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REVIEW

Proteinases involved in matrix turnover during cartilage and bone breakdown

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Abstract The joint is a discrete unit that consists of cartilage, bone, tendon and ligaments. These tissues are all composed of an extracellular matrix made of collagens, proteoglycans and specialised glycoproteins that are actively synthesised, precisely assembled and subsequently degraded by the resident connective tissue cells. A balance is maintained between matrix synthesis and degradation in healthy adult tissues. Different classes of proteinases play a part in connective tissue turnover in which active proteinases can cleave matrix protein during resorption, although the proteinase that predominates varies between different tissues and diseases. The metalloproteinases are potent enzymes that, once activated, degrade connective tissue and are inhibited by tissue inhibitors of metalloproteinases (TIMPs); the balance between active matrix metalloproteinases and TIMPs determines, in many tissues, the extent of extracellular matrix degradation. The serine proteinases are involved in the initiation of activation cascades and some, such as elastase, can directly degrade the matrix. Cysteine proteinases are responsible for the breakdown of collagen in bone following the removal of the osteoid layer and the attachment of osteoclasts to the exposed bone surface. Various growth factors increase the synthesis of matrix and proteinase inhibitors, whereas cytokines (alone or in combination) can inhibit matrix synthesis and stimulate proteinase production and matrix destruction.

Keywords Cartilage · Collagen · Extracellular matrix · Metalloproteinase · Arthritis

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Abbreviations

ADAM	a disintegrin and metalloproteinase
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
BMP-1	bone morphogenetic protein-1
ECM	extracellular matrix
GPI	glycosylphosphatidyl inositol
HDAC	histone deacetylase
IGFBP	insulin-like growth factor binding protein
IL	interleukin
Jak-STAT	Janus kinase-signal transducer and activator of transcription
MAPK	mitogen-activated protein kinase
MMPs	matrix metalloproteinases
NF-κB	nuclear factor kappa B
OA	osteoarthritis
OSM	oncostatin M
RA	rheumatoid arthritis
RANKL	receptor activator of nuclear factor κB ligand
TNF	tumour necrosis factor
TIMPs	tissue inhibitors of metalloproteinases
TGF	transforming growth factor

Introduction

Cartilage tissue consists of a single cell type, chondrocytes (Goldring 2000), which are embedded within an extracellular matrix (ECM) of aggrecan, type II collagen and other minor components that are precisely arranged within an interactive matrix. The rod-shaped collagen molecules aggregate in a staggered array to form cross-linked fibres giving connective tissues strength and rigidity. Trapped between these collagen fibres are the aggrecan molecules

(Iozzo 1998) that, in the presence of hyaluronic acid, form highly charged aggregates that attract water into the tissue and allow cartilage to resist compression. Chondrocytes in normal adult cartilage maintain a steady state in which the rate of matrix synthesis equals the rate of degradation. Any change in this steady state will affect the functional integrity of the cartilage. During growth and development, the synthesis of matrix components exceeds the rate of degradation; a reduction in the rate of matrix synthesis and an increase in the rate of degradation occurs during matrix resorption (Mort and Billington 2001).

Bone is a metabolically active tissue that is constantly formed and removed throughout life. The processes are carefully coordinated by bone cells that respond to a variety of external factors. These include genetic, mechanical, hormonal and nutritional factors and a large number of growth factors and cytokines. The cells contained in bone belong to three types: osteoblasts, osteocytes and osteoclasts. These are all contained within a highly mineralised matrix of type I collagen and other highly specialised proteins such as osteocalcin, osteonectin and proteoglycan. The mineral is present mainly as a mixture of calcium and phosphate in the form of hydroxyapatite. Two anatomical types of bone exist, namely trabecular and cortical. Trabecular bone exhibits more metabolically active surfaces on which the basic multicellular units act, whereas these multicellular units operate through resorbing channels in cortical bones. The cells of bone occupy a central role in this active metabolism. Osteoclasts are haemopoietic in origin and responsible for the resorption of bone and form following the activation of macrophage-like mononuclear cells.

In childhood, more bone is formed than is resorbed, whereas in the young adult, when the bone mass is constant, these two processes are balanced. In later life, more resorption than formation leads to diseases such as osteoporosis. Many of the activities of the osteoclast depend on the osteoblast. Osteocytes are formed from osteoblasts that become isolated in bone and surrounded by matrix. The osteocytes communicate with each other through extended cellular processes that link cells allowing them to respond to stimuli such as changes in mechanical forces.

In severe cases of arthritis, both cartilage and the underlying bone are destroyed and this prevents joints from functioning normally. The primary cause of cartilage and bone destruction in joint pathology involves elevated levels of active proteinases that are secreted from a variety of cells and that degrade the ECM. These proteinases are regulated by various cytokines and growth factors acting on cells found within the joint. In osteoarthritis (OA), the proteinases produced by chondrocytes play a major role (Takaishi et al. 2008; van den Berg 2000). In a highly inflamed rheumatoid joint, proteinases produced primarily by synovial and inflammatory cells contribute to the loss of tissue matrix (Firestein 2003). This review describes the proteolytic enzymes that are implicated in the destruction of cartilage and bone tissue and considers the inhibition of matrix metalloproteinases (MMPs) as an effective strategy for the prevention of joint destruction.

Role of proteolytic enzymes in matrix breakdown

The five main classes of proteinases are classified according to the chemical group which participates in the hydrolysis of peptide bonds (Barrett et al. 1998). Cysteine, aspartate, and threonine proteinases are predominantly active at acid pH and act intracellularly; the serine and metalloproteinases are active at neutral pH and act extracellularly (Fig. 1). Some proteinases are membrane-bound rather than secreted from the cell and such enzymes are associated with cytokine processing, receptor shedding and the removal of proteins that are associated with cell–cell or cell–matrix interactions (Becherer and Blobel 2003). Some enzymes, such as elastase are released when neutrophils are stimulated, whereas others might not participate in the cleavage of matrix proteins but activate proenzymes that then proceed to degrade the matrix. All classes of proteinase play a part in the turnover of connective tissues and one proteinase pathway may precede another. For example, in bone, the removal of the osteoid layer by metalloproteinases precedes the attachment of the osteoclast and subsequent breakdown of the ECM by cysteine proteinases (Everts et al. 1992). A close apposition of intra- and extra-cellular pathways will be found in many conditions involving connective tissue turnover.

Neutral proteinases

Metzincin superfamily

These metalloproteinases are distinguished by a highly conserved motif containing three histidines that bind zinc at the catalytic site and a conserved methionine turn that lies beneath the active-site zinc (Stocker et al. 1995). Metalloproteinases are further divided into four multigene families: the serralysins, the astacins, ADAMs (a disintegrin and metalloproteinase)/adamalysins and MMPs (Egeblad and Werb 2002). These families are classified according to the sequence around the three conserved histidines that bind zinc. A fifth group, the pappalysins, have been proposed (Boldt et al. 2001) that cleave insulin-like growth factor binding protein-4 and -5 (Overgaard et al. 2001).

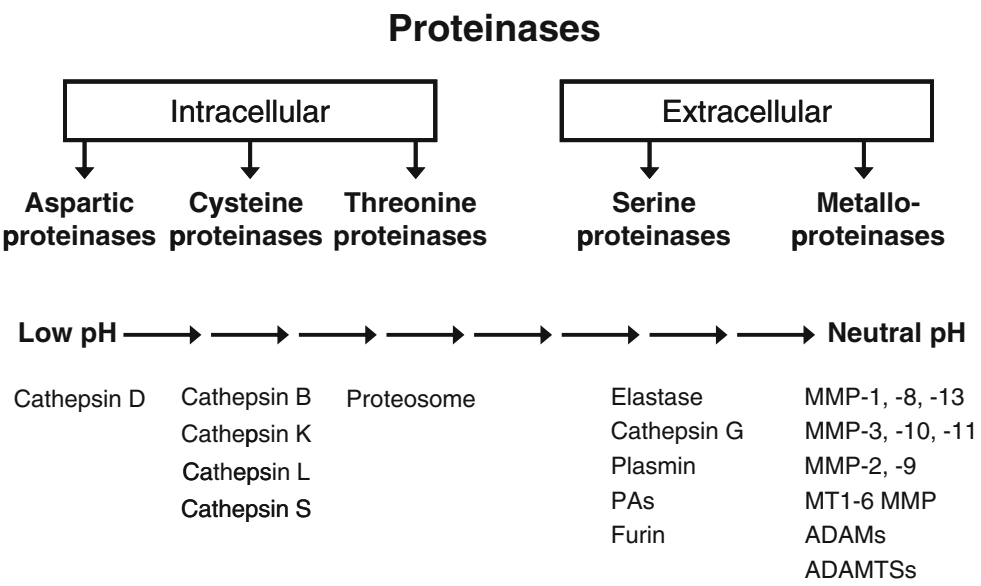

Fig. 1 The five classes of proteinase, three of which act predominantly intracellularly (aspartate, cysteine and threonine) and two predominantly extracellularly (metallo and serine). Examples are shown of representative enzymes from each class (*MMP* matrix metalloproteinase, *ADAM* a disintegrin and metalloproteinase, *ADAMTS* a disintegrin and metalloproteinase with thrombospondin motifs)

Matrix metalloproteinases

The MMPs constitute a multigene family of over 23 secreted and cell-surface zinc-dependent endopeptidases that process or degrade numerous substrates at neutral pH (Nagase and Woessner 1999; Tallant et al. 2009). All MMPs contain common domains (Fig. 2), zinc is present at the catalytic centre and all are produced in a proenzyme form. Latency of the proMMP is maintained by the interaction of a conserved cysteine residue in the prodomain with the catalytic zinc in the active site (Springman et al. 1990; Van Wart and Birkedal-Hansen 1990). The MMP family are best known for their ability to cleave components of the ECM but they also cleave other proteinases, proteinase inhibitors, latent growth factors, chemotactic molecules, growth factor binding proteins, cell surface receptors and cell-cell adhesion molecules (Fig. 3; Sternlicht and Werb 2001).

Traditionally MMPs have been divided into various groups, according to the ECM substrates that they cleaved: the stromelysins, collagenases, gelatinases (Nagase and Woessner 1999). MMP-3 and MMP-10 (stromelysin-1 and -2, respectively) have a broad and similar substrate specificity (Nagase 1995) but the expression pattern of these enzymes is often distinct. Their natural substrates are probably proteoglycans, fibronectin and laminin. Both enzymes are able to activate latent collagenases (Knäuper et al. 1993, 1996b; Murphy et al. 1987) and are present in articular cartilage and synovium from patients with either rheumatoid arthritis (RA) or OA (Hembry et al. 1995; Okada et al. 1992; Wolfe et al. 1993).

