much weaker than that of ADAMTS-5 (Durigova et al. 2011). Collagen II fibrils are, on the other hand, degraded primarily by MMPs (Dodge and Poole 1989). Potential candidate enzymes are MMP-1 and MMP-13 in interterritorial collagenolysis and MMP-2 and MMP-14 in pericellular collagenolysis. Collagen IX is cleaved by MMP-3 at NC2 and NC domains. This cleavage of collagen IX may affect the susceptibility of collagen fibrils by collagenolytic MMPs. Ablation of MMP-13 in mice protects the cartilage from degradation upon induction of OA, but not chondrocyte hypertrophy nor osteophyte formation (Little et al. 2009). Prevention of cartilage and bone destruction in animal models of RA and OA by synthetic MMP inhibitors (Conway et al. 1995; Ishikawa et al. 2005; Sabatini et al. 2005) also indicates that MMPs play a key role in joint destruction. However, it is still unclear which MMPs plays key roles in cartilage destruction in humans.

13.3 ADAMTSs and Aggrecanases

The human gemone encodes 19 ADAMTSs. The first ADAMTS (ADMTS1) was discovered by Kuno et al. (1997) in mouse colon adenocarcinoma cells as a cDNA which encodes a signal peptide, a pro-domain and metalloproteinase (M) domain, a disintegrin (Dis) domain, a thrombospondin type I (TS) domain, a cysteine-rich (CysR) domain, a spacer (Sp) domain and two additional TS domains at the C-terminus (Fig. 13.1). The M domain and the Dis domain are similar in amino acid sequence to those of ADAMs, but TS domains are unique. Thus, the terms, "ADAMTS" was coined. The proteolytic activity of ADAMTS1 was first demonstrated as its ability to cleave α2-macroglobulin (Kuno et al. 1999). The second enzyme cloned was ADAMTS2, which process the N-propetide of procollagens I, II, and III (Colige et al. 1997). This enzyme has a unique domain specific to procollagen N-proteinase and a proteinase and lacunin (PLAC) domain at the C-teminus. ADAMTS3 (Fernandes et al. 2001) and ADAMTS14 (Bolz et al. 2001; Colige et al. 2002) are also characterized as procollagen N-proteinase with similar domain arrangements.

ADAMTS4 was the first enzyme characterized as an aggrecanase that cleaves the Glu^{373}–Ala^{374} bond in the interglobular domain of aggrecan core protein and called aggrecanase 1 (Tortorella et al. 1999). Aggrecanase 2 was also reported soon after and originally assigned to ADAMTS11 (Abbaszade et al. 1999), but it was found to be identical to ADAMTS5 (Hurskainen et al. 1999) and therefore ADAMTS11 is no longer used. However, the detection of this unique aggrecan-cleaving activity was reported in the early 1990s in normal and interleukin 1 (IL-1)-treated cartilage (Ilic et al. 1992; Loulakis et al. 1992; Sandy et al. 1991).

Several other fragments of aggrecan resulting from cleavage of Glu^{1666}–Gly^{1667}, Glu^{1771}–Ala^{1771}, and Glu^{1871}–Leu^{1871} were also found (Ilic et al. 1992; Loulakis et al. 1992). Since these fragments were found in synovial fluid and serum of age-matched animals (Ilic et al. 1992), it was suggested that the enzyme responsible for these cleavages was important in both physiological and pathological catabolism of aggrecan. Aggrecan fragments resulting from the cleavage of the Glu^{373}–Ala^{374} bond accumulate in synovial fluids of patients with OA and inflammatory joint injury (Lohmander et al. 1993; Sandy et al. 1992).

Another notable member of the family is ADAMTS13 that selectively cleaves ultra-large von Willebrand factor under sheer flow stress. ADAMTS13 is unique in both structure and enzymatic activity. It is the only ADAMTS that has two C-terminal CUB (complement C1r/C1s—urchin epidermal growth factor-bone morphogenetic protein 1) domains along with seven TS domains after the Sp domain. The control of the size of multimeric von Willebrand factor by ADAMTS13 is important to maintain normal hemostasis, and larger multimers are hemostatically more active (Sadler 1998). Reduced ADAMTS13 activity in plasma causes thrombotic thrombocytopenic purpura (TTP) which characterized by thrombocytopenia, microangiopathic haemolytic anaemia, renal failure, neurologic dysfunction and fever resulting from blood clots formed in micro-capillaries. The deficiency of its activity is caused either by genetic mutation of the gene (Levy et al. 2001; Lotta et al. 2010) or by autoantibodies (Luken et al. 2006) that inhibit the ADAMT13 activity.

13.3.1 3D Structures of ADAMTSs

ADAMTSs are synthesized as pre-proenzymes, and they are intracellularly processed and secreted from the cell. The pro-domain of these enzymes may assist folding of the proteinase (Koo et al. 2007; Longpre and Leduc 2004) and they are most likely processed by a pro-protein convertase intracellularly as they have furin-like proteinase processing sites (Longpre and Leduc 2004; Wang et al. 2004), although proADAMTS5 (Longpre et al. 2009) and proADAMTS9 (Koo et al. 2006) may be activated on the cell surface. The first 3D structure reported in this family was the M domain with the Dis domain of ADAMTS1 in 2007 (Gerhardt et al. 2007). In the following year, the structures of the M-Dis domains of ADAMTS4 and ADAMTS5 (Mosyak et al. 2008) and the M domain of ADAMTS-5 (Shieh et al. 2008) were also reported. Full-length ADATMTS protein structures have not been solved yet, but Akiyama et al. (2009) reported the crystal structure of the Dis-TS-CysR-Sp domains of ADAMTS13. Figure 13.6 shows a composite structure of the M-Dis domain of ADAMTS4 (Mosyak et al. 2008) and the TS-CysR-Sp of ADAMTS13 (Akiyama et al. 2009), illustrating a possible topological arrangement of the domains in this family of enzymes. Since the residues located at the domain-domain interface are concerved among ADAMTSs, the general topological arrangements of Dis-TS-CysR-Sp in ADAMTSs are considered to be similar to that of ADAMTS13 (Akiyama et al. 2009).

