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Review

The role of proteases in pathologies of the synovial joint

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Abstract

Synovial (diarthrodial) joints are employed within the body to provide skeletal mobility and have a characteristic structure adapted to provide a smooth almost frictionless surface for articulation. Pathologies of the synovial joint are an important cause of patient morbidity and can affect each of the constituent tissues. A common feature of these pathologies is degenerative changes in the structure of the tissue which is mediated, at least in part, by proteolytic activity. Most tissues of the synovial joint are composed primarily of extracellular matrix and key pathological roles in the degeneration of this matrix are performed by metalloproteinases such as matrix metallproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). However, other proteases such as cathepsin K are likely to play an important role, especially in bone turnover. In addition to the cleavage of structural proteins, proteolytic activities are employed to regulate the activity of other proteases, growth factors, cytokines and other inflammatory mediators. Proteases combine to form complex regulatory networks, the correct functioning of which is required for tissue homeostasis and the imbalance of which may be a feature of pathology. A precise understanding of the proteases involved in these networks is required for a true understanding of the associated pathology.
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Keywords: Protease; Pathology; Arthritis; Tendinopathy; Joint

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Abbreviations: ADAM, a disintegrin and metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; Ala, alanine; Asp, aspartic; CD, cluster of differentiation; CLIP, class II invariant chain-associated peptide; COMP, cartilage oligomeric matrix protein; CTx, type I collagen C-telopeptide fragments; Cys, cysteine; ECM, extracellular matrix; Glu, glutamate; ICE, interleukin converting enzyme; ICTP, cross-linked carboxyterminal telopeptide of type I collagen; IGD, interglobular domain; IL, interleukin; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; mRNA, messenger ribonucleic acid; MT-MMP, membrane-type matrix metalloproteinase; PAR, protease-activated receptor; Ser, serine/threonine; TGFβ, transforming growth factor β; tPA, tissue plasminogen activator; TNF, tumour necrosis factor; uPA, urokinase plasminogen activator.

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1. Overview

Synovial (diarthrodial) joints are employed within the body to provide skeletal mobility and have a characteristic structure adapted to provide a smooth, almost frictionless surface for articulation. To the articulating surfaces of the bones is attached articular hyaline cartilage—a highly hydrated tissue resistant to compression that is maintained by a resident population of cells called chondrocytes. Surrounding the entire joint, inserting into the bones close to the articulating surface, is a dense connective tissue known as the fibrous capsule, the inner surface of which is lined with a specialised layer of connective tissue cells, synoviocytes, which secrete synovial fluid rich in hyaluronate into the joint space. The combination of synovial fluid and hyaline cartilage provides a smooth friction-free surface for articulation. This arrangement is stabilised through the combined influence of the bony configuration of the joint, the ligamentous and capsular tissue that bind the bones of the joint together, and the muscles and tendons controlling the joint.

Synovial joint pathologies are an important cause of patient morbidity, particularly within developed countries. They span a wide clinical range of conditions including arthropathies (e.g. rheumatoid arthritis, osteoarthritis and spondylarthropies) and soft tissue pathologies (e.g. tendinopathy, capsulitis), each with a characteristic presentation and affecting specific tissues of the joint. However, there is clear overlap in the biochemical changes accompanying different pathologies and each of the component tissues of the synovial joint may be affected. A common feature of these pathologies is degenerative changes in the structure of the affected tissue(s) and peptidases (proteases) are central mediators of these processes. Ligament shares structural features with tendon but clinical referrals are generally concerned with acute injury rather than chronic pathology.

In concert with regulated synthesis, the controlled enzymatic cleavage of proteins by proteases is of critical importance to tissue homeostasis. An indication of this, based on the analysis of completed genomes, is the recognition that approximately 2% of the total genes present within a eukaryotic genome encode peptidases (Rawlings & Barrett, 1999). A search of the Ensembl (http://www.ensembl.org) and MEROPS (http://www.merops.sanger.ac.uk) databases indicates that of the 21,858 protein-encoding genes (26,008 genes in total) identified in the human genome, 612 (2.80% (2.35%)) were known or predicted to encode peptidases. A further 115 (0.53% (0.44%)) encode known or predicted inhibitors of peptidases.