The three mammalian collagenases, viz. MMP-1, MMP-8 and MMP-13 (collagenase-1, -2 and -3 respectively), cleave fibrillar collagens producing three-quarter- and one-quarter-sized fragments; MMP-2 and MMP-14 (MT1-MMP) can also cleave at this site (Aimes and Quigley 1995; Ohuchi et al. 1997). The enzymes differ in their specificity for different collagens; MMP-13 prefers to cleave type II collagen (Knäuper et al. 1996a), whereas MMP-1 and MMP-8 prefer type III and I, respectively. Both MMP-1 and MMP-13 are synthesised by macrophages, fibroblasts and chondrocytes when these cells are stimulated with inflammatory mediators. MMP-8 is predominantly released from intracellular storage granules within neutrophils upon stimulation but can also be produced by chondrocytes. All three collagenases are present in diseased cartilage (Tetlow et al. 2001), although their control can be different; for example, retinoic acid, which downregulates MMP-1, is known to upregulate MMP-13 in some cells (Shingleton et al. 2000).

The two gelatinases cleave denatured collagens, type IV and V collagen and elastin (Aimes and Quigley 1995; Fosang et al. 1992). MMP-2 (gelatinase A) is the most widespread of all the MMPs and can activate proMMP-13 (Knäuper et al. 1996b). MMP-9 (gelatinase B) is expressed in a wide variety of transformed and tumour-derived cells (Murphy and Crabbe 1995). MMP-2 and MMP-9 protein levels are elevated in RA synovial fluids and tissues (Ahrens et al. 1996; Gruber et al. 1996; Yoshihara et al. 2000).

With the increasing numbers, complexity and range of substrates, MMPs are now often grouped according to their domain structure (Clark and Parker 2003; Fig. 2). Most MMPs resemble MMP-1; MMP-2 and MMP-9 have fibronectin-like inserts, whereas MMP-21 has a vitronectin-like domain insert. MMP-17 and MMP-25 both have a cytoplasmic glycosylphosphatidyl inositol (GPI)

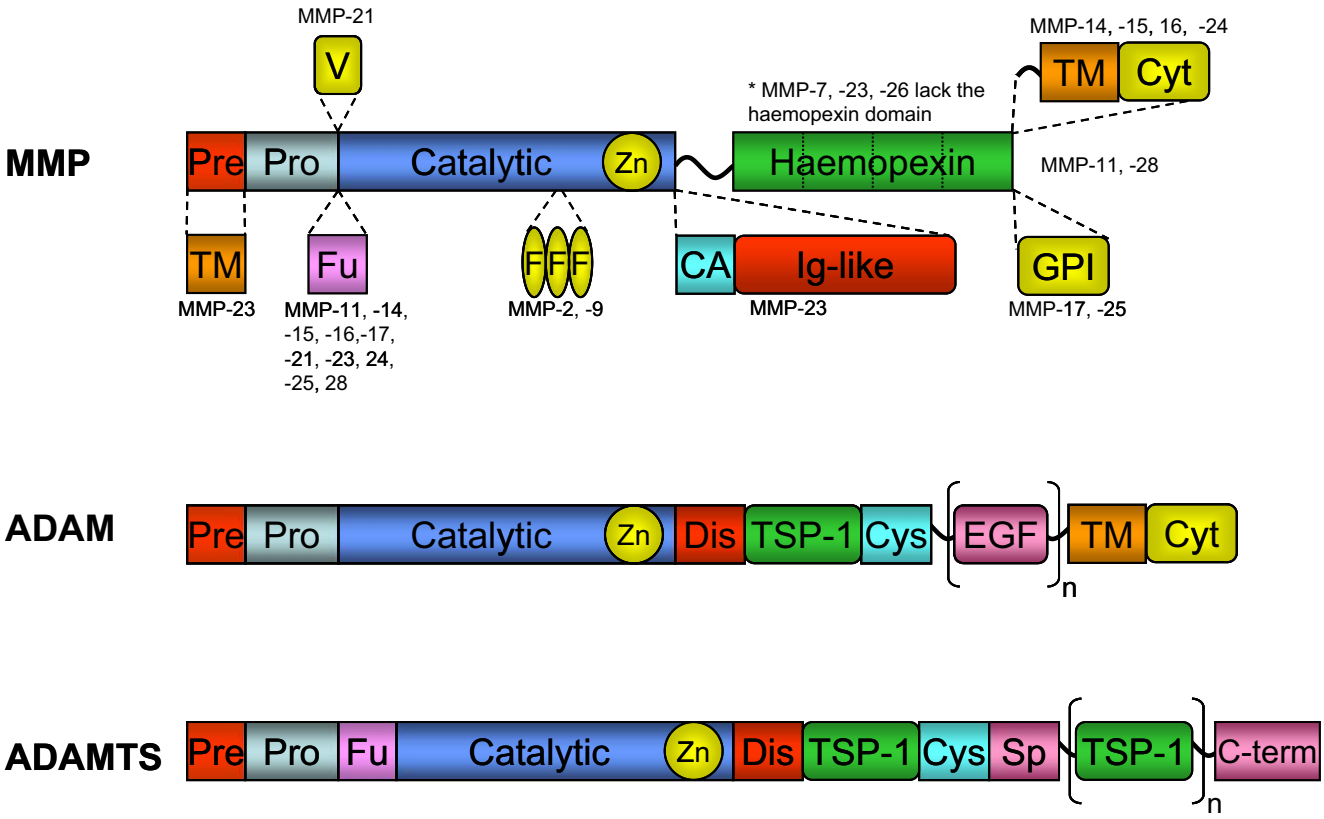

Fig. 2 Domain structures of metalloproteinases. MMPs, ADAMs and ADAMTSs have a domain structure, with several common domains across the family that influences the behaviour of the protein. *Top* All MMPs have a catalytic domain containing the active site zinc (*Zn*). Some MMPs contain a furin recognition motif (*Fu*) that allows intracellular activation by furin-like proteinases. Apart from MMP-7, -26 and -23, all MMPs contain a haemopexin domain that often determines substrate specificity. Other domains found within the MMPs are the fibronectin-like domains (*F*) in MMP-2 and -9 and the vitronectin-like domain (*V*) in MMP-21. Some MMPs are anchored to the cell surface via a trans membrane domain (*TM*) with cytoplasmic tail (*Cyt*) or via a glycosylphosphatidyl inositol (*GPI*) anchor. MMP-23 is structurally unique amongst the MMPs and contains an N-terminal TM (actually an N-terminal signal anchor), a cysteine array (*CA*) and a immunoglobulin-like domain (*Ig-like*). Adapted from Egeblad and Werb 2002). *Middle* The ADAMs contain a disintegrin (*Dis*) and a metalloprotease domain. The metalloprotease domains of ADAMs can induce ectodomain shedding and cleave extracellular matrix (ECM) proteins (*EGF* epidermal growth factor-like). The ADAMs disintegrin (*Dis*) and cysteine-rich (*Cys*) domains have adhesive activities. All ADAMs contain a trans membrane domain (*TM*) and their activities may be controlled in part via phosphorylation of their cytoplasmic tails (*Cyt*). *Bottom* ADAMTS also contain a disintegrin (*Dis*) and a metalloprotease domain but uniquely contain a thrombospondin type-1 (*TSP-1*) repeat, then a Cys domain and one or more additional TSP-1 repeats, except ADAMTS-4 (*Sp* signal peptide). This is frequently followed by a C-terminal domain often containing a recently described protease and lacunin motif (Clark and Parker 2003)

anchor, MMP-23 has a C-terminal immunoglobulin-like domain and neither MMP-7 (matrilysin) nor MMP-26 have a haemopexin domain (Egeblad and Werb 2002).

Levels of different MMPs are increased in rheumatoid synovial fluid, in conditioned culture media from rheumatoid synovial tissues and cells, in synovial tissue at the cartilage–pannus junction from rheumatoid joints, in osteoarthritic cartilage and in animal models of arthritis (Cawston 1996; Konttinen et al. 1999; Murphy and Crabbe 1995; Tetlow et al. 2001). In OA, both the rate of matrix synthesis and breakdown are increased leading to the formation of excess matrix in some regions (such as osteophytes) with focal loss of the ECM in other areas.

MMPs are controlled at different levels

MMPs regulate many biological processes and are precisely controlled at a number of critical steps that include synthesis and secretion, activation of the proenzymes, inhibition of the active enzymes and localisation and clearance of MMPs (Fig. 4; Clark et al. 2008).

Synthesis and secretion

Cytokines such as interleukin (IL)-1, tumour necrosis factor (TNF)-α and IL-17 stimulate numerous cell types to produce many MMPs (Goldring et al. 2008; Koshy et al. 2002a; van den Berg 1999; Yan and Boyd 2007). Within

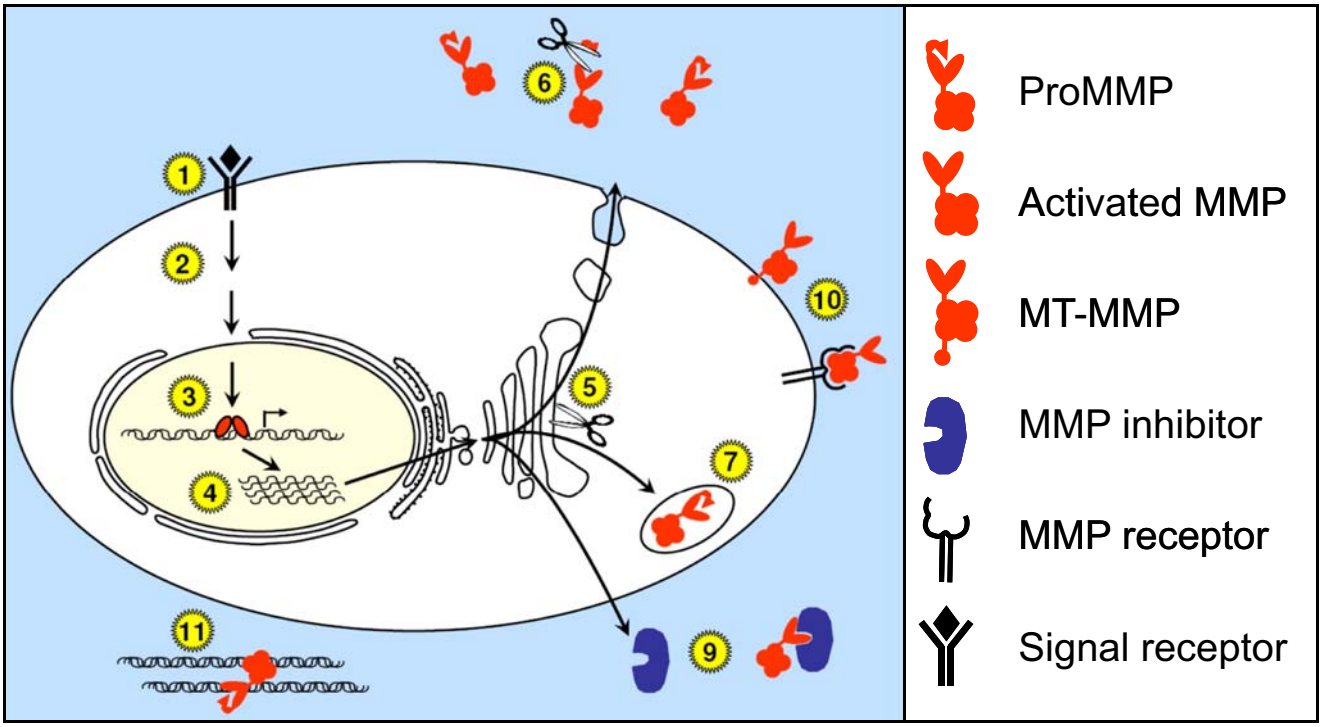

Fig. 3 Control of MMP activity (*IGFBP* insulin-like growth factor binding protein, *TGFB* transforming growth factor β, *EMPRIN* EMMPRIN extracellular matrix metalloproteinase inducers). Cytokines and growth factors can up-regulate or down-regulate MMP expression (*1*). Different intracellular signalling pathways combine (*2*) to activate or suppress transcription (*3*). RNA can be unstable and rapidly processed (*4*). ProMMPs can be activated intracellularly by furin (*5*) or after they have left the cell (*6*). Some MMPs are stored in granules within the cell (*7*) prior to secretion. Secreted MMPs can be expressed on the cell surface (*9*), bound to cell surface receptor proteins or sequested by ECM proteins (*10*). All active MMPs can be inhibited by tissue inhibitors of metalloproteinases (*11*)

the arthritic joint, different cell types produce specific cytokines and growth factors that can be found in synovial fluids from RA patients. These cytokines often differ in their action on individual cell types and many cytokines can synergise to increase the production of MMPs by cells.