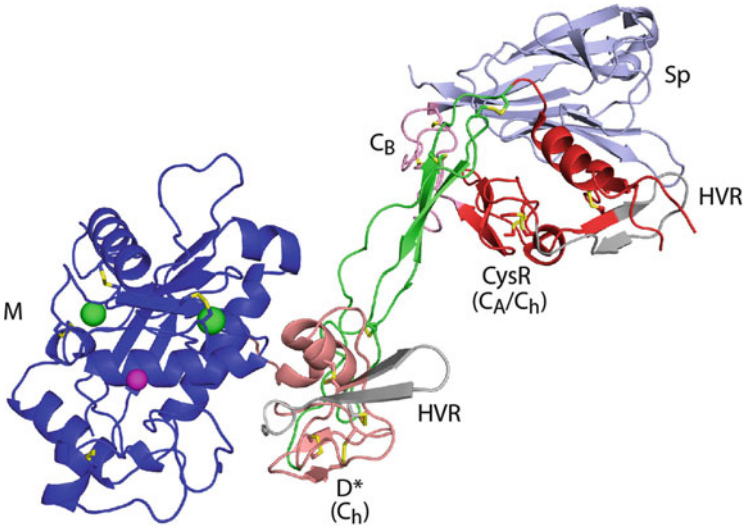


Fig. 13.6 A 3D structural model of ADAMTS. The structure was modelled based on the M and D* domain of ADMTS-5 (PDB ID: 2RJQ) and the D*, TS, CysR and Sp domains of ADMTS13 (PDB ID: 3GHN). Catalytic zinc ion is in *purple sphere*; calcium ions are in *green sphere*; and disulfide bonds in *yellow*. The image was prepared by Robert Visse

13.3.1.1 M Domain

The M domain of ADAMTS1 consists of a twisted central β-sheet of five strands, five α helices and connecting loops. The overall polypeptide fold of the M domain is a typical metzincin metalloproteinase fold (Gerhardt et al. 2007), similar to those of MMPs and ADAMs. The catalytic zinc ion interacts with three His residues in the HEXXHXXGXXH motif, but there is no structural zinc. There are, however, four disulfide bridges and the fourth bridge Cys^{379}-Cys^{462} connects the loop between strands β4 and β5 and a 22-residue connector loop after helix α5 which wraps around the back of the M domain and connects with the Dis domain. This arrangement places the Dis domain on the sites, so-called "primed sites", which interact with substrate residues located at C-terminal side of the scissile bond. An interesting feature noted for the M domains of ADAMTS4 and ADAMTS5 (Mosyak et al. 2008) is that, in the presence of an active site-directed inhibitor, the unique S2′-loop defined by a short disulfide-containing loop with the sequence CGXXXCDTL (322–330 in ADAMTS4) adopts a compact β-turn structure which is stabilized by the disulfide bond and a Ca^{2+}. Without the inhibitor, the S2′-loop is in a markedly different conformation and loses a Ca^{2+}. These changes bring Asp^{328} and Thr^{329} closer to the catalytic Zn^{2+}, and the carboxylate group of Asp^{328} chelates the Zn^{2+} and Thr^{329} fills the space at the mouth of the S1′ pocket. The S2′-loop therefore behaves like an auto-inhibitor of the metalloproteinases. This interaction is reminiscent of the "cysteine switch" found in the pro-domains of MMPs that interacts with the catalytic Zn^{2+} and maintains their inactive zymogen form.

Non-catalytic domains of ADAMTSs play important roles in substrate recognition and catalysis. It may be speculated that the binding of a natural substrate through the non-catalytic ancillary domains of the enzyme may induce such changes of the S2′-loop open and activate the enzyme. This may also explain why only certain substrates that interact with the non-catalytic domain are substrates of this group of enzymes.

13.3.1.2 Dis Domain

The 3D structure of the Dis domain of ADAMTS1, 4 and 5 (Gerhardt et al. 2007; Mosyak et al. 2008) did not show structural homology in 3D to the Dis domain of snake venom metalloproteinase VAP1 (Takeda et al. 2006), or that of ADAM10 (Janes et al. 2005). It is similar to the structure of the "hand" (C_h) segment of the CysR domain of VAP1. The CysR domain of VAP1 is structurally subdivided into "wrist" (C_w) and "hand" (C_h) subdomains (Takeda 2009; Takeda et al. 2006). The C_h subdomain has a core of α/β-fold structure consisting of two antiparallel β strands packed against two of the three α helices and five disulfide bonds (Takeda et al. 2006). The C_h fold of VAP1 is unique, but it is similar to the fold of the Dis domain and an N-terminal part of the CysR domain called "C_A" subdomain of ADAMTS13 (illustrated in red in Fig. 13.6), even though their amino acid sequence identity is low (Akiyama et al. 2009). The Dis domain of ADAMTSs is designated as "D*" by Takeda et al. (2012) to distinguish its structure from the canonical Dis domains found in snake venom metalloproteinases and ADAMs, and this distinction is adopted here. The D* domain in ADAMTS13 contains the loop region encompassing the central core of the D* domain. The sequences corresponding to this region in ADAMs and ADAMTSs are the most divergent and variable in length (11–55 amino acids) and it is referred as the hyper-variable (HVR) region. The HVR is located in close proximity to the active site and forms a part of the S3′ pocket and therefore participates in substrate interaction (Fig. 13.6). This may be the reason why the M domain alone has little proteolytic activity (Gendron et al. 2007; Kashiwagi et al. 2004).

13.3.1.3 TS Domain

The TS domain has an anti-parallel 3-stranded fold with three disulfide bonds, (Akiyama et al. 2009), a very similar structure of that of the prototypical thrombospondin type I repeat in thrombospondin-1 (Tan et al. 2002).