According to the International Union of Biochemistry and Molecular Biology (IUBMB) classification system (1992), proteases (mechanistically better described as peptide bond hydrolases), are grouped by the reaction catalysed. However, the naming and classifying of proteases by this means is problematic since they all essentially catalyse the same chemical reaction. In addition, such a system does not infer any evolutionary relationship between potentially homologous proteins (Barrett, 1994). Therefore an alternative classification based on catalytic mechanism and evolutionary relationship has been proposed and developed (Barrett, 1994), and is available as the MEROPS database (Rawlings, Tolle, & Barrett, 2004). Four distinct types of catalytic mechanism are employed by proteases: serine/threonine, cysteine, aspartic, and metallo (see Fig. 1) (Barrett, 1994). Within the human genome the mechanistic distribution of proteases is approximately 3% aspartic (Asp), 23% cysteine (Cys), 32% serine (Ser) and 36% metallo (Southan, 2001). Amongst these the three largest families are the chymotrypsin family of serine proteases (S1), the ubiquitin-specific cysteine proteases (C19) and the adamalysin metalloproteases (M12) (Southan, 2001).

2. Role of proteases in joint pathologies

This review will concentrate on the roles of proteases in the arthritidies (a group of pathologies with an overlapping spectrum of biochemical changes), specifically rheumatoid and osteoarthritis, which have been the focus of a large body of research. The roles of proteases in the pathological changes observed in each of the major component tissues will be investigated in turn. The roles of proteases in tendinopathies, which are often distinct from arthritides, will also be examined briefly.