Although IL-1 and TNFα are able to initiate cartilage collagen resorption alone, when these cytokines are combined with oncostatin M (OSM), a rapid and reproducible release of collagen is found in bovine and porcine cartilage (Cawston et al. 1998). Human cartilage also responds to this combination of cytokines (Morgan et al. 2006). Synthetic MMP inhibitors and two tissue inhibitors of metalloproteinases, viz. TIMP-1 and TIMP-2, are able to prevent this release, strongly implicating the collagenolytic MMPs in this process (Ellis et al. 1994); chondrocytes are known to synthesise collagenases-1, -2 and -3 (Kevorkian et al. 2004).

Activation of proenzymes

The control of the activation of the proenzyme form of MMPs is important in connective tissue breakdown (Kleiner and Stetler-Stevenson 1993; Milner et al. 2001). Some MMPs (Fig. 2) have a furin recognition sequence between the propeptide and the catalytic domain and these enzymes are often activated within the Golgi. Recent data show that cartilage explant cultures, treated with cytokines and an inhibitor of furin, have reduced levels of active collagenases and low collagen release (Milner et al. 2003). For those MMPs without a furin site, the proteolytic removal of the propeptide is likely to be achieved in a tightly controlled environment close to the cell surface. Plasmin and other serine proteinases can activate some proMMPs (Eeckhout and Vaes 1977; He et al. 1989; Knäuper et al. 1996b; Werb et al. 1977) and are involved in the activation cascades of the pro-collagenases (Milner et al. 2001). Active MMP-3 can activate pro-collagenases and other proMMPs (Knäuper et al. 1993, 1996a; Murphy et al. 1987; Ogata et al. 1992). Several members of the membrane-type MMP family (MMP-14, -16, -24 and 25) can activate proMMP-2 (Butler et al. 1997; Pei 1999; Sato et al. 1994; Takino et al. 1995; Velasco et al. 2000) and MMP-14 can also activate proMMP-13 (Knäuper et al. 1996b).

Active enzyme inhibition

All active MMPs are inhibited by TIMPs (Brew et al. 2000; Cawston 1996), which bind tightly to active MMPs in a 1:1

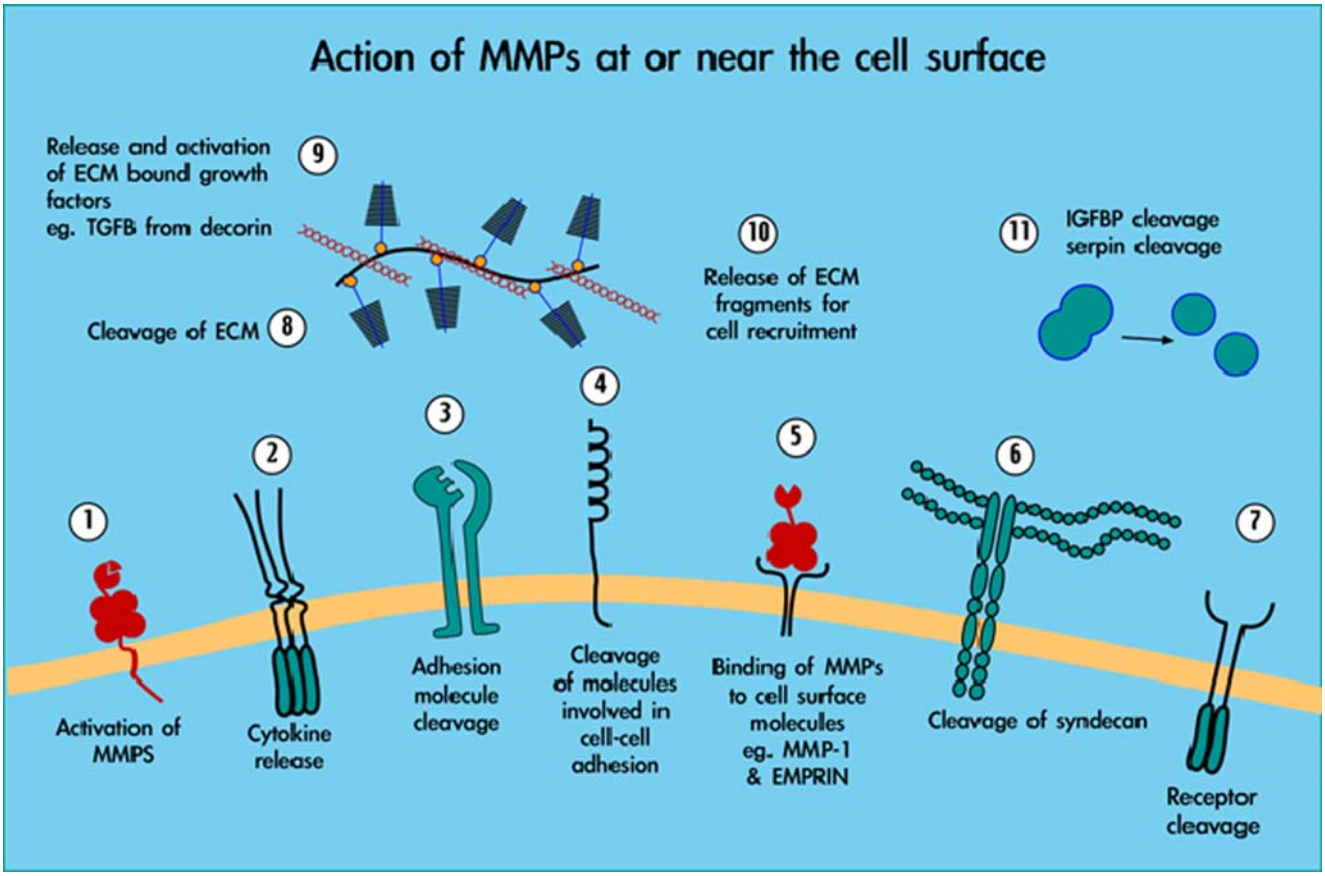

Fig. 4 Action of MMPs at or near the cell surface (*MT-MMP* membrane-type matrix metalloproteinase). MMPs can be activated at the cell surface (*1*) and also cleave and release cytokines (*2*), adhesion molecules (*3*) and proteins involved in cell-cell adhesion (*4*). Some cell surface proteins can bind MMPs localising them to cleave proteins such as syndecan (*6*) and various receptors (*7*). Active MMPs cleave ECM macromolecules (*8*) leading to damage to the tissue structure. Breakdown of matrix molecules leads to the release of growth factors (*9*) and the release of matrix fragments that act on local cells (*10*). MMPs can also cleave inhibitors or serine proteinases such as serpins (*11*)

ratio (Fig. 3) and so can control connective tissue breakdown. If TIMP levels exceed those of active enzyme, then connective tissue turnover is prevented. TIMP-2 is known to be associated with the activation of proMMP-2. TIMP-3 is bound by the ECM after secretion and inhibits some members of the ADAM family, whereas TIMP-4 is predominantly localised in the heart but can be produced by joint tissues (Greene et al. 1996). MMP-14 is known to be poorly inhibited by TIMP-1. TIMP-1 and -3 are up-regulated by growth factors such as transforming growth factor (TGF) β, insulin-like growth factor-1 and OSM and these agents also induce matrix synthesis (Varga et al. 1987). All active MMPs bind to the protease inhibitor α2-macroglobulin and these complexes are rapidly cleared via endocytosis and degradation within the lysosomal system.

Control of the localisation and clearance of MMPs

Proteolysis often occurs in the immediate vicinity of the cell in peri-cellular pockets close to the cell membrane where MMPs can be secreted to specific areas at the cell surface (Fig. 4; Zucker et al. 2003). This allows a high degree of control and these localisation mechanisms can enhance MMP activity, prevent access of MMP inhibitors, concentrate MMPs to their precise target substrate and limit the extent of proteolysis to a discrete region. Although the MMPs with transmembrane domains are the most important cell surface enzymes, some MMPs bind to cell surface receptors, to cell surface activating enzymes or to pericellular matrix proteins. Cell surface heparan sulphate can bind MMPs such as MMP-7 (Yu and Woessner 2000) and also TIMP-3, whereas MMP-1 can bind to the cell-surface protein EMMPRIN (extracellular matrix metalloproteinase inducer; Guo et al. 2000).

Adam family of proteinases

To date, over 25 ADAM genes and 19 ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) genes have been described. ADAMs are usually membrane-anchored proteinases with diverse functions

conferred by the addition of their different protein domains (Becherer and Blobel 2003; Primakoff and Myles 2000; Fig. 2). The disintegrin domain can bind to integrins and prevent cell-cell interactions; cysteine-rich, epidermal growth factor-like, transmembrane and cytoplasmic tail domains are also found (Fig. 2). ADAM-17 is known for its ability to release TNFα from the cell surface (Black et al. 1997). Not only ADAM-17, but also ADAM-10, -12 and -15 have been described in cartilage (McKie et al. 1997). The ADAMTS family members are distinguished from the ADAMs in that they lack these latter three domains but have additional thrombospondin-1 (TSP-1) domains (predominantly at the C-terminus), which are thought to mediate interactions with the ECM (Porter et al. 2005).

The major aggrecan fragments from resorbing cartilage are cleaved at a specific Glu(373)-Ala(374) bond (Sandy et al. 1992). ADAMTS-1, -4, -5, -8, -9, -15 , -16 and -18 are all able to cleave proteoglycan at this bond, although with dramatically different efficiencies in vitro (Caterson et al. 2000; Collins-Racie et al. 2004; Mort and Billington 2001; Porter et al. 2005; Rodriguez-Manzaneque et al. 2002; Tortorella et al. 2001, 2002; Yamanouchi-Pharmaceutical 2001; Zeng et al. 2006). Recent compelling data from mouse knock-out studies indicate that ADAMTS-5 is the pathophysiological mediator of murine aggrecan catabolism (Glasson et al. 2005; Stanton et al. 2005), although ADAMTS-4/ADAMTS-5 double-knock-out studies indicate that a further aggrecan-degrading activity remains to be identified (Rogerson et al. 2008). Interestingly, bovine and porcine studies have led to the proposal that ADAMTS-4 is responsible for cartilage-aggrecan cleavage (Powell et al. 2007; Tortorella et al. 2001), whereas in humans, the depletion of either ADAMTS-4 or ADAMTS-5 protects cartilage from aggrecan degradation (Song et al. 2007). Purified chondrocyte membranes are also able to cleave aggrecan at the Glu(373)-Ala(374) and this activity is not associated with ADAMTS-4 or ADAMTS-5 expression (Billington et al. 1998; Hui et al. 2005).