13.3.1.4 CysR Domain

The CysR domain of ADAMTS13 consists of two subdomains, the N-terminal C_A subdomain and the C-terminal C_B subdomain (Fig. 13.6) (Akiyama et al. 2009).

The structure of the C_A domain is similar to that of D* (or C_h), although they have only 17 % identity in amino acid sequence. The C_B subdomain does not have an apparent secondary structure (shown in pink in Fig. 13.6), but it has a series of turns stabilized by a pair of disulfide bonds at its N- and C-termini and forms a rod shape. The C_A subdomain contains an RGD (498–500) sequence and an HVR, but the side-chain of Arg^{498} of RGD is buried and unavailable for protein-protein interaction, but Asp^{500} is exposed to the solvent.

13.3.1.5 Sp Domain

The Sp domain is a long segment without cysteines and the primary structure shows no apparent homology to known structural motifs, but it folds into a single globular domain with 10 β-strands in a jelly-roll topology, forming two anti-parallel β-sheets that lie almost parallel to each other (Akiyama et al. 2009). Many key residues of the Sp domains are conserved among ADAMTS proteins and it is predicted that ADAMTSs share a similar domain architecture. In addition, the N- and C-termini of the Sp domain are in close proximity and therefore the TS2 domain after the Sp domain should be close to the C_A/Sp domain junction.

13.3.2 The Role of Non-catalytic Domain of Aggrecanases (ADAMTS4 and ADAMTS5) in Aggrecanolysis

There are eight ADAMTSs (ADAMTS 1, 4, 5, 8, 9, 15, 17 and 18) that have been reported to cleave aggrecan core protein at the aggrecanase sites (Stanton et al. 2011). Among them ADAMTS4 and ADAMTS5 have been extensively characterized biochemically as they are much more active than other ADAMTSs on aggrecan cleavage. The mature full-length ADAMTS4 and ADAMTS5 cleave aggrecan core protein effectively, but the M-D* domains alone show little aggrecananse activity (Gendron et al. 2007; Kashiwagi et al. 2004), suggesting that non-catalytic ancillary domains regulate their aggrecanolytic activities.

Domain deletion studies of ADAMTS4 by Kashiwagi et al. (2004) showed that the full-length ADAMTS4 has the greatest aggrecan-degrading activity, and the deletion of the Sp domain reduces the activity by 20 %. Further deletion of the CysR reduces it by about 60 %. The subsequent deletion of the TS domain yields a form with only 3 % of the full activity. However, it was reported that full-length ADAMTS4 showed only a very weak ability to cleave the Glu^{373}–Ala^{374} bond in the interglobular domain of aggrecan (Gao et al. 2004; Kashiwagi et al. 2004). Upon removal of the Sp domain by proteolysis (Gao et al. 2004) or mutagenesis (Kashiwagi et al. 2004), ADAMTS4 gained the ability to cleave the Glu^{373}–Ala^{374} bond and activity towards S-carboxymethylated transferrin, fibromodulin and decorin (Kashiwagi et al. 2004). Gao et al. (2004) found that MT4-MMP

(MMP-17) on the cell surface processes the C-terminal Sp domain and activates ADAMTS4 to cleave the Glu^{373}–Ala^{374} bond. Those data were interpreted to mean that the Sp domain inhibits ADAMTS4 activity towards specific cleavage sites. Later, however, Fushimi et al. (Fushimi et al. 2008) found that the suppressed cleavage of the Glu^{373}–Ala^{374} bond by full-length ADAMTS4 was due to the presence of heparin that was used to prevent the full-length enzyme from binding to ECM. Once heparin was chromatographically removed, ADAMTS4 revealed about 20-times higher activity on the Glu^{373}–Ala^{374} bond than the Sp domain-deleted form. It was found that heparin preferentially masks the activity of full-length ADAMTS4 towards the Glu^{373}–Ala^{374} bond, but it is less effective on its activity for the chondroitin sulfate-rich region. This suggests that full-length ADAMTS4 bound to heparan sulfate proteoglycan on the cell surface or the ECM has greatly reduced activity towards the Glu^{373}–Ala^{374} bond in the interglobular damain. When the Sp domain is removed, the enzyme can be released from the cell surface or ECM, and it gains broader proteolytic activity and digest decorin, biglycan, fibromodulin. However, the truncation of the Sp domain from ADAMTS4 results in about 95 % loss of aggrecanase activity including the Glu^{373}–Ala^{374} bond (Fushimi et al. 2008). Thus, removal of the Sp domain from ADAMTS4 shifts the preference of its substrates as well as the site of its action in the tissue.

ADAMTS5 also binds to the cell surface and the ECM. The main ECM binding site of ADAMTS5 is located in the CysR domain (Gendron et al. 2007). Full-length ADAMTS5 is about 30-times more active on aggrecan than full-length ADAMTS4. Domain deletion studies have indicated that the C-terminal TS domain has little effect on aggrecanase activity, but the deletion of the Sp domain lowers the activity by about 99 % for the chondroitin sulfate region, but only by about 50 % for the Glu^{373}–Ala^{374} bond. Further deletion of the CysR domain reduced the activity on the Glu^{373}–Ala^{374} bond by further 25 % (Gendron et al. 2007). Thus, non-catalytic domains are important controllers of the aggrecanolytic activity.