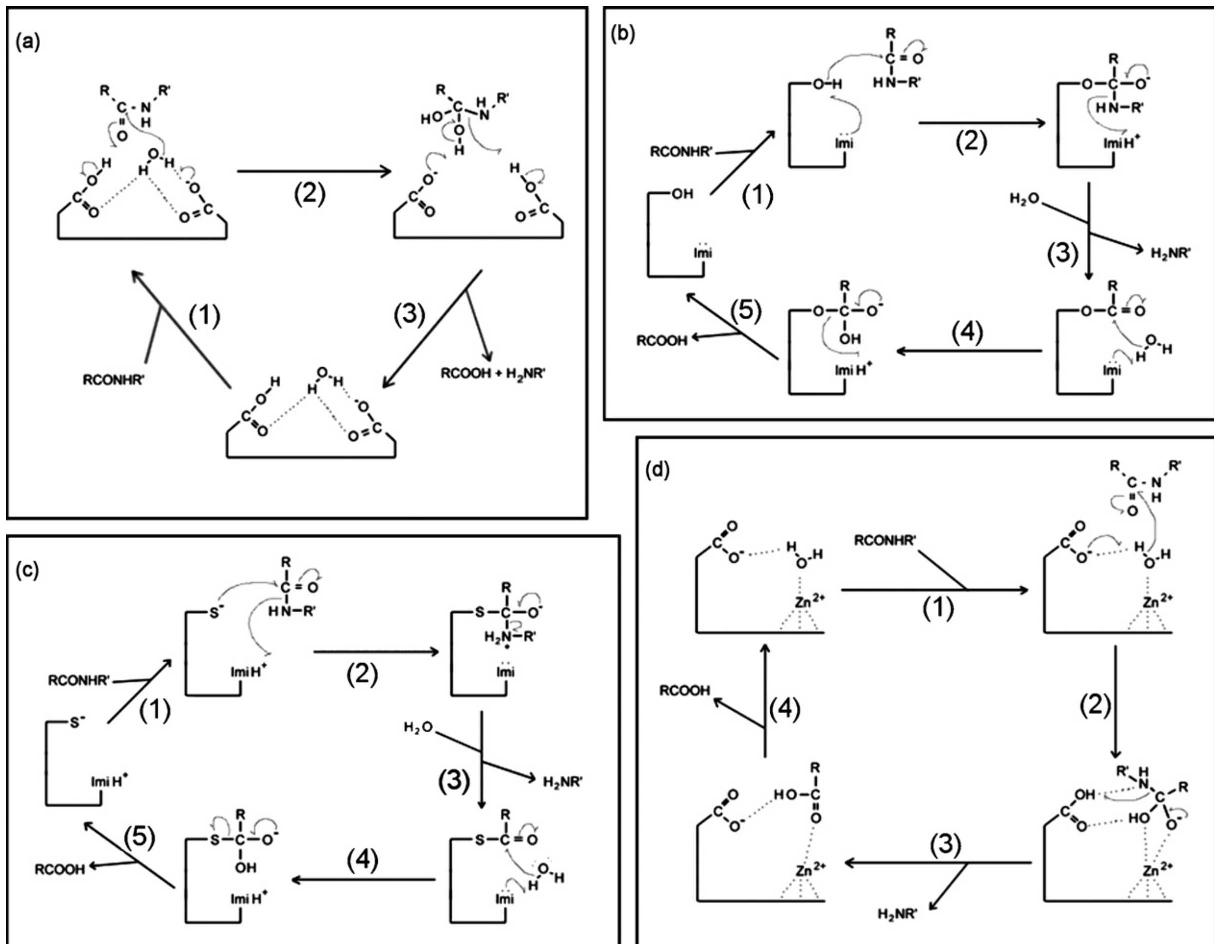

Fig. 1. Schematic outline of the reaction pathways of the four classes of peptidases. (a) Aspartic reaction mechanism: (1) Substrate binding, (2) nucleophilic attack by a water molecule on the carbonyl group of the scissile bond resulting in formation of tetrahedral transition state, (3) re-arrangement of transition state completes hydrolysis and N and C-terminal peptides released. (b) Serine reaction mechanism: (1) Substrate binding, (2) nucleophilic attack by hydroxyl group of serine on the carbonyl group of the scissile bond resulting in the formation of tetrahedral transition state, (3) re-arrangement resulting in the formation of an acyl-enzyme intermediate and the release of the C-terminal peptide. A water molecule then binds into the active site, (4) nucleophilic attack by water on the acyl intermediate resulting in the formation of tetrahedral transition state, (5) re-arrangement resulting in the release of the N-terminal fragment and reformation of the active site. (c) Cysteine reaction mechanism: (1) Substrate binding, (2) nucleophilic attack by thiol group of cysteine on the carbonyl group of the scissile bond resulting in the formation of tetrahedral transition state, (3) re-arrangement resulting in the formation of acyl-enzyme intermediate and the release of the C-terminal peptide. A water molecule then binds into the active site, (4) nucleophilic attack by water on the acyl intermediate resulting in the formation of tetrahedral transition state, (5) re-arrangement resulting in the release of the N-terminal fragment and reformation of the active site. (d) Metallo reaction mechanism: (1) substrate binding, (2) nucleophilic attack by zinc-bound water on the carbonyl group of the scissile bond resulting in the formation of a tetrahedral transition state, (3) proton donation by glutamate to the C-terminal leaving group and re-arrangement resulting in release of C-terminal peptide, (4) release of N-terminal peptide. Schematics were adapted from the Prolysis website (http://delphi.phys.univ-tours.fr/Prolysis/introprotease.html). Dotted lines indicate hydrogen bonding. Arrows represent net electron movements per step.

2.1. Arthritides

2.1.1. Activation of cellular and molecular inflammation

Both rheumatoid and osteoarthritis display inflammatory properties and are associated with the production of cytokines such as tumour necrosis factor (TNF) and interleukin-1 (IL-1) (Brennan, Maini, & Feldmann, 1992; Deleuran et al., 1992; Fernandes, Martel-Pelletier, & Pelletier, 2002; Hulejová et al., 2007). A number of cytokines are known to exist within tissues in inactive or sequestered forms that can be cleaved by proteases to generate functionally active forms. TNF has been implicated as an important regulator of arthritic development and anti-TNF therapies are effective in treating rheumatoid arthritis in the majority of patients (Moreland et