Proteoglycan release from cartilage occurs following stimulation with a variety of mediators such as IL-1, TNFα, IL-17, retinoic acid and fibronectin fragments (Arner et al. 1998; Dudler et al. 2000; Stanton et al. 2002). Levels of ADAMTS-4 are upregulated in cartilage in response to IL-1 and TNFα and in synovial fibroblasts in response to TGFβ (Caterson et al. 2000; Yamanishi et al. 2002), whereas ADAMTS-5 appears to be unaffected. In an immortalised chondrocyte line, ADAMTS-1, -4, -5 and -9 are all regulated by a mixture of IL-1 and OSM, although the speed of induction differs between these enzymes (Koshy et al. 2002b; Young et al. 2005). Aggrecanase activity can be blocked by specific synthetic inhibitors (Ellis et al. 1994) and by TIMP-3 (Kashiwagi et al. 2001). A role for neprilysin-induced aggrecanase activity via the generation of regulatory peptides has also been proposed (Chevrier et al. 2001).

Serine proteinases

Early studies that investigated the role of serine proteinases in matrix resorption focused on the role of elastase and cathepsin G, both these enzymes being contained within intracellular granules inside leukocytes. Later in vitro experiments with tissue or cells demonstrated that a variety of serine proteinases were upregulated by proinflammatory stimuli and implicated the plasminogen–plasmin system in the activation of proMMPs (Campbell et al. 1994; Nagase and Woessner 1999). IL-1- and TNFα-induced proteoglycan release can be blocked with an inactivator of urokinase-type plasminogen activator (Bryson et al. 1998). Inclusion of α_1 proteinase inhibitor to resorbing cartilage effectively blocks the release of collagen implicating serine proteinase (s) in the activation cascades of pro-inflammatory cytokine-induced proMMPs (Milner et al. 2001). Both tissue- and urokinase-type plasminogen activators are found in cartilage and cleave plasminogen to plasmin.

Other serine proteinase activities have been implicated in arthritis. Granzyme B can initiate proteoglycan degradation (but not collagen); granzyme B-positive cells can be detected in synovium and at the invasive front in RA (Ronday et al. 2001). The serine proteinase fibroblast activation protein alpha has been shown to be upregulated following cytokine stimulation of chondrocytes and this serine proteinase is elevated in osteoarthritic cartilage (Milner et al. 2006). This study has also identified a number of serine proteinases that are upregulated in chondrocytes by using an active site probe and the role of serine proteinases in tissue turnover in arthritic tissues has been reviewed (Milner et al. 2008). The proteinase profile, including serine proteinases, of normal and osteoarthritic chondrocytes has recently been reported (Swingler et al. 2009).

Acid proteinases

Cysteine proteinases and bone resorption

Bone is also destroyed in RA (Walsh et al. 2005) and both the MMPs and cysteine proteinases are involved (Skoumal et al. 2005). Osteoblasts respond to parathyroid hormone and other agents that induce bone resorption, such as IL-1 and TNF-α, by increasing the secretion of MMPs to remove the osteoid layer on the bone surface. Osteoclast precursors then adhere to the exposed bone surface, differentiate and form a low pH microenvironment beneath their lower surface. This removes mineral, and lysosomal

proteinases then resorb the exposed matrix. There is clear evidence for a central role for receptor activator of nuclear factor κB ligand (RANKL) in the bone destruction seen in RA. This member of the TNF ligand family of cytokines is abundantly produced by T cells and synovial fibroblasts in RA synovial membrane and stimulates the formation of multinucleate osteoclasts. It is upregulated by a variety of cytokines including IL-1, TNF-α, IL-11, OSM, parathyroid hormone-related peptide, macrophage colony-stimulating factor and IL-17. It binds to a specific receptor, RANK, on the surface of osteoclast precursors. Increased levels of RANK and RANKL, and of multinucleate cells, are evident in arthritis models associated with bone erosions. The potent activity of IL-17 in osteoclastogenesis is mediated by the upregulation of RANKL and its action is antagonised by the decoy receptor osteoprotegerin. This molecule is effective in blocking bone resorption (Walsh et al. 2005) and, in rat adjuvant arthritis and the arthritis of TNFtg mice (Schett et al. 2003), it protects against the development of bone and cartilage destruction.

Cysteine proteinases can degrade type I collagen at acidic pH (Burleigh et al. 1974; Etherington 1972) and specific inhibitors prevent the resorption of bone explants (Delaisse et al. 1980, 1984) suggesting an involvement of lysosomal cysteine proteinases (Turk et al. 2001) in matrix resorption. Cathepsin B is elevated in OA tissue and raised levels of cathepsins B, L and H are found in antigen-induced rat arthritis models and within the rheumatoid joint. Incubation of resorbing cartilage with specific cathepsin B inhibitors blocks the release of proteoglycan fragments (Buttle et al. 1995) suggesting an involvement in cartilage proteoglycan breakdown. Everts et al. (1996) have shown that substantial amounts of fibrillar collagen accumulate intracellularly in the presence of cysteine proteinase inhibitors (Everts et al. 1985). Cathepsin K plays a key role in collagen turnover and subsequent bone resorption (Bossard et al. 1996; Inaoka et al. 1995). It cleaves type I collagen at the N-terminal end of the triple helix at pH values as high as pH 6.5 (Kafienah et al. 1998) and is produced by synovial fibroblasts, contributing to synovium-initiated bone destruction in the rheumatoid joint (Hummel et al. 1998). Both cathepsins K and S are expressed in RA and OA synovia (Hou et al. 2002) and evidence has been presented that cathepsin K, whose expression is elevated in OA cartilage (Swingler et al. 2009), is localised to sites of cartilage erosion (Li et al. 2000). Cathepsin K has potent aggrecan-degrading activity and the resulting degradation products potentiate the collagenolytic activity of cathepsin K toward types I and II collagen (Hou et al. 2001). Bone resorption is impaired in situations in which cathepsin K is deficient, evidence that has made cathepsin K a drug target for the treatment of osteoporosis in which bone resorption is excessive.

Calpain (calcium-dependent neutral cysteine proteinase) can cleave proteoglycan (Suzuki et al. 1992) and its presence correlates with arthritis and tissue destruction (Szomor et al. 1995). However, the significance of calpain in arthritic disease is currently unclear (Ishikawa et al. 1999).

Threonine proteinases

Threonine proteinases represent a relatively new class of proteinases (Wlodawer 1995). The proteasome is a ubiquitously expressed, essential, intracellular protease complex belonging to this new proteinase class. It performs many intracellular roles including the degradation of phosphorylated and ubiquitinated inhibitor of kappa B (Tanaka et al. 2001).

Inhibition of proteinases as a therapeutic target

Synthetic MMP inhibitors

Considerable interest has been shown in the designing of inhibitors of proteinases as a therapy for preventing tissue breakdown (Roycik et al. 2009). Early MMP inhibitors were designed to avoid modification within the gut whilst retaining potency. Initial inhibitors were broad-range inhibitors produced by using conventional pharmaceutical screening processes and many caused musculoskeletal side-effects. As the crystal structures of the catalytic domains of many MMPs became available, they explained in part the variation in substrate specificity amongst MMPs and have allowed the design of more specific synthetic inhibitors (Borkakoti 2004).

The challenges for MMP inhibition in the arthritides is to decide whether broad spectrum or targeted inhibition is best and whether proteoglycan or collagen release should be the focus. Other considerations might involve the inclusion or avoidance of sheddase inhibition and the prevention of the inhibition of MMPs that have essential and beneficial effects on tissue integrity.

MMP inhibitors and arthritis: animal and clinical trials

Pfizer, Kureha and Sanyo, Ono, Pharmacia, Wyeth and Proctor & Gamble have all reported preclinical evaluations of MMP inhibitors for the treatment of arthritis (Clark and Parker 2003) and shown the efficacy of MMP inhibitors in animal models of arthritis. Sabatini et al. (2005) have described a wide-spectrum MMP inhibitor that has preferential activity against MMP-13 compared with MMP-1 and that prevents the loss of cartilage *ex vivo* and in a guinea pig model of OA. Ishikawa et al. (2005a, 2005b; Fujisawa

Pharmaceuticals) have established that broad-spectrum metalloproteinase inhibitors suppress joint destruction in adjuvant and collagen-induced arthritis rat models, respectively, and have suggested these inhibitors as novel anti-rheumatic drugs.

Synthetic MMP inhibitors have not been shown to be effective in terms of their ability to prevent joint destruction in patients with arthritis, even though they are effective in animal models. Trocade (Ro 32-3555), a selective collagenase inhibitor, has a low nanomolar inhibition constant (Ki) against MMP-1, -8 and -13 with approximately 10- to 100-fold lower potency against MMP-2, -3 and -9. It blocks IL-1α-induced collagen release from cartilage explants and, in vivo, prevents cartilage degradation in a rat granuloma model, a *P. acnes*-induced rat arthritis model and OA model in the SRT/ORT mouse (Lewis et al. 1997). Over 1000 RA patients were treated with Trocade in a large scale trial that was terminated after 1 year because of a lack of efficacy, although this drug was reported to be well tolerated in patients with RA (Hemmings et al. 2001).

An orally active, broad-spectrum MMP inhibitor with nanomolar Ki against MMP-1, -2, -3, -9, -12 and -13 was chondroprotective in both the rabbit menisectomy model of OA and the guinea pig model of spontaneous OA (MacPherson et al. 1997; O'Byrne et al. 1999). However, phase I clinical trials with this compound were halted because of concerns of toxicity with musculoskeletal side-effects.

Tanomastat, a synthetic MMP inhibitor of MMP-3, -2, -8, -9 and -13 with low activity against MMP-1, is effective in guinea pig and canine models of OA (Chau et al. 1998). Tanomastat was given to 300 OA patients for 3 months and no musculoskeletal side-effects were reported. The drug could be detected in the cartilage of treated patients undergoing joint replacement (Leff et al. 2003). However, this compound was withdrawn from a 1800-patient phase III trial in OA following negative results in a separate trial of the same drug in cancer patients (1999; see also MMP inhibitors: safety and toxicity).

The antibiotic doxycycline is known to inhibit MMPs. Periostat a modified doxycycline is currently the only US FDA approved MMP inhibitor to be licensed for the treatment of periodontal disease at subantimicrobial doses.