13.3.3 ADAMTSs in Arthritis

The major role of ADAMTSs in arthritis is considered to be their ability to cleave aggrecan and both ADAMTS 4 and 5 are much more effective in this activity than MMPs (Durigova et al. 2011). They also cleave other ECM molecules such as fibromodulin, biglycan and decorin in cartilage in vitro. Again, MMPs are not effective enzymes to cleave decorin (Geng et al. 2006). The treatment of human cartilage with IL-1 or TNFα increases aggrecanase activity, but it has no effect on mRNA levels for ADAMTS1, 4 and 5 (Flannery et al. 1999). mRNA levels for ADAMTS4 and ADAMTS5 in human OA cartilage are not significantly elevated compared to that in normal cartilage (Kevorkian et al. 2004), but ADAMTS5 mRNA levels are higher than ADAMTS4 mRNA (Bau et al. 2002). Treatment of human OA chondrocytes in culture with IL-1 increased ADAMTS4 mRNA levels,

but did not alter the levels of ADAMTS5 mRNA (Bau et al. 2002). While some studies confirmed the unaltered ADAMTS5 mRNA level in human chondrocytes upon IL-1 treatment, other studies reported its increase [see Fosang et al. (2008) for review]. Inconsistency among these reports may be due to the age of the tissue, culture conditions of isolated chondrocytes, the time of the transcriptional activity measurement and stability of the mRNA. Nonetheless, an increase in the aggrecan fragments generated by aggrecanases were found in RA and OA cartilage (Lark et al. 1997) and in synovial fluids (Lohmander et al. 1993; Sandy et al. 1992; Struglics et al. 2006), suggesting that ADAMTSs are important enzymes in aggrecanolysis. ADAMTS4-null mice and ADAMTS5-null mice show no obvious abnormality, but when challenged either via surgically induced OA (Glasson et al. 2004, 2005) or antigen-induced inflammatory arthritis (Stanton et al. 2005), the degradation of aggrecan in the cartilage of ADAMTS5-null mice was protected, but not that of ADAMTS4-null mice, indicating that ADAMTS5 is a major aggrecan-degrading enzyme in cartilage, at least in mice. Whether ADAMTS5 is a key aggrecanase in the development of human OA will only be determined by further investigation, as the levels of ADAMTS4 expression in both mRNA and protein correlate with OA progression (Naito et al. 2007).

While the importance of aggrecanases in aggrecanolysis is well recognised, using neoepitope antibodies that detect either MMP cleaved fragments or ADAMTS cleaved fragments, Lark et al. (Lark et al. 1997) showed that both RA and OA cartilages contain aggrecan fragments generated by MMPs (G1-NITEGE^{373} fragment) and ADAMTSs (G1-VDIPEN^{360} fragment). Struglics et al. (2006) confirmed these observations in OA cartilage and suggested that MMP-mediated aggrecanolysis is mostly pericellular while ADAMTSs are both pericellular and in the intraterritorial regions. Based on the fact that MT1-MMP-null mice and MMP-9-null mice cause destruction of articular cartilage, impairment of endochondral-ossification and fracture repair, it is suggested that some MMPs may be important in cartilage matrix homeostasis (Sandy 2006). Kevorkian et al. (2004) showed that ADAMTS16 is elevated in late human OA cartilage, but its function is not known. More recently, Yamamoto et al. (2013) reported that ADAMTS5 activity is regulated by endocytosis mediated by low density-lipoprotein receptor related protein 1 (LRP1) in normal cartilage, but this endocytic pathway is impaired in OA cartilage due to increased shedding of LRP1. This is considered one of the mechanisms that increase aggrecanolytic activity in OA cartilage without changing mRNA levels of ADAMTS5.

13.4 ADAMs

The ADAMs are type I transmembrane proteins with a characteristic domain structure comprised of a pro-peptide, a metalloproteinase (M) domain, a disintegrin (Dis) domain, a cysteine-rich (CysR) domain, an EGF-like (or other) domain, a transmembrane domain and a cytoplasmic domain (Fig. 13.1) (White 2003).

Homologous M, Dis and CysR domains are also found in snake venom metalloproteinases (SVMPs) (Gomis-Rüth 2003; Takeda et al. 2012). Thirteen out of a total of 21 ADAMs in the human genome have a potentially functional catalytic domain with the characteristic metzincin zinc-binding motif, but other ADAMs do not have proteolytic activity as they lack a complete zinc-binding motif. The pro-peptide acts as a molecular chaperone during synthesis (Leonard et al. 2005) and interacts with the catalytic cleft of the M domain, forming the inactive precursor (Gonzales et al. 2004). Proteolytic cleavage of the pro-peptide by a furin-like proteinase in the secretory pathway generates a mature active form of the ADAM in many cases. The isolated pro-domain of ADAM17 inhibits the proteolyic activity of the M domain, but the cysteine residue found in the putative cysteine switch in not important for the inhibition of the enzyme activity (Gonzales et al. 2004). Many of the mammalian ADAM metalloproteinases have been shown to cleave cell surface proteins, such as cytokines, growth factors, growth factor receptors and binding proteins and cell adhesion molecules, a process referred to as "shedding" (Becherer and Blobel 2003; Edwards et al. 2008; Weber and Saftig 2012; White 2003). In rheumatoid arthritis, ADAM17, also called tumor necrosis factor α converting enzyme (TACE), releases the inflammatory mediator TNFα from the plasma membrane, which in turn stimulates synovial cells to produce ECM-degrading enzymes. ADAM9, ADAM10 and ADAM15 are also expressed in rheumatoid synovial cells (Komiya et al. 2005) and ADAM9, ADAM 12 and 15 in OA cartilage (Okada et al. 2008).