al., 1999). TNF can be cleaved to its active form by the metalloprotease a disintegrin and metalloproteinase (ADAM)-17, also known as TNFα converting enzyme (TACE), which is reportedly up-regulated in arthritis (Black et al., 1997; Ohta et al., 2001). IL-1 is a potent stimulator of cartilage breakdown and is recognized as a pivotal destructive factor in arthritis (Dayer, 2003; Pettipher, Higgs, & Henderson, 1986). It is synthesized as a pro-form without a secretion signal peptide in two forms encoded by different genes, IL-1α and IL-1β. The β form is secreted in an active mature form and is processed intracellularly by the cysteine proteinase caspase-1 (interleukin-1 converting enzyme, ICE) (Thornberry et al., 1992). In contrast, IL-1α is secreted in its pro-form and can be activated proteolytically by the cysteine proteinase calpain (Kobayashi et al., 1990).

Post-traumatic activation of the complement cascade is associated with the pathophysiology of injured tissue degradation and repair in general and activation of the complement cascade has been implicated in inflammatory arthritis and cartilage injury (John, Stahel, Morgan, & Schulze-Tanzil, 2007; Oleesky, Daniels, Williams, Amos, & Morgan, 1991). Many of the components of the complement cascade have been identified within the synovial fluid and may be derived from synovial cells or chondrocytes, which have been demonstrated to synthesize many of these components (Bradley et al., 1996; Gulati, Guc, Lemercier, Lappin, & Whaley, 1994). Infiltrating leukocytes and the synovial vasculature may also contribute to the synovial fluid pool of complement. Complement activation in cartilage may contribute to pathology by affecting inflammatory responses via C5a (receptors for which have been identified on chondrocytes) and inducing cellular lysis via the formation of the membrane attack complex (Onuma et al., 2002). Addition of anti-C5a antibodies to a collagen-induced arthritis model have been reported to reduce the level of pro-inflammatory cytokines suggesting that the complement system represents a positive amplification loop of inflammatory cytokine production (Banda et al., 2002). In rheumatoid arthritis and active osteoarthritis evidence of complement activation has been observed in the synovial vasculature and sub-lining matrix, whereas in chronic osteoarthritic conditions it was not apparent, suggesting complement activation is a feature of the inflammatory stage of disease (Konttinen et al., 1996).

Central to the activation of the complement cascade are a number of serine proteases: C1r, C1s, C2a in the classical pathway; factor B and factor D in the alternative pathway; MASP-1, -2 and -3 and C2a in the lectin pathway (Walport, 2001). In each case the initiation of the cascade requires the formation of multimeric complexes containing these proteases upon an activating surface; archetypal surfaces of the classical, alternative and lectin pathways are ligand-bound antibodies (IgG or IgM), C3b, and mannose lectins, respectively (Walport, 2001). Extracellular matrix (ECM) components such as fibromodulin and fibronectin are also reportedly able to activate complement pathways through interactions with C1q (Barilla & Carsons, 2000; Sjoberg, Onnerfjord, Mörgelin, Heinegård, & Blom, 2005), suggesting that in the arthritides ECM fragments released during pathology may provide an activating surface. Consistent with this mechanism, fibronectin fragments produced in osteoarthritis are known to accelerate cartilage and synovial inflammation (Yasuda, 2006). In addition to its role in complement activation, studies of C1s have identified proteolytic activity toward ECM components including type I and II collagens (Yamaguchi, Sakiyama, Matsumoto, Moriya, & Sakiyama, 1990). Studies of C1s have shown that it is up-regulated and activated in rheumatoid arthritis within cartilage and may contribute to matrix degradation (Nakagawa, Sakiyama et al., 1999) (Table 1).