Some recent derivatives can be shown to inhibit MMPs but have no antibiotic activity and have been proposed as a treatment to prevent cartilage damage in the arthritides (Ryan et al. 1996). These compounds are effective in animal models (de Bri et al. 1998) but their effectiveness in RA patients is currently unclear (Stone et al. 2003; van der Laan et al. 2001). A trial of 430 OA patients randomly assigned to receive either doxycycline or placebo (Mazzuca et al. 2003) showed that, when the X-rays of the two groups were compared at 30 months, the affected joint had been protected in the treated patients.

A variety of explanations have been offered to explain the lack of success of metalloproteinase inhibitors in clinical trials in patients with joint diseases. There is no doubt that MMPs are present and active in joint diseases but, if compounds are unable to penetrate the cartilage/bone/synovial interface, they will be ineffective. Early inhibitors were originally screened against a limited set of available MMPs and so may not inhibit some MMPs that have subsequently been discovered. Further studies are required to demonstrate the effectiveness of MMP inhibitors in the prevention of joint destruction, although the clinical evaluation of these drugs is difficult and expensive. Radiographs are still the most reliable measure of joint damage but any change in joint damage is impossible to detect over short periods of time. Whereas some progress has been made with the use of magnetic resonance imaging (MRI) to image joints, this technology is still to be proven and routine centres do not have access to validated methods for quantitation. There are currently no reliable biomarkers that predict the onset or progression of joint destruction (Felson and Lohmander 2009).

MMP inhibitors: safety and toxicity

MMPs are involved in many physiological processes (Vu and Werb 2000) and so their inhibition could affect the rate of wound healing, growth and fetal development. Metalloproteinases are involved in the activation and/or release of cytokines and growth factors from the ECM (Sternlicht and Werb 2001). These released factors have a myriad of effects on cellular proliferation, migration and behaviour. Inhibition of these enzymes could lead to fibrosis although dose-ranging studies should avoid such complications. The most advanced safety data available concern the musculoskeletal pain and tendonitis identified as a reversible side-effect in treated patients (Nemunaitis et al. 1998). These effects commence in the small joints of the hand and upper limbs and the symptoms are time- and dose-dependent and reversible. These symptoms were seen with a Roche compound Ro 31-9790 and led to its development as an arthritis treatment being stopped. All new compounds can be effectively screened in rodent models to eliminate those that cause musculoskeletal events. A Bayer compound was withdrawn as it was associated with increased tumour growth and poor survival times in small cell lung cancer but no other cases of such effects have been reported. It is not necessarily logical to assume that an effect seen with one member of this class of compounds will automatically be seen by all and there are significant differences in chemical structure and metabolism of individual inhibitors. Recent reviews have been published (Pavlaki and Zucker 2003).

Future prospectives for the inhibition of proteinase activity and expression

Signalling pathway inhibitors and proteinase expression

The efficacy of anti-cytokine biotherapies in the treatment of RA patients provides supporting evidence that the inhibition of a signal-transduction pathway could be a potential therapeutic target. Cytokine-mediated transcriptional regulation has been shown to be a key mechanism in the control of the expression of many MMPs. The four main pathways involved in the inflammatory response are believed to be those acting through nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 protein kinase and Janus kinase-signal transducer and activator of transcription (Jak-STAT). These pathways are activated by a variety of stimuli and recent studies have shown that the Toll-like receptors are also involved (Zhang et al. 2008). Both synthetic and natural inhibitors, together with biologics, of these pathways have been developed and tested both in vitro and in vivo with variable degrees of success (Morgan and Kalsheker 1997). For example, SP600125, a pharmacological inhibitor of the MAPK JNK (c-Jun N-terminal kinase) pathway decreases joint destruction in an adjuvant arthritis model, in part by diminishing the production of MMP-1 (Han et al. 2001). Inhibition of the MAPK p38 reduces rodent collagen-induced arthritis (Medicherla et al. 2006; Mihara et al. 2008; Nishikawa et al. 2003). However, the p38 inhibitor Pamapimod has proved less efficacious in human RA (Cohen et al. 2009). IL-1α and OSM signal via the NF-κB and Jak-STAT pathways, respectively, a cytokine combination that in vivo causes a RA-like phenotype and rapid joint destruction concomitantly with an upregulation of specific MMPs (Rowan et al. 2003). Gene therapy with inhibitors of both these pathways appears efficacious in arthritis animal models (Shouda et al. 2001; Tak et al. 2001) and represent excellent potential methodologies to prevent the induction of the degradative MMPs.

Acetylation is a key post-translational protein modification that controls signal transduction and gene transcription events (Kouzarides 2000). Substrates for acetylation include NF-κB and STATs, transcription factors that represent the end points of IL-1 and OSM signalling, respectively. Deacetylation is mediated by a family of eleven enzymes, the histone deacetylases (HDACs). Many structurally divergent HDAC inhibitors (HDACi) have been developed as cancer therapies as they cause cancer cells specifically to undergo growth arrest, differentiation or apoptosis in vivo and in vitro (Johnstone 2002). HDAC inhibitors are also showing therapeutic promise in animal models of inflammatory diseases such as arthritis (Halili et al. 2009). Several reports demonstrate that HDACi modulate gene expression in synovial cells in vivo (Chung et al. 2003; Mori et al. 2003; Nishida et al. 2004). Structurally different HDACi block the proliferation of synovial fibroblasts, all probably by a similar mechanism involving the upregulation of cell cycle inhibitors ($p16^{INK4}$ and $p21^{Cip1}$). In vivo, this is mirrored by the inhibition of TNFα expression, leading to an abrogation of cartilage destruction (Chung et al. 2003; Nishida et al. 2004). These and other results suggest that HDACi represent a new class of compounds for the treatment of inflammatory diseases (Blanchard and Chipoy 2005; Choo et al. 2008; Chung et al. 2003).

We have demonstrated that the HDACi trichostatin A and sodium butyrate potently inhibit cartilage degradation in an explant assay. These compounds decrease the level of collagenolytic enzymes in explant-conditioned culture and block the cytokine (IL-1 and OSM) induction of key MMPs (e.g. MMP-1, -3, -8 and -13) and aggrecanases (e.g. ADAMTS4, ADAMTS5 and ADAMTS9) at the mRNA level (Young et al. 2005). Thus, our current data indicate that HDACi function as potent repressors of metalloproteinase expression in chondrocytes and may therefore not only be a new treatment for RA, but also potentially for any of the destructive arthritides mediated by metalloproteinases.

MMP-substrate interactions

As more detailed information about the interaction of MMPs with their substrates becomes available, we might be able to design inhibitors that target areas of the enzyme other than the active site. For example, the C-terminal haemopexin-like domain of collagenases has long been known to be required for collagenolysis, presumably because of interactions with the substrate. The activation of the proenzyme is also a valid target, again requiring a detailed knowledge of the underlying biology (Nagase and Brew 2003; Tallant et al. 2009).

Modification of TIMP function or expression

One further possibility for inhibiting metalloproteinase activity is to induce the expression of their natural inhibitors, viz. the TIMPs, or exogenously to deliver modified TIMPs that are specifically targeted to inhibit specific enzymes (Lee et al. 2004; Lee et al. 2005; Nagase and Brew 2003). Both TIMP-1 and TIMP-2 are capable of preventing cartilage destruction *ex vivo*, whereas the N-terminal domain of TIMP-3 in a similar system can prevent aggrecan release. Adenoviral delivery of TIMP-1 and -3 prevents cartilage degradation and invasion by rheumatoid synovial fibroblasts in vitro (van der Laan et al. 2003). However, their efficacy in arthritis-animal model studies require further confirmation.

Finally, like many metalloproteinases, TIMP-1, -3 and -4 are regulated at the transcriptional level and can be induced by a number of growth factors and cytokines. Modulation of these cytokine pathways may re-address the local balance of metalloproteinase and TIMP activities believed to be pivotal in determining the extent of ECM turnover in disease.

Concluding remarks

Inhibition of cartilage collagen destruction still remains an important and viable target to prevent joint damage in arthritic disease. Although the trials of proteinase inhibitors in patients have been disappointing, new agents are still under development and these may overcome some of the problems of both delivery and side-effects. A key to future success is to identify the specific proteinases that are responsible for the destruction of both bone and cartilage within arthritic joints in various diseases. This will allow highly specific inhibitors that target individual enzymes and potentially reduce side-effects.

The blocking of MMPs will probably be more effective when combined with treatments that target earlier steps in inflammation. Furthermore, as noted above, MMPs are not alone in being implicated in joint disease. Serine proteinases are involved in MMP activation and cysteine proteinases have been shown to degrade collagen particularly in the resorption of bone. Combination of proteinase inhibitors, either in sequence or with other agents that hit other specific steps in the pathogenesis, might be necessary before the chronic cycle of joint destruction found in these diseases can be broken.

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References

Ahrens D, Koch AE, Pope RM, Stein-Picarella M, Niedbala MJ (1996) Expression of matrix metalloproteinase 9 (96-kd gelatinase B) in human rheumatoid arthritis. Arthritis Rheum 39:1576–1587

Aimes RT, Quigley JP (1995) Matrix metalloproteinase-2 is an interstitial collagenase. Inhibitor-free enzyme catalyzes the cleavage of collagen fibrils and soluble native type I collagen generating the specific 3/4 and 1/4-length fragments. J Biol Chem 270:5872–5876

Arner EC, Hughes CE, Decicco CP, Caterson B, Tortorella MD (1998) Cytokine-induced cartilage proteoglycan degradation is mediated by aggrecanase. Osteoarthritis Cartilage 6:214–228

Barrett AJ, Rawlings ND, Woessner JF Jr (1998) Handbook of proteolytic enzymes. Academic Press, New York

Becherer JD, Blobel CP (2003) Biochemical properties and functions of membrane-anchored metalloprotease-disintegrin proteins (ADAMs). Curr Top Dev Biol 54:101–123

Berg WB van den (1999) The role of cytokines and growth factors in cartilage destruction in osteoarthritis and rheumatoid arthritis. Z Rheumatol 58:136–141

Berg WB van den (2000) Pathophysiology of osteoarthritis. Joint Bone Spine 67:555–556

Billington CJ, Clark IM, Cawston TE (1998) An aggrecan-degrading activity associated with chondrocyte membranes. Biochem J 336:207–212

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. Nature 385:729–733

Blanchard F, Chipoy C (2005) Histone deacetylase inhibitors: new drugs for the treatment of inflammatory diseases? Drug Discov Today 10:197–204

Boldt HB, Overgaard MT, Laursen LS, Weyer K, Sottrup-Jensen L, Oxvig C (2001) Mutational analysis of the proteolytic domain of pregnancy-associated plasma protein-A (PAPP-A): classification as a metzincin. Biochem J 358:359–367

Borkakoti N (2004) Matrix metalloprotease inhibitors: design from structure. Biochem Soc Trans 32:17–20

Bossard MJ, Tomaszek TA, Thompson SK, Amegadzie BY, Hanning CR, Jones C, Kurdyla JT, McNulty DE, Drake FH, Gowen M, Levy MA (1996) Proteolytic activity of human osteoclast cathepsin K. Expression, purification, activation, and substrate identification. J Biol Chem 271:12517–12524