13.4.1 3D Structure of the ADAMs

The first 3D structure of this family member solved was adamalysin II from eastern diamondback rattlesnake venom (Gomis-Rüth et al. 1993). Since then numerous structures of snake venom metalloproteinases have been solved including those with the M, Dis and CysR domains, which belong to P-II and P-III SVMPs (Takeda et al. 2012). Among the mammalian ADAMs, structures of the M domains of ADAM17 (Maskos et al. 1998) and ADAM33 (Orth et al. 2004), the Dis-CysR domains of ADAM10 (Janes et al. 2005), and the full ectodomain of ADAM22 (Liu et al. 2009) have been reported. ADAM22 lacks proteolytic activity as its zinc-binding motif of the M domain lacks two out of the three essential histidines. However, the M domain has a typical metalloproteinase fold even without a zinc ion. ADAM22 functions as a postsynaptic receptor for the secreted neurotransmission modulators LGI1 and LGI4 (Fukata et al. 2006; Sagane et al. 2008). It is predominantly expressed in the neuronal tissues and the mice deficient in ADAM22 exhibit ataxia, seizure and hypomyelination in the peripheral nerves (Sagane et al. 2005). Figure 13.7 shows the structure of ADAM22, illustrating that the M, Dis, CysR, EGF (E) domains which are typically found in ADAMs are arranged like a four-leaf clover, each leaf representing one of the four domains. The Dis, CysR and E domains have a continuous hydrophobic core and they appear as an

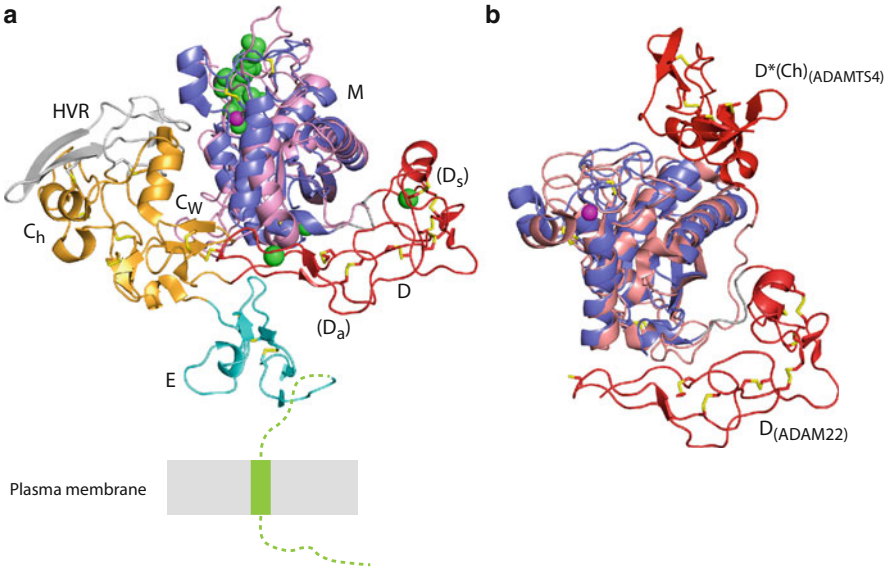


Fig. 13.7 3D structures of ADAMs. (**a**) Superimposition of the M domains of ADAM17 (PDB ID: 1BKC) that of the ectodomain of ADAM22 (PDB ID: 3G5C). The catalytic domain of ADAM17 is in *pink* and that of ADAM22 in *blue*. A hydroxamate inhibitor boud to the active site of ADAM17 is shown as a *green sphere*. The disintegrin (D), cysteine-rich domain consisting of C_w C_h, and hypervariable region (HVR) subdomains, and EGF (E) domain in the ectodomains are shown in *red*, *gold* and *grey*, and *cyan*, respectively. The catalytic zinc ion is in *purple sphere* and calcium ions in *green sphere*. The linker, transmembrane domain and the cytoplasmic tail are schematically shown in *green*. (**b**) Superimposition of the catalytic domains of ADAM22 shown in *blue* and that of ADAMTS4 (PDF ID: 2RJP) in *salmon* shows that the location of the disintegrin domains (D and D*) are located at the opposite sides of the catalytic domains. The images were prepared by Robert Visse

integral module mediated by extensive interface between the domains. The M domain is held in a concave face of the Dis-CysR-E module, and there is a long stretch of interactions between the M domain and the Dis domain and a small patch of interaction between the M domain and the CysR domain. The location of the M, Dis and CysR domains are resemble that in SVMPs, but vascular apoptosis-inducing protein-1 (VAP1) (Takeda et al. 2006) and VAP2B (Igarashi et al. 2007) have an open C-shaped conformation. However, the location of the D* domain of ADAMTSs is essentially opposite from that of the Dis domain of ADAMs (Fig. 13.7b).

13.4.1.1 M Domain

The M domain of ADAM22 and that of ADAM17 which has proteolytic activity are essentially identical, indicating that the catalytic zinc ion is not critical for folding of the M domain. Besides the catalytic Zn^{2+} the M domain has one Ca^{2+} ion.

Topological arrangement of Dis-CysR suggests that ADAM proteinases have the putative substrate binding site at the top of the molecule away from the plasma membrane. However, while ADAM17 has an extended substrate binding grove, one end of the groove of ADAM22 is blocked.

13.4.1.2 Dis Domain

The term "disintegrin" was initially used for a group of cysteine-rich RGD-containing small proteins (49–84 amino acids) from viper venom that inhibit platelet aggregation and integrin-mediated cell adhesion (Gould et al. 1990). The Dis structures of SVMPs and ADAM22 revealed similar structure as the RGD-containing disintegrin trimestatin (Fujii et al. 2003), but the disintegrin loop of ADAMs is not exposed for protein-protein interaction (Takeda 2009). The Dis domain of ADAMs and SVMPs is different from that of the D* domain of ADAMTSs as discussed above, and it is divided into two structurally distinct sub-segments, the 'shoulder' (D_s) and the 'arm' (D_a) subdomains. Both segments consist largely of a series of turns and two short regions of antiparallel β-sheet and constitute a continuous C-shaped 'arm' structure together with the N-terminal region of the CysR domain, designated as the 'wrist' (C_w) subdomain. Both D_s and D_a subdomains have three disulfide bonds and one structural calcium-binding site in each subdomain, which are essential for the structural rigidity of the C-shaped 'arm' structure. The D_a subdomain is connected to the C_w subdomain by an additional disulfide bond. ADAM10 and ADAM17 are atypical members of the ADAM family and they lack calcium-binding sites in the M domain and in D_a subdomain. Instead they have an additional disufide bond in the M domain. They also lack the E domain.