2.1.2. Cartilage: chondrocyte apoptosis and extracellular matrix degradation

A key characteristic and functional change observed in both osteo- and rheumatoid arthritis is the erosion of the articular cartilage, which results in a narrowing of the joint space and the impairment of joint articulation. In addition, chondrocyte apoptosis is a feature of both osteo- and rheumatoid arthritis and is probably a contributory factor to the ineffective repair of the tissue (Hashimoto, Ochs, Komiya, & Lotz, 1998a; Kim & Song, 1999; Lotz, Hashimoto, & Kühn, 1999). The degree of apoptosis is reported to correlate with the clinical grade of osteoarthritic disease and may be initiated by loss of cell–matrix interactions as has been demonstrated in several cell systems (Hashimoto, Takahashi, Amiel, Coutts, & Lotz, 1998b; Meredith, Fazeli, & Schwartz, 1993). Alternatively, apoptosis may be signalled by Fas ligand derived from inflammatory cells within the synovium or by nitric oxide (Kim & Song, 1999; Lotz et al., 1999). Central to the apoptotic process is a proteolytic cascade comprising a series of evolutionarily related caspase cysteine proteases (Fan, Han, Cong, & Liang, 2005). These caspases are present within cells in a latent pro-form which is activated by auto-proteolysis following oligomerization or by proteases such as granzyme B (Fan et al., 2005). Apoptotic signals such as Fas ligand or cellular stress leads to the activation of one or more apoptotic initiator caspases (caspase-2, -8, -9 or

Table 1
Summary of proteases identified with signalling roles in joint pathology

Enzyme	Class	Pathological role	Reference
Generation of inflammatory mediators			
ADAM17	Metallo	TNF activation	Black et al. (1997)
Calpain	Cys	IL-1α activation	Kobayashi et al. (1990)
Caspase-1	Cys	IL-1β activation	Thornberry et al. (1992)
Complement cascade proteases (C1r, C1s, C2a, factor B, factor D, MASP-1, MASP-2 and MASP-3)	Ser	Pro-inflammatory signalling via formation of C5a and cell lysis through formation of membrane attack complex	Banda et al. (2002), Barilla and Carsons (2000), Onuma et al. (2002), Sjoberg et al. (2005)
Cleavage of extracellular matrix components			
C1s	Ser	Cleavage of extracellular matrix components	Nakagawa, Sakiyama et al. (1999), Yamaguchi et al. (1990)

-10) which in turn activate apoptotic mediator caspases (-3, -6, -7) (Fan et al., 2005). The mediator caspases then cleave cellular proteins that trigger apoptosis (Fan et al., 2005).

Articular cartilage is a predominantly extracellular tissue maintained by the resident chondrocyte cell population, which occupies 2–5% of the tissue volume (Poole, Alini, & Hollander, 1995). The major components of the tissue matrix are type II collagen, aggrecan and hyaluronan. Type II collagen fibrils confer tensile strength to the tissue and form a network that gives the tissue structure. Aggrecan binds hyaluronan to form large, highly anionic aggregates that attract water and, when restrained within the collagen framework, provide resistance to compression (Hardingham, Fosang, & Dudhia, 1994; Kiani, Chen, Wu, Yee, & Yang, 2002).

Models of articular pathologies have demonstrated that aggrecan breakdown and removal from the tissue is an early and reversible event in cartilage degeneration that is followed by the loss of collagen (van Meurs, van Lent, Holthuysen, Singer et al., 1999; Woolley, 1995). The reversible nature of aggrecan loss from the tissue suggests that the key pathological activity is the degradation of type II collagen. However, recent studies have suggested that aggrecan protects the collagen network from proteolysis and have demonstrated that prevention of aggrecan cleavage diminishes both aggrecan loss and cartilage erosion in arthritic model systems (Little et al., 2007; Pratta et al., 2003). Aggrecan proteolysis may therefore be a critical initiating step in these pathologies.

Aggrecan possesses approximately 100 chondroitin sulphate chains of around 20 kDa in size and up to 60 keratan sulphate chains of 5–15 kDa covalently attached to its protein core predominantly between the second and third of three globular domains (Hardingham et al., 1994; Kiani et al., 2002). The large number of negative groups associated with the sulphates and hydroxyls of the chondroitin sulphate chains attract water molecules that are functionally important in conferring compressive resistance to the tissue (Hardingham et al., 1994; Kiani et al., 2002). The N-terminal globular domain of aggrecan mediates the interaction between aggrecan and hyaluronan, an interaction that is stabilised by link protein (Day, 1999). Although numerous proteolytic cleavages of aggrecan have been identified *in vitro*, the most studied are cleavages occurring within the interglobular domain (IGD) that separates the N-terminal globular domains from the main keratan sulphate and chondroitin sulphate attachment domains. The well-known "aggrecanase" cleavage site is at Glu^{373}-Ala^{374} (Lohmander, Neame, & Sandy, 1993; Sandy, Flannery, Neame, & Lohmander, 1992; Sandy, Neame, Boynton, & Flannery, 1991). This activity generates a major fragment containing the chondroitin sulphate chains that is unable to bind hyaluronan and is subsequently lost from the tissue (Campbell, Roughley, & Mort, 1986).