Brew K, Dinakarpandian D, Nagase H (2000) Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 1477:267–283

Bri E de, Lei W, Svensson O, Chowdhury M, Moak SA, Greenwald RA (1998) Effect of an inhibitor of matrix metalloproteinases on spontaneous osteoarthritis in guinea pigs. Adv Dent Res 12:82–85

Bryson H, Bunning RAD, Feltell R, Kam CM, Kerrigan J, Powers JC, Buttle DJ (1998) A serine proteinase inactivator inhibits chondrocyte-mediated cartilage proteoglycan breakdown occurring in response to proinflammatory cytokines. Arch Biochem Biophys 355:15–25

Burleigh MC, Barrett AJ, Lazarus GS (1974) Cathepsin B1. A lysosomal enzyme that degrades native collagen. Biochem J 137:387–398

Butler GS, Will H, Atkinson SJ, Murphy G (1997) Membrane-type-2 matrix metalloproteinase can initiate the processing of progelatinase A and is regulated by the tissue inhibitors of metalloproteinases. Eur J Biochem 244:653–657

Buttle DJ, Bramwell H, Hollander AP (1995) Proteolytic mechanisms of cartilage breakdown: a target for arthritis therapy? Clin Pathol Mol Pathol 48:M167–M177

Campbell IK, Wojta J, Novak U, Hamilton JA (1994) Cytokine modulation of plasminogen activator inhibitor-1 (PAI-1) production by human articular cartilage and chondrocytes. Down-regulation by tumor necrosis factor α and up-regulation by transforming growth factor-β and basic fibroblast growth factor. Biochim Biophys Acta 1226:277–285

Caterson B, Flannery CR, Hughes CE, Little CB (2000) Mechanisms involved in cartilage proteoglycan catabolism. Matrix Biol 19:333–344

Cawston TE (1996) Metalloproteinases inhibitors and the prevention of connective tissue breakdown. Pharmacol Ther 70:163–182

Cawston TE, Curry VA, Summers CA, Clark IM, Riley GP, Life PF, Spaull JR, Goldring MB, Koshy PJ, Rowan AD, Shingleton WD (1998) The role of oncostatin M in animal and human connective tissue collagen turnover and its localization within the rheumatoid joint. Arthritis Rheum 41:1760–1771

Chau T, Jolly G, Plym MJ, McHugh M, Bortolon E, Wakefield J, Gianpaolo-Ostravage C, Maniglia C (1998) Inhibition of articular cartilage degradation in dog and guinea-pig models of osteoarthritis by the stromelysin inhibitor, BAY-12–9566. Arthritis Rheum 41:S300

Chevrier A, Mort JS, Crine P, Hoemann CD, Buschmann MD (2001) Soluble recombinant neprilysin induces aggrecanase-mediated cleavage of aggrecan in cartilage explant cultures. Arch Biochem Biophys 396:178–186

Choo QY, Ho PC, Lin HS (2008) Histone deacetylase inhibitors: new hope for rheumatoid arthritis? Curr Pharm Des 14:803–820

Chung YL, Lee MY, Wang AJ, Yao LF (2003) A therapeutic strategy uses histone deacetylase inhibitors to modulate the expression of genes involved in the pathogenesis of rheumatoid arthritis. Mol Ther 8:707–717

Clark IM, Parker AE (2003) Metalloproteinases: their role in arthritis and potential as therapeutic targets. Expert Opin Ther Targets 7:19–34

Clark IM, Swingler TE, Sampieri CL, Edwards DR (2008) The regulation of matrix metalloproteinases and their inhibitors. Int J Biochem Cell Biol 40:1362–1378

Cohen SB, Cheng TT, Chindalore V, Damjanov N, Burgos-Vargas R, Delora P, Zimany K, Travers H, Caulfield JP (2009) Evaluation of the efficacy and safety of pamapimod, a p38 MAP kinase inhibitor, in a double-blind, methotrexate-controlled study of patients with active rheumatoid arthritis. Arthritis Rheum 60:335–344

Collins-Racie LA, Flannery CR, Zeng W, Corcoran C, Annis-Freeman B, Agostino MJ, Arai M, DiBlasio-Smith E, Dorner AJ, Georgiadis KE, Jin M, Tan XY, Morris EA, LaVallie ER (2004) ADAMTS-8 exhibits aggrecanase activity and is expressed in human articular cartilage. Matrix Biol 23:219–230

Delaisse JM, Eeckhout Y, Vaes G (1980) Inhibition of bone resorption in culture by inhibitors of thiol proteinases. Biochem J 192:365–368

Delaisse JM, Eeckhout Y, Vaes G (1984) In vivo and in vitro evidence for the involvement of cysteine proteinases in bone resorption. Biochem Biophys Res Commun 125:441–447

Dudler J, Renggli-Zulliger N, Busso N, Lotz M, So A (2000) Effect of interleukin 17 on proteoglycan degradation in murine knee joints. Ann Rheum Dis 59:529–532

Eeckhout Y, Vaes G (1977) Further studies on the activation of procollagenase, the latent precursor of bone collagenase. Effects of lysomal cathepsin B, plasmin and kallikrein and spontaneous activation. Biochem J 166:21–31

Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2:161–174

Ellis AJ, Curry VA, Powell EK, Cawston TE (1994) The prevention of collagen breakdown in bovine nasal cartilage by TIMP-1, TIMP-2 and a low molecular weight synthetic inhibitor. Biochem Biophys Res Commun 201:94–101

Etherington DJ (1972) The nature of the collagenolytic cathepsin of rat liver and its distribution in other rat tissues. Biochem J 127:685–692

Everts V, Beertsen W, Tigchelaar-Gutter W (1985) The digestion of phagocytosed collagen is inhibited by the proteinase inhibitors leupeptin and E-64. Collagen Relat Res 5:315–336

Everts V, Delaisse JM, Korper W, Niehof A, Vaes G, Beertsen W (1992) Degradation of collagen in the bone-resorbing compartment underlying the osteoclast involves both cysteine-proteinases and matrix metalloproteinases. J Cell Physiol 150:221–231

Everts V, Van der Zee E, Creemers L, Beertsen W (1996) Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling. Histochem J 28:229–245

Felson DT, Lohmander LS (2009) Whither osteoarthritis biomarkers? Osteoarthritis Cartilage 17:419–422

Firestein GS (2003) Evolving concepts of rheumatoid arthritis. Nature 423:356–361

Fosang AJ, Neame PJ, Last K, Hardingham TE, Murphy G, Hamilton JA (1992) The interglobular domain of cartilage aggrecan is cleaved by PUMP, gelatinases, and cathepsin B. J Biol Chem 267:19470–19474

Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, Flannery CR, Peluso D, Kanki K, Yang Z, Majumdar MK, Morris EA (2005) Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 434:644–648

Goldring MB (2000) The role of the chondrocyte in osteoarthritis. Arthritis Rheum 43:1916–1926

Goldring MB, Otero M, Tsuchimochi K, Ijiri K, Li Y (2008) Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. Ann Rheum Dis 67 (Suppl 3):iii75–iii82

Greene J, Wang M, Liu YE, Raymond LA, Rosen C, Shi YE (1996) Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. J Biol Chem 271:30375–30380

Gruber BL, Sorbi D, French DL, Marchese MJ, Nuovo GJ, Kew RR, Arbeit LA (1996) Markedly elevated serum MMP-9 (gelatinase B) levels in rheumatoid arthritis: a potential useful laboratory marker. Clin Immunol Immunopathol 78:161–171

Guo H, Li R, Zucker S, Toole BP (2000) EMMPRIN (CD147), an inducer of matrix metalloproteinase synthesis, also binds interstitial collagenase to the tumor cell surface. Cancer Res 60:888–891

Halili MA, Andrews MR, Sweet MJ, Fairlie DP (2009) Histone deacetylase inhibitors in inflammatory disease. Curr Top Med Chem 9:309–319

Han Z, Boyle DL, Chang L, Bennett B, Karin M, Yang L, Manning AM, Firestein GS (2001) c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. J Clin Invest 108:73–81

He C, Wilhelm SM, Pentland AP, Marmer BL, Grant GA, Eisen AZ, Goldberg GI (1989) Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. Proc Natl Acad Sci USA 86:2632–2636

Hembry RM, Bagga MR, Reynolds JJ, Hamblen DL (1995) Immunolocalisation studies on six matrix metalloproteinases and their inhibitors, TIMP-1 and TIMP-2, in synovia from patients with osteo- and rheumatoid arthritis. Ann Rheum Dis 54:25–32

Hemmings FJ, Farhan M, Rowland J, Banken L, Jain R (2001) Tolerability and pharmacokinetics of the collagenase-selective inhibitor Trocade in patients with rheumatoid arthritis. Rheumatology (Oxford) 40:537–543

Hou WS, Li Z, Gordon RE, Chan K, Klein MJ, Levy R, Keysser M, Keyszer G, Bromme D (2001) Cathepsin k is a critical protease in synovial fibroblast-mediated collagen degradation. Am J Pathol 159:2167–2177

Hou WS, Li W, Keyszer G, Weber E, Levy R, Klein MJ, Gravallese EM, Goldring SR, Bromme D (2002) Comparison of cathepsins K and S expression within the rheumatoid and osteoarthritic synovium. Arthritis Rheum 46:663–674

Hui W, Barksby HE, Young DA, Cawston TE, McKie N, Rowan AD (2005) Oncostatin M in combination with tumour necrosis factor alpha induces a chondrocyte membrane-associated aggrecanase that is distinct from ADAMTS aggrecanase-1 or -2. Ann Rheum Dis 64:1624–1632

Hummel KM, Petrow PK, Franz JK, Muller-Ladner U, Aicher WK, Gay RE, Bromme D, Gay S (1998) Cysteine proteinase cathepsin K mRNA is expressed in synovium of patients with rheumatoid arthritis and is detected at sites of synovial bone destruction. J Rheumatol 25:1887–1894