13.4.1.3 CysR Domain

The CysR domain is comprised of the "wrist" C_w subdomain and the "hand" (C_h) subdomain. The C_w subdomain consists of a pair of antiparallel β-sheets and loops. It is tightly associated with the D_a subdomain of the Dis domain on one side and with the C-terminal region of the C_h segment on the other side. The C_h subdomain has a unique fold with no structural homology to currently known proteins, except the corresponding segments of ADAMs and ADAMTSs. It has a core of αβ-fold structure consisting of two antiparallel β-stands packed against two of the three α-helices, five disulfide bonds which stabilize the core, and peripheral loops including an HVR which has a relatively small number of direct contacts with the core, suggesting that they are flexible in solution. Because of its location, opposed to the catalytic site, it is suggested that this region participates in substrate recognition (Takeda 2009).

13.4.1.4 EGF Domain

The E domain located after the C domain is divided into an N-subdomain and a C-subdomain. The N-subdomain consists of a loop region, a 3_{10} helix and a pair of antiparallel β-strands. The C-subdomain consists of two short antiparallel β-strands and the C-terminal flexible region which connect to a linker region and a transmembrane domain of the protein. There are three disulfide bonds in the E domain that stabilize its secondary structure. The alignment of E domains of ADAMs indicates that the loop regions are most diverged, but the overall structures of E domains are similar.

13.4.2 ADAMs in Arthritis

An imbalance in pro- and anti-inflammatory cytokines is thought to be a major feature of RA and may have some role in OA. The major pro-inflammatory cytokines TNFα and IL-1 are produced by synovial macrophages and ADAM17 is thought to be responsible for the solubilisation of the membrane-bound pro form of TNFα, hence it is also called TNFα converting enzyme or TACE. An endogenous inhibitor TIMP-3 inhibits ADAM17 (Amour et al. 1998) and TIMP-3-null mice have shown the importance of ADAM17 in the regulation of TNFα in systemic inflammation (Smookler et al. 2006). When antigen induced arthritis was studied in TIMP-3-null mice, compared to wild-type animals they showed a drastic increase in the initial inflammatory response to intra-articular antigen injection, and serum TNF-α levels were greatly elevated, although these differences in clinical features disappeared by days 7–14 (Mahmoodi et al. 2005). More recently the importance of ADAM17 in inflammatory arthritis has also been shown by the analysis of mice lacking iRHOM2, a catalytically inactive member of the rhomboid family, which controls maturation of ADAM17 in myeloid cells (Adrain et al. 2012; McIlwain et al. 2012). Mice deficient in iRHOM2 were protected from the K/BxN inflammatory arthritis model to a similar level as mice lacking ADAM17 in myeloid cells, or those deficient in TNFα (Issuree et al. 2013).

ADAM17 is activated by a variety of stimuli, including phorbol esters (Reddy et al. 2000), TNFα (Le Gall et al. 2010), IL-1β (Xu and Derynck 2010), LPS (Rousseau et al. 2008), activation of G protein-coupled receptors (Zhang et al. 2006) or FGF receptor 2 (Maretzky et al. 2011), and disulfide isomerization (Willems et al. 2010). ADAM17 also releases a number of other key effectors including TRANCE, fractalkine and various EGF receptor ligands, which may play roles in the development of arthritic disease. In addition, ADAM17 is potentially responsible for the proteolysis of receptors such as TNFR-I and TNFR-II, c-Met, IL1 receptor-II, trkA, IL-6 receptor, etc. The generation of soluble forms of receptors, e.g., IL-6 receptor, may be critical for the down-regulation of ligand function (Scheller et al. 2006). The shedding of cell adhesion molecules such as

L-selectin, VCAM-1, ICAM-1, CD30 and CD40 by ADAM17 may also have implications for cell behaviour within inflammatory and immune pathways.

While the role of ADAM17 is well recognized in RA, potential roles of other ADAMs in arthritic joint tissues have been reported. Examining the mRNA expression levels of 10 different ADAMs in synovial tissues of patients with RA or OA, Komiya et al. (Komiya et al. 2005) reported that ADAM15 mRNA was more elevated in RA samples compared with those in OA, but that negligible mRNA for ADAM8 and ADAM28 could be found whilst ADAM9, 10, and 17 were constitutively expressed in both RA and OA synovial tissues. Immunohistochemistry and *in situ* hybridisation have demonstrated a high level of expression of ADAM15 in RA synovial tissue, compared with normal or OA synovial tissue. It is found in macrophage-like and fibroblast-like synoviocytes, as well as in plasma cells (Böhm et al. 2001). Its expression was shown to have a direct correlated with vascular density and upregulated by VEGF165 (Komiya et al. 2005). The study of aging ADAM15-null mice exhibited accelerated development of OA lesions compared with wild-type mice (Böhm et al. 2005). The results were interpreted as showing that ADAM-15 had a protective role in the maintenance of joint integrity. More recent studies have shown that ADAM15 contributes anti-apoptotic pathway in OA chondrocytes upon serum- and matrix-withdrawal or under genotoxic stress-induced by camptothecin exposure (Böhm et al. 2010). This is through the cytoplasmic domain-mediated activation of survival signal-transducing kinase FAK and concurrent activation of Src (Fried et al. 2012).

In OA cartilage, elevated mRNA levels were reported for ADAM9 and membrane bound ADAM12m, as well as ADAM15, compared to those in normal human articular cartilage, but mRNAs for ADAM8, ADAM17 and ADAM28 were not detected, and ADAM19 was constitutive (Okada et al. 2008). Okada et al. (2008) also found that ADAM12m cleaves insulin-like growth factor-binding protein 5 and promote chondrocyte proliferation in OA cartilage. The production of ADAM12m was increased by TGFβ, which explains an anabolic effect of TGFβ which enhances matrix production and chondrocyte proliferation (Grimaud et al. 2002).

13.5 Endogenous Inhibitors of MMPs, ADAMTSs and ADAMs

Well-characterized endogenous inhibitors of metalloproteinases are α_2-macroglobulin and the TIMPs.