Members of a phylogenetically related sub-group of the a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) metalloproteases including ADAMTS-1, -4 (aggrecanase-1) and -5 (aggrecanase-2) have been identified as possessing aggrecanase activity and cleave the aggrecan core protein at several glutamyl bonds (Abbaszade et al., 1999; Jones & Riley, 2005; Kuno et al., 2000; Tortorella et al., 1999) (Fig. 2). Their involvement in cartilage aggrecan degradation has been demonstrated using both cleavage site-specific antibodies and N-terminal sequencing of proteolytic fragments (Malfait, Liu, Ijiri, Komiya, & Tortorella, 2002; Sandy & Verscharen, 2001; Vankemmelbeke et al., 2003). In studies of osteoarthritis ADAMTS-4 and -5 have been implicated as the responsible aggrecanases on the basis of mRNA and protein expression and investigations into their relative contributions in models of arthritis have recently been conducted in mice using catalytic

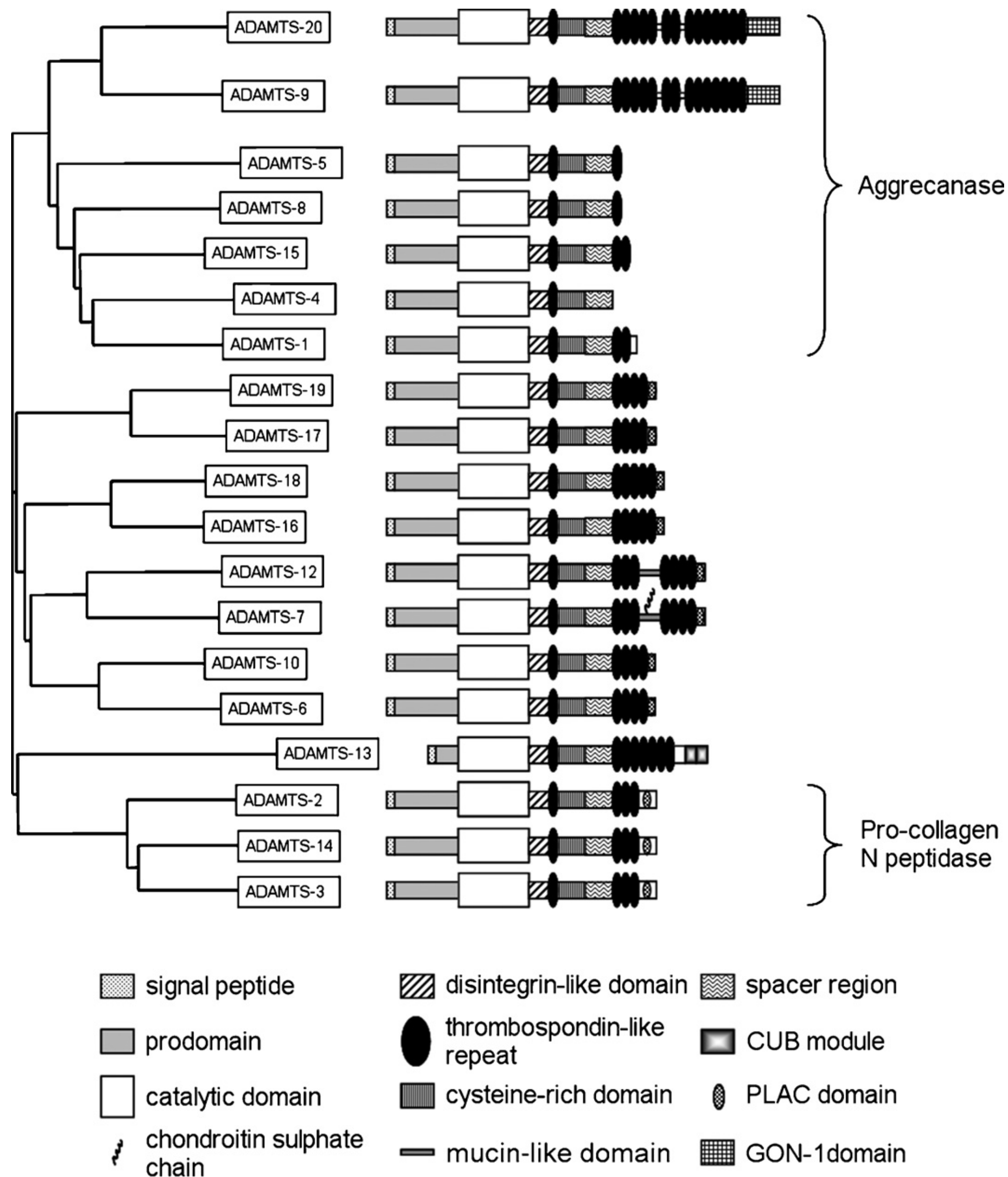

Fig. 2. Schematic representation of the structural and evolutionary relationship of the 19 human ADAMTS gene products. The dendrogram was calculated using ClustalW 1.7 (Thompson, Higgins, & Gibson, 1994). The structural representation of ADAMTS proteins were adapted from Jones and Riley (2005). Where applicable the long form of splice variants is shown. PLAC, protease and lacunin domain; CUB, complement C1r/C1s, Uegf (epidermal growth factor-related sea urchin protein) and BMP-1 (bone morphogenic protein-1).

domain knockouts of each gene (Glasson et al., 2004, 2005; Malfait et al., 2002). Both ADAMTS-4 and -5 knockout mice were phenotypically normal and indistinguishable from wild-type littermates, indicating that each enzyme was dispensable for normal development (Glasson et al., 2004, 2005; Stanton et al., 2005). In a surgically induced OA model there was a major reduction in the severity of induced OA in ADAMTS-5 knockout mice, but no difference in progression or severity in ADAMTS-4 knockout mice (Glasson et al., 2004, 2005). In a model of inflammatory arthritis, ADAMTS-5, but not ADAMTS-4, knockout mice were protected against aggrecan loss (Stanton et al., 2005). Furthermore, aggrecanase activity was inducible in articular cartilage explants from wild-type and ADAMTS-4 knockout mice but not from ADAMTS-5 knockout littermates (Glasson