Inaoka T, Bilbe G, Ishibashi O, Tezuka K, Kumegawa M, Kokubo T (1995) Molecular cloning of human cDNA for cathepsin K:

novel cysteine proteinase predominantly expressed in bone. Biochem Biophys Res Commun 206:89–96
Iozzo RV (1998) Matrix proteoglycans: from molecular design to cellular function. Annu Rev Biochem 67:609–652
Ishikawa H, Nakagawa Y, Shimizu K, Nishihara H, Matsusue Y, Nakamura T (1999) Inflammatory cytokines induced down-regulation of m-calpain mRNA expression in fibroblastic synoviocytes from patients with osteoarthritis and rheumatoid arthritis. Biochem Biophys Res Commun 266:341–346
Ishikawa T, Nishigaki F, Miyata S, Hirayama Y, Minoura K, Imanishi J, Neya M, Mizutani T, Imamura Y, Naritomi Y, Murai H, Ohkubo Y, Kagayama A, Mutoh S (2005a) Prevention of progressive joint destruction in collagen-induced arthritis in rats by a novel matrix metalloproteinase inhibitor, FR255031. Br J Pharmacol 144:133–143
Ishikawa T, Nishigaki F, Miyata S, Hirayama Y, Minoura K, Imanishi J, Neya M, Mizutani T, Imamura Y, Ohkubo Y, Mutoh S (2005b) Prevention of progressive joint destruction in adjuvant induced arthritis in rats by a novel matrix metalloproteinase inhibitor, FR217840. Eur J Pharmacol 508:239–247
Johnstone RW (2002) Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. Nat Rev Drug Discov 1:287–299
Kafienah W, Bromme D, Buttle DJ, Croucher LJ, Hollander AP (1998) Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. Biochem J 331:727–732
Kashiwagi M, Tortorella M, Nagase H, Brew K (2001) TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5). J Biol Chem 276:12501–12504
Kevorkian L, Young DA, Darrah C, Donell ST, Shepstone L, Porter S, Brockbank SM, Edwards DR, Parker AE, Clark IM (2004) Expression profiling of metalloproteinases and their inhibitors in cartilage. Arthritis Rheum 50:131–141
Kleiner DE Jr, Stetler-Stevenson WG (1993) Structural biochemistry and activation of matrix metalloproteases. Curr Opin Cell Biol 5:891–897
Knäuper V, Wilhelm SM, Seperack PK, DeClerck YA, Langley KE, Osthues A, Tschesche H (1993) Direct activation of human neutrophil procollagenase by recombinant stromelysin. Biochem J 295:581–586
Knäuper V, López-Otin C, Smith B, Knight G, Murphy G (1996a) Biochemical characterization of human collagenase-3. J Biol Chem 271:1544–1550
Knäuper V, Will H, López-Otin C, Smith B, Atkinson SJ, Stanton H, Hembry RM, Murphy G (1996b) Cellular mechanisms for human procollagenase-3 (MMP-13) activation. J Biol Chem 271:17124–17131
Konttinen YT, Ainola M, Valleala H, Ma J, Ida H, Mandelin J, Kinne RW, Santavirta S, Sorsa T, López-Otin C, Takagi M (1999) Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis 58:691–697
Koshy PJ, Henderson N, Logan C, Life PF, Cawston TE, Rowan AD (2002a) Interleukin 17 induces cartilage collagen breakdown: novel synergistic effects in combination with proinflammatory cytokines. Ann Rheum Dis 61:704–713
Koshy PJ, Lundy CJ, Rowan AD, Porter S, Edwards DR, Hogan A, Clark IM, Cawston TE (2002b) The modulation of matrix metalloproteinase and ADAM gene expression in human chondrocytes by interleukin-1 and oncostatin M: a time-course study using real-time quantitative reverse transcription-polymerase chain reaction. Arthritis Rheum 46:961–967
Kouzarides T (2000) Acetylation: a regulatory modification to rival phosphorylation? EMBO J 19:1176–1179
Laan W van der, Molenaar E, Ronday K, Verheijen J, Breedveld F, Greenwald R, Dijkmans B, TeKoppele J (2001) Lack of effect of doxycycline on disease activity and joint damage in patients with rheumatoid arthritis. A double blind, placebo controlled trial. J Rheumatol 28:1967–1974
Laan WH van der, Quax PH, Seemayer CA, Huisman LG, Pieterman EJ, Grimbergen JM, Verheijen JH, Breedveld FC, Gay RE, Gay S, Huizinga TW, Pap T (2003) Cartilage degradation and invasion by rheumatoid synovial fibroblasts is inhibited by gene transfer of TIMP-1 and TIMP-3. Gene Ther 10:234–242
Lee MH, Rapti M, Knauper V, Murphy G (2004) Threonine 98, the pivotal residue of tissue inhibitor of metalloproteinases (TIMP)-1 in metalloproteinase recognition. J Biol Chem 279:17562–17569
Lee MH, Rapti M, Murphy G (2005) Total conversion of tissue inhibitor of metalloproteinase (TIMP) for specific metalloproteinase targeting: fine-tuning TIMP-4 for optimal inhibition of TNF-a converting enzyme (TACE). J Biol Chem 280:15967-15975
Leff RL, Elias I, Ionescu M, Reiner A, Poole AR (2003) Molecular changes in human osteoarthritic cartilage after 3 weeks of oral administration of BAY 12–9566, a matrix metalloproteinase inhibitor. J Rheumatol 30:544–549
Lewis EJ, Bishop J, Bottomley KM, Bradshaw D, Brewster M, Broadhurst MJ, Brown PA, Budd JM, Elliott L, Greenham AK, Johnson WH, Nixon JS, Rose F, Sutton B, Wilson K (1997) Ro 32–3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. Br J Pharmacol 121:540–546
Li Z, Hou WS, Bromme D (2000) Collagenolytic activity of cathepsin K is specifically modulated by cartilage-resident chondroitin sulfates. Biochemistry 39:529–536
MacPherson LJ, Bayburt EK, Capparelli MP, Carroll BJ, Goldstein R, Justice MR, Zhu L, Hu S, Melton RA, Fryer L, Goldberg RL, Doughty JR, Spirito S, Blancuzzi V, Wilson D, O'Byrne EM, Ganu V, Parker DT (1997) Discovery of CGS 27023A, a non-peptidic, potent, and orally active stromelysin inhibitor that blocks cartilage degradation in rabbits. J Med Chem 40:2525–2532
Mazzuca SA, Brandt KD, Lane KA, Katz BP (2003) Subject retention and adherence to dosing regimen in a 30-month clinical trial of doxycycline (doxy) as a disease-modifying osteoarthritis drug (DMOARD). Arthritis Rheum 48:294
McKie N, Edwards T, Dallas DJ, Houghton A, Stringer B, Graham R, Russell G, Croucher PI (1997) Expression of members of a novel membrane linked metalloproteinase family (ADAM) in human articular chondrocytes. Biochem Biophys Res Comm 230:335–339
Medicherla S, Ma JY, Mangadu R, Jiang Y, Zhao JJ, Almirez R, Kerr I, Stebbins EG, O'Young G, Kapoun AM, Luedtke G, Chakravarty S, Dugar S, Genant HK, Protter AA (2006) A selective p38 alpha mitogen-activated protein kinase inhibitor reverses cartilage and bone destruction in mice with collagen-induced arthritis. J Pharmacol Exp Ther 318:132–141
Mihara K, Almansa C, Smeets RL, Loomans EE, Dulos J, Vink PM, Rooseboom M, Kreutzer H, Cavalcanti F, Boots AM, Nelissen RL (2008) A potent and selective p38 inhibitor protects against bone damage in murine collagen-induced arthritis: a comparison with neutralization of mouse TNFalpha. Br J Pharmacol 154:153–164
Milner JM, Elliott SF, Cawston TE (2001) Activation of procollagenases is a key control point in cartilage collagen degradation: interaction of serine and metalloproteinase pathways. Arthritis Rheum 44:2084–2096
Milner JM, Rowan AD, Elliott SF, Cawston TE (2003) Inhibition of furin-like enzymes blocks interleukin-1alpha/oncostatin M-stimulated cartilage degradation. Arthritis Rheum 48:1057–1066
Milner JM, Kevorkian L, Young DA, Jones D, Wait R, Donell ST, Barksby E, Patterson AM, Middleton J, Cravatt BF, Clark IM, Rowan AD, Cawston TE (2006) Fibroblast activation protein alpha is expressed by chondrocytes following a pro-inflammatory

stimulus and is elevated in osteoarthritis. Arthritis Res Ther 8: R23
Milner JM, Patel A, Rowan AD (2008) Emerging roles of serine proteinases in tissue turnover in arthritis. Arthritis Rheum 58:3644–3656
Morgan K, Kalsheker NA (1997) Regulation of the serine proteinase inhibitor (SERPIN) gene α_1-antitrypsin: a paradigm for other SERPINs. Int J Biochem Cell Biol 29:1501–1511
Morgan TG, Rowan AD, Dickinson SC, Jones D, Hollander AP, Deehan D, Cawston TE (2006) Human nasal cartilage responds to oncostatin M in combination with interleukin 1 or tumour necrosis factor alpha by the release of collagen fragments via collagenases. Ann Rheum Dis 65:184–190
Mori H, Abe F, Furukawa S, Sakai F, Hino M, Fujii T (2003) FR235222, a fungal metabolite, is a novel immunosuppressant that inhibits mammalian histone deacetylase (HDAC) II. Biological activities in animal models. J Antibiot (Tokyo) 56:80–86
Mort JS, Billington CJ (2001) Articular cartilage and changes in arthritis: matrix degradation. Arthritis Res 3:337–341
Murphy G, Cockett MI, Stephens PE, Smith B, Docherty AJP (1987) Stromelysin is an activator of procollagenase. J Biochem 248:265–268
Murphy G, Crabbe T (1995) Gelatinases A and B. Methods Enzymol 248:470–484
Nagase H (1995) Stromelysins 1 and 2. Methods Enzymol 248:449–470
Nagase H, Woessner JF Jr (1999) Matrix metalloproteinases. J Biol Chem 274:21491–21494
Nagase H, Brew K (2003) Designing TIMP (tissue inhibitor of metalloproteinases) variants that are selective metalloproteinase inhibitors. Biochem Soc Symp 70:201–212
Nemunaitis J, Poole C, Primrose J, Rosemurgy A, Malfetano J, Brown P, Berrington A, Cornish A, Lynch K, Rasmussen H, Kerr D, Cox D, Millar A (1998) Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: selection of a biologically active and tolerable dose for longer-term studies. Clin Cancer Res 4:1101–1109
Nishida K, Komiyama T, Miyazawa S, Shen ZN, Furumatsu T, Doi H, Yoshida A, Yamana J, Yamamura M, Ninomiya Y, Inoue H, Asahara H (2004) Histone deacetylase inhibitor suppression of autoantibody-mediated arthritis in mice via regulation of p16INK4a and p21(WAF1/Cip1) expression. Arthritis Rheum 50:3365–3376
Nishikawa M, Myoui A, Tomita T, Takahi K, Nampei A, Yoshikawa H (2003) Prevention of the onset and progression of collagen-induced arthritis in rats by the potent p38 mitogen-activated protein kinase inhibitor FR167653. Arthritis Rheum 48:2670–2681
O'Byrne EM, Blancuzzi V, Singh H, MacPherson LJ, Parker DT, Roberts ED (1999) Chondroprotective activity of a matrix metalloproteinase inhibitor, CGS 27023A in animal models of osteoarthritis. Springer, Tokyo
Ogata Y, Enghild JJ, Nagase H (1992) Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. J Biol Chem 267:3581–3584
Ohuchi E, Imai K, Fujii Y, Satio H, Seiki M, Okada Y (1997) Membrane type 1 matrix metalloproteinase digests interstitial collagenase and other extracellular macromolecules. J Biol Chem 272:2446–2451
Okada Y, Shinmei M, Tanaka O, Naka K, Kimura A, Nakanishi I, Bayliss MT, Iwata K, Nagase H (1992) Localization of matrix metalloproteinase 3 (stromelysin) in osteoarthritic cartilage and synovium. Lab Invest 66:680–690
Overgaard MT, Boldt HB, Laursen LS, Sottrup-Jensen L, Conover CA, Oxvig C (2001) Pregnancy-associated plasma protein-A2 (PAPP-A2), a novel insulin-like growth factor-binding protein-5 proteinase. J Biol Chem 276:21849–21853
Pavlaki M, Zucker S (2003) Matrix metalloproteinase inhibitors (MMPIs): the beginning of phase I or the termination of phase III clinical trials. Cancer Metastasis Rev 22:177–203
Pei D (1999) Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. J Biol Chem 274:8925–8932
Porter S, Clark IM, Kevorkian L, Edwards DR (2005) The ADAMTS metalloproteinases. Biochem J 386:15–27
Powell AJ, Little CB, Hughes CE (2007) Low molecular weight isoforms of the aggrecanases are responsible for the cytokine-induced proteolysis of aggrecan in a porcine chondrocyte culture system. Arthritis Rheum 56:3010–3019
Primakoff P, Myles DG (2000) The ADAM gene family: surface proteins with adhesion and protease activity. Trends Genet 16:83–87
Rodriguez-Manzaneque JC, Westling J, Thai SN, Luque A, Knauper V, Murphy G, Sandy JD, Iruela-Arispe ML (2002) ADAMTS1 cleaves aggrecan at multiple sites and is differentially inhibited by metalloproteinase inhibitors. Biochem Biophys Res Commun 293:501–508
Rogerson FM, Stanton H, East CJ, Golub SB, Tutolo L, Farmer PJ, Fosang AJ (2008) Evidence of a novel aggrecan-degrading activity in cartilage: studies of mice deficient in both ADAMTS-4 and ADAMTS-5. Arthritis Rheum 58:1664–1673
Ronday HK, Laan WH van der, Tak PP, Roos JA de, Bank RA, TeKoppele JM, Froelich CJ, Hack CE, Hogendoorn PC, Breedveld FC, Verheijen JH (2001) Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the invasive front of the synovium in rheumatoid arthritis. Rheumatology (Oxford) 40:55–61
Rowan AD, Hui W, Cawston TE, Richards CD (2003) Adenoviral gene transfer of interleukin-1 in combination with oncostatin M induces significant joint damage in a murine model. Am J Pathol 162:1975–1984
Roycik MD, Fang X, Sang QX (2009) A fresh prospect of extracellular matrix hydrolytic enzymes and their substrates. Curr Pharm Des 15:1295–1308
Ryan ME, Greenwald RA, Golub LM (1996) Potential of tetracyclines to modify cartilage breakdown in osteoarthritis. Curr Opin Rheumatol 8:238–247
Sabatini M, Lesur C, Thomas M, Chomel A, Anract P, Nanteuil G de, Pastoureau P (2005) Effect of inhibition of matrix metalloproteinases on cartilage loss in vitro and in a guinea pig model of osteoarthritis. Arthritis Rheum 52:171–180
Sandy JD, Flannery CR, Neame PJ, Lohmander LS (1992) The structure of aggrecan fragments in human synovial fluid. Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond. J Clin Invest 89:1512–1516
Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M (1994) A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 370:61–65
Schett G, Redlich K, Hayer S, Zwerina J, Bolon B, Dunstan C, Gortz B, Schulz A, Bergmeister H, Kollias G, Steiner G, Smolen JS (2003) Osteoprotegerin protects against generalized bone loss in tumor necrosis factor-transgenic mice. Arthritis Rheum 48:2042–2051
Shingleton WD, Ellis AJ, Rowan AD, Cawston TE (2000) Retinoic acid combines with interleukin-1 to promote the degradation of collagen from bovine nasal cartilage: matrix metalloproteinases-1 and - 13 are involved in cartilage collagen breakdown. J Cell Biochem 79:519–531
Shouda T, Yoshida T, Hanada T, Wakioka T, Oishi M, Miyoshi K, Komiya S, Kosai K, Hanakawa Y, Hashimoto K, Nagata K, Yoshimura A (2001) Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis. J Clin Invest 108:1781–1788
Skoumal M, Haberhauer G, Kolarz G, Hawa G, Woloszczuk W, Klingler A (2005) Serum cathepsin K levels of patients with