α_2M is a major plasma proteinase inhibitor of 725 kDa, consisting of four identical subunits. It inhibits most endopeptidases from different catalytic classes of proteinase regardless of their substrate specificities (Barrett 1981). The interaction of a proteinases with α_2M is initiated by proteolysis of the so-called "bait" region located in the middle of the subunits. This triggers a conformational change in α_2M that, in turn, entraps the enzyme without blocking the active site (Barrett and

Starkey 1973). The resulting α_2M-proteinase complexes are rapidly endocytosed via a scavenger receptor, LRP1 and degraded (Lillis et al. 2008). The activities of MMPs (Nagase et al. 1994), ADAMTS-1 (Kuno et al. 1999), ADAMTS-4 (Tortorella et al. 2004), and soluble form of ADAM12 (Loechel et al. 1998) have been shown to be regulated by α_2M. Indeed most, if not all, MMPs and ADAMTSs and soluble form of ADAMs are likely to be inhibited by α_2M. The site of action of α_2M is thought to be primarily in the fluid phase. The levels of α_2M in synovial fluids of RA patients are 0.7–1.0 mg/ml, approximately one-third of the normal plasma level (Flory et al. 1982).

In the tissue, TIMPs are considered to be key inhibitors of metalloproteinases. They form 1:1 enzyme-inhibitor complexes. Four TIMPs (TIMPs-1–4) are found in the human genome and they are 21–29 kDa proteins consisting of the N-terminal metalloproteinase inhibitory domain (about 125 amino acids) and the C-terminal domain (about 65 amino acids). The C-terminal domain mediates specific interaction with some MMP zymogens through their C-terminal Hpx domains. The complex formation between proMMP-2 and TIMP-2 through their C-terminal domain is important for proMMP-2 activation by MMP-14 on the cell surface. Four TIMPs have been characterized as MMP inhibitors, but some TIMPs inhibit ADAMTSs and ADAMs (Brew and Nagase 2010): TIMP-3 has the broadest activity and inhibits ADAMTS1, 4, 5, ADAM 10, 12, 28 and 33, and weakly ADAMTS2; TIMP-1 inhibits ADAM10; TIMP-2 inhibits ADAM12; and TIMP-4 inhibits ADAM28 and weakly ADAM33.

Early work by Dean et al. (1989) showed that both MMP levels and TIMP levels were elevated in an early stage of OA cartilage compared with unaffected cartilage, but the total amount of MMPs was slightly higher than that of TIMPs, whereas this balance was reversed in the unaffected cartilage. This subtle difference in the ratio of MMPs and TIMPs is considered to be a cause of the gradual degradation of the cartilage matrix.

13.5.1 Inhibition Mechanisms of TIMPs

The first 3D structure of TIMP reported was the NMR solution structure of the N-terminal inhibitory domain of TIMP-2 (N-TIMP-2), which revealed a five-stranded OB-fold β-barrel and two α-helices (Williamson et al. 1994). Elucidation of the inhibition mechanism of TIMPs had to wait for the crystal structures of the TIMP-1-MMP-3 catalytic domain complex (Gomis-Rüth et al. 1997) (see Fig. 13.8). The structure showed that TIMP is a "wedge-shaped" molecule and where Cys^1 and Cys^{70} and Cys^3-Cys^{99} are disulfide bonded and form a rigid structure, slots into the active site of MMP. The N-terminal α-amino and carboxyl groups of Cys^1 bidentately coordinate the catalytic Zn^{2+} of the enzyme and the N-terminal segment binds to the active site cleft subsite S1–S4′ of the enzyme like the substrate, and Ser^{68} and Val^{69} are orientated towards the S_2 and S_3 subsites, respectively, nearly opposite to the direction of a substrate. The side chain of Thr^2

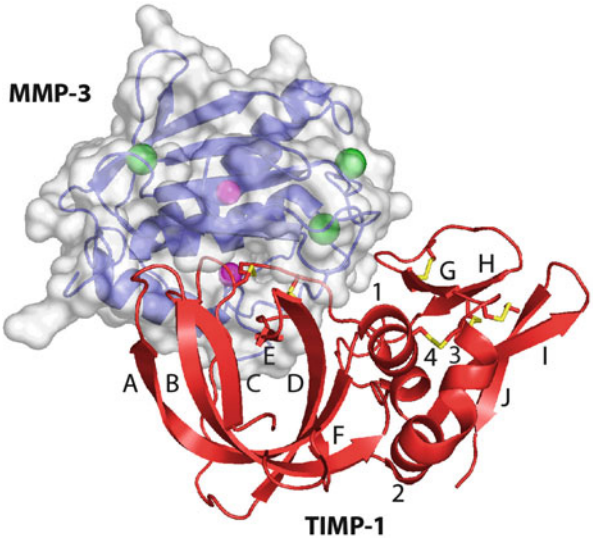


Fig. 13.8 3D structure of the TIMP-1-MMP-3 catalytic domain. TIMP-1 is shown in *red* and disulfide bonds in *yellow*; α-helices and β-strand are indicated. MMP-3 is shown in *blue* ribbon and *grey* surface. Zinc ions and calcium ions in MMP-3 are shown as *purple* and *green spheres*. The image were prepared by Robert Visse based on PDB ID: 1UEA

extends into the large S1′ specificity pocket of the MMP. The structure of the N-TIMP-3-ADAM17 catalytic domain (Wisniewska et al. 2008) has indicated that ADAMs and ADAMTSs may interact with TIMPs in a similar binding mode.