et al., 2004, 2005; Stanton et al., 2005). Together these studies suggest that, at least in these mouse models, ADAMTS-5 is primarily responsible for the increased aggrecanase activity and subsequent development of arthritis. Although aggrecan is likely to be the major substrate of the aggrecanases in cartilage, it is known that other macromolecules can also be cleaved. These include other hyalectins such as versican (Westling et al., 2004) and cartilage oligomeric matrix protein (COMP; thrombospondin-5) (Dickinson et al., 2003).

The ADAMTS enzymes are multi-domain proteins that are synthesized as zymogens but which undergo constitutive removal of the pro-domain in the secretory pathway by pro-protein convertases such as furin (Wang et al., 2004). Secreted ADAMTS proteinases can then undergo additional processing at their C-terminal end (Cal, Arguelles, Fernández, & López-Otín, 2001; Flannery et al., 2002; Rodriguez-Manzaneque, Milchanowski, Dufour, Leduc, & Iruela-Arispe, 2000). In the case of ADAMTS-4, two such events have been identified to date, one resulting in the removal of the majority of the spacer region, the other in the removal of the spacer region and the majority of the cysteine-rich domain (Flannery et al., 2002). These processing events have been shown to alter the activity of ADAMTS-4 against aggrecan: the processed ADAMTS-4 acquires the ability to cleave aggrecan within the interglobular domain in addition to sites within the CS attachment region that are also cleaved by the full-length proteinase (Gao et al., 2002, 2004) Although C-terminal processing of ADAMTS-4 has been demonstrated to occur through an autocatalytic mechanism (Flannery et al., 2002), cell-based experiments have suggested that such cleavages are mediated by MMP (Gao et al., 2002, 2004). A processing pathway in which the full-length mature enzyme is bound at the cell surface and cleaved by membrane-type 4-MMP (MT4-MMP, also termed MMP-17) to generate both identified truncated forms has been proposed (Gao et al., 2004). The processed form retaining the cysteine-rich domain appears to be maintained at the cell surface by syndecan-1 through interactions with both CS and heparan sulphate chains, whereas the shorter form is released into the medium (Gao et al., 2004). Analagous studies of ADAMTS-5 have not been reported. However, it is likely that the regulation of aggrecanase activity through proteolytic processing of ADAMTS aggrecanases is of importance in the arthritides. Processing of this type has the capacity to transform protease activities from the relatively benign trimming of the C-terminus of aggrecan to a more destructive activity capable of disaggregating aggrecan-hyaluronan complexes through IGD proteolysis, resulting in the loss of aggrecan from the tissue.