longstanding rheumatoid arthritis: correlation with radiological destruction. Arthritis Res Ther 7:R65–R70

Song RH, Tortorella MD, Malfait AM, Alston JT, Yang Z, Arner EC, Griggs DW (2007) Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. Arthritis Rheum 56:575–585

Springman EB, Angleton EL, Birkedal-Hansen H, Van Wart HE (1990) Multiple modes of activation of latent human fibroblast collagenase: evidence for the role of a Cys^{73} active-site zinc complex in latency and a "cysteine switch" mechanism for activation. Proc Natl Acad Sci USA 87:364–368

Stanton H, Ung L, Fosang AJ (2002) The 45 kDa collagen-binding fragment of fibronectin induces matrix metalloproteinase-13 synthesis by chondrocytes and aggrecan degradation by aggrecanases. Biochem J 364:181–190

Stanton H, Rogerson FM, East CJ, Golub SB, Lawlor KE, Meeker CT, Little CB, Last K, Farmer PJ, Campbell IK, Fourie AM, Fosang AJ (2005) ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. Nature 434:648–652

Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17:463–516

Stocker W, Grams F, Baumann U, Reinemer P, Gomis-Ruth FX, McKay DB, Bode W (1995) The metzincins–topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases. Protein Sci 4:823–840

Stone M, Fortin PR, Pacheco-Tena C, Inman RD (2003) Should tetracycline treatment be used more extensively for rheumatoid arthritis? Metaanalysis demonstrates clinical benefit with reduction in disease activity. J Rheumatol 30:2112–2122

Suzuki K, Shimizu K, Hamamoto T, Nakagawa Y, Murachi T, Yamamuro T (1992) Characterization of proteoglycan degradation by calpain. Biochem J 285:857–862

Swingler TE, Waters JG, Davidson RK, Pennington CJ, Puente XS, Darrah C, Cooper A, Donell ST, Guile GR, Wang W, Clark IM (2009) Degradome expression profiling in human articular cartilage. Arthritis Res Ther 11:R96

Szomor Z, Shimizu K, Fujimori Y, Yamamoto S, Yamamuro T (1995) Appearance of calpain correlates with arthritis and cartilage destruction in collagen induced arthritic knee joints of mice. Ann Rheum Dis 54:477–483

Tak PP, Gerlag DM, Aupperle KR, Geest DA van de, Overbeek M, Bennett BL, Boyle DL, Manning AM, Firestein GS (2001) Inhibitor of nuclear factor kappaB kinase beta is a key regulator of synovial inflammation. Arthritis Rheum 44:1897–1907

Takaishi H, Kimura T, Dalal S, Okada Y, D'Armiento J (2008) Joint diseases and matrix metalloproteinases: a role for MMP-13. Curr Pharm Biotechnol 9:47–54

Takino T, Sato H, Shinagawa A, Seiki M (1995) Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library—MT-MMPs form a unique membrane-type subclass in the MMP family. J Biol Chem 270:23013–23020

Tallant C, Marrero A, Gomis-Ruth FX (2009) Matrix metalloproteinases: fold and function of their catalytic domains. Biochim Biophys Acta (in press)

Tanaka K, Kawakami T, Tateishi K, Yashiroda H, Chiba T (2001) Control of IkappaBalpha proteolysis by the ubiquitin-proteasome pathway. Biochimie 83:351–356

Tetlow LC, Adlam DJ, Woolley DE (2001) Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage. Arthritis Rheum 44:585–594

Tortorella MD, Malfait AM, Deccico C, Arner E (2001) The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation. Osteoarthritis Cartilage 9:539–552

Tortorella MD, Liu RQ, Burn T, Newton RC, Arner E (2002) Characterization of human aggrecanase 2 (ADAM-TS5): substrate specificity studies and comparison with aggrecanase 1 (ADAM-TS4). Matrix Biol 21:499–511

Turk V, Turk B, Turk D (2001) Lysosomal cysteine proteases: facts and opportunities. EMBO J 20:4629–4633

Van Wart HE, Birkedal-Hansen H (1990) The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci USA 87:5578–5582

Varga J, Rosenbloom J, Jimenez SA (1987) Transforming growth factor beta (TGF beta) causes a persistent increase in steady-state amounts of type I and type III collagen and fibronectin mRNAs in normal human dermal fibroblasts. Biochem J 247:597–604

Velasco G, Cal S, Merlos-Suárez A, Ferrando AA, Alvarez S, Nakano A, Arribas J, López-Otín C (2000) Human MT6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. Cancer Res 60:877–882

Vu TH, Werb Z (2000) Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 14:2123–2133

Walsh NC, Crotti TN, Goldring SR, Gravallese EM (2005) Rheumatic diseases: the effects of inflammation on bone. Immunol Rev 208:228–251

Werb Z, Mainardi CL, Vater CA, Harris ED (1977) Endogenous activation of latent collagenase by rheumatoid synovial cells. Evidence for a role of plasminogen activator. N Engl J Med 296:1017–1023

Wlodawer A (1995) Proteasome: a complex protease with a new fold and a distinct mechanism. Structure 3:417–420

Wolfe GC, MacNaul KL, Buechel FF, McDonnell J, Hoerrner LA, Lark MW, Moore VL, Hutchinson NI (1993) Differential in vivo expression of collagenase messenger RNA in synovium and cartilage: quantitative comparison with stromelysin messenger RNA levels in human rheumatoid arthritis and osteoarthritis patients and in two animal models of acute inflammatory arthritis. Arthritis Rheum 36:1540–1547

Yamanishi Y, Boyle DL, Clark M, Maki RA, Tortorella MD, Arner EC, Firestein GS (2002) Expression and regulation of aggrecanase in arthritis: the role of TGF-beta. J Immunol 168:1405–1412

Yamanouchi-Pharmaceutical (2001) Patent WO0134785

Yan C, Boyd DD (2007) Regulation of matrix metalloproteinase gene expression. J Cell Physiol 211:19–26

Yoshihara Y, Nakamura H, Obata K, Yamada H, Hayakawa T, Fujikawa K, Okada Y (2000) Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis 59:455–461

Young DA, Lakey RL, Pennington CJ, Kevorkian L, Edwards DR, Cawston TE, Clark IM (2005) Histone deacetylase inhibitors modulate metalloproteinase gene expression in chondrocytes and block cartilage resorption. Arthritis Res Ther 7:R503-R512

Yu WH, Woessner JF Jr (2000) Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase 7). J Biol Chem 275:4183–4191

Zeng W, Corcoran C, Collins-Racie LA, Lavallie ER, Morris EA, Flannery CR (2006) Glycosaminoglycan-binding properties and aggrecanase activities of truncated ADAMTSs: comparative analyses with ADAMTS-5, -9, -16 and -18. Biochim Biophys Acta 1760:517–524

Zhang Q, Hui W, Litherland GJ, Barter MJ, Davidson R, Darrah C, Donell ST, Clark IM, Cawston TE, Robinson JH, Rowan AD, Young DA (2008) Differential Toll-like receptor-dependent collagenase expression in chondrocytes. Ann Rheum Dis 67:1633–1641

Zucker S, Pei D, Cao J, Lopez-Otin C (2003) Membrane type-matrix metalloproteinases (MT-MMP). Curr Top Dev Biol 54:1–74