Because the N-terminal α-amino group is essential to chelate the catalytic Zn^{2+} and it plays an important role in inhibition of MMPs, any extension or blocking of the N-terminal drastically reduces the inhibitory activity for MMPs (Higashi and Miyazaki 1999; Troeberg et al. 2002; Wingfield et al. 1999). Similarly the mutation of Thr^2 to Gly leads to greatly reduced inhibitory activity for MMPs (Meng et al. 1999). However, this mutation has a relatively small effect on the affinity of N-TIMP-1 for MMP-9 (Hamze et al. 2007), suggesting that interaction outside of the S1′ pocket are more important with MMP-9 than with other MMPs. Mutation of Thr^2 to Gly of N-TIMP-3 also reduced inhibitory activity for most MMPs, but this mutant retains good inhibitory activity for ADAM17 (Wei et al. 2005) and ADAMTS-4 and -5 (Lim et al. 2010). Addition of one or two extra Ala to the N-terminus ([-1A]N-TIMP-3 or [-2A]N-TIMP-3) also retained inhibitory activity for ADAM17 and ADAMTS-4 and -5 (Lim et al. 2010; Wei et al. 2005). Those mutants are useful to discriminate activities derived from MMPs and those from ADAM17 or ADAMTS-4 and -5. TIMP-1, TIMP-2 and TIMP-4 do not inhibit ADAM17. A series of studies by Lee et al. mutated the TIMPs to inhibit ADAM17, further delineating the segments that can dictate specificity of each TIMP molecule (Lee et al. 2004a, b, 2005b).

13.5.2 *Pentosan Polysulfate Is an Exosite Inhibitor of ADAMTS4 and ADAMTS5*

Pentosan polysulfate (PPS) is a chemically sulfated xylosan extracted from beechwood with a molecular mass of 4–6 kDa. It has been shown to be an effective anti-arthritic agent in animal models (Ghosh 1999). PPS inhibits ADAMTS4 and ADAMTS5 activity on native aggrecan, but not non-aggrecan substrates, by binding to the Sp domain of ADAMTS4 and the Sp and CysR domains of ADAMTS5, but not to the M domain of the enzymes (Troeberg et al. 2008). PPS is therefore an exosite inhibitor of aggrecanases. Other interesting activities of PPS include its ability to increase the affinity between aggrecanases and TIMP-3 more than 100-fold (Troeberg et al. 2008). A single chain of PPS binds to the Sp domain of ADAMTS5 and to TIMP-3 by forming a trimolecular complex. This interaction depends on the size and 11 saccharide length is sufficient for the trimolecular complex formation, which is driven electrostatically (Troeberg et al. 2012). PPT also bocks the LRP1-mediated endocytosis of TIMP-3 by chrondrocytes or other cell types (Troeberg et al. 2008). The treatment of the cartilage with IL-1 induces aggrecanase activity and aggrecan degradation. This activity is blocked by PPS, which is associated with an increased amount of TIMP-3 in the cartilage without changing its mRNA levels. This is due to blocking TIMP-3 endocytosis and increasing the affinity of TIMP-3 and aggrecanases. PPS is ineffective in blocking of the IL-1-induced aggrecan degradation of the cartilage from TIMP-3-null mice, indicating that chondroprotective activity of PPS is dependent on the presence of TIMP-3 (Troeberg et al. 2008).

13.6 Future Prospects

A number of MMPs, ADAMTSs and ADAMs have been implicated in the progression of RA and OA and they are involved in degradation of cartilage ECM, altering cellular environments and trafficking cells. Therefore, the agents that regulate these metalloproteinase activities are potential therapeutics for RA and OA. The structural studies have assisted to design numerous potent inhibitors and some have been advanced to clinical studies for arthritis, and also for cancer and cardiovascular disease, but it has turned out to be challenging. Whilst all compounds showed good efficacy and tolerability in animal models of arthritis, all have been discontinued citing either lack of efficacy or appearance of musculoskeletal syndrome (MSS) in humans. This is due to poor pharmacokinetics in man and/or poor selectivity. Good advancement has been made in the case of MMP-13 inhibitors that do not have a chelating moiety, but enter into the deep S1′ pocket of the active site (Engel et al. 2005; Johnson et al. 2007). This greatly increases the selectivity of the inhibitor. Another important consideration is the exosites of these enzymes. MMPs, ADAMs and ADAMTSs are multi-domain proteinases and in many cases their activities for

the natural ECM substrates are regulated by non-catalytic ancillary domains. Deletion of such domains critically impairs their activity, suggesting molecules that modify their functions (allosteric inhibitors) may specifically affect the enzymatic activity on natural substrates. An example may be seen in autoantibodies found in patients with thrombotic thrombocytopenic purpura that inhibit the activity of ADAMTS-13 to cleave ultra-large von Willebrand factor. Those antibodies are directed to the non-catalytic domains of the proteinase, not toward the catalytic domain (Soejima et al. 2003). Specific antibodies and allosteric inhibition are ideal for selective inhibition of a target proteinase, and such antibodies have been engineered for ADAM17 (Tape et al. 2011; Richards et al. 2012) and MMP-14 (Basu et al. 2012). Therefore, this direction of research will be further developed.

The activities of these metalloproteinases are regulated at the level of transcription, activation from precursors or inactive forms, inhibition by endogenous inhibitors. More recently, it has been shown that the synthesis of ADAM17 is regulated by iRHOMs, and that some of MMPs, ADAMTSs and TIMPs are endocytosed via a scavenger receptor LRP1. Furthermore, under inflammatory conditions shedding of LRP1 increased and the levels of soluble LRP1 is elevated in plasma of patients of rheumatoid arthritis, systemic lupus erythematous (Gorovoy et al. 2010) and liver disease (Quinn et al. 1997). LRP1 protein is largely lost in OA cartilage (Yamamoto et al. 2013). These findings indicate further complexity of regulation of these metalloproteinases, but they also suggest numerous possibilities to develop therapeutic agents for RA and OA, and for other diseases associated with aberrant ECM turnover.

Acknowledgements We thank Ngee Han Lim, Alan Lyons and Rob Visse for preparation of the figures. This work is supported by grant from Arthritis Research UK, European FP7 LIVIMODE programme, NIH/NIAMS grant AR40994 (to HN), and Cancer Research UK and Medical Research Council (to GM).

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