Although the aggrecanase-mediated cleavage of aggrecan appears to be the initial and functionally most important activity against aggrecan in pathology a large number of additional proteinases are capable of cleaving this proteoglycan. These include a number of the MMPs (Cawston, 1995), the aspartic protease cathepsin D (Handley et al., 2001) and the cysteine proteases cathepsins B, L, K and S (Hou et al., 2001; Nguyen, Mort, & Roughley, 1990). Within cartilage the apparent protection of collagen proteolysis conferred by aggrecan and the requirement of aggrecanase-mediated aggrecan IGD cleavage for pathological progression may reflect in part an inability of the tissue to activate MMP whilst aggrecan aggregates are intact. Consistent with such a hypothesis, the appearance of MMP-mediated cleavages of the aggrecan IGD correlates with the cleavage of type II collagen (van Meurs, van Lent, Stoop et al., 1999).

Link protein is essential for the maintenance of the hyaluronan-aggrecan framework of cartilage. Without the presence of link protein, the non-covalent association of hyaluronan and aggrecan is weakened considerably. Surprisingly little is known about the metabolism of this important component of the cartilage ECM. Early studies demonstrated molecular heterogeneity of link protein in cartilage (Roughley, Poole, & Mort, 1982), possibly as a result of proteolytic processing. It is known that certain MMPs have the capacity to degrade link protein (Nguyen, Murphy, Hughes, Mort, & Roughley, 1993; Nguyen, Murphy, Roughley, & Mort, 1989) although proteolysis may also involve cellular uptake (Tester, Ilic, Robinson, & Handley, 1999). Proteolysis of link protein may also be involved in regulatory pathways of matrix homeostasis (Liu, McKenna, & Dean, 2000).

In addition to aggrecan loss, advanced arthritic pathologies are associated with the cleavage and loss of type II collagen (Dodge & Poole, 1989). In osteoarthritis cartilage appears to be lost initially from the superficial layer (adjacent to the synovial fluid), whereas in RA damage to the type II collagen is first seen at pericellular sites adjacent to the subchondral bone and also at sites immediately adjacent to the invading pannus (Dodge & Poole, 1989; Poole et al., 1995).

In mature cartilage type II collagen triple helices are assembled into fibrils that are stabilised by covalent cross-links at the non-helical termini of individual proteins (Cawston, 1995). Triple helical collagen degradation is associated with an activity, termed collagenase, which cleaves the triple helical collagen to produce

characteristic one quarter and three quarter fragments (Dioszegi, Cannon, & Van Wart, 1995). Five phylogenetically related members of the MMP have been identified as possessing collagenase activity including the 'collagenases' MMP-1, -8 and -13 (collagenase-1, -2 and -3, respectively) (Dioszegi et al., 1995; Freije et al., 1994), and MMP-2 and MMP-14 (MT1-MMP) (Aimes & Quigley, 1995; Ohuchi et al., 1997). These proteases differ in their specificity for different collagen types; MMP-1 is most active against type III, MMP-8 is most active against type I and MMP-13 most active against type II (Cawston & Wilson, 2006). All of the collagenases are present in cartilage pathology and may play a role in type II collagen degradation (Kevorkian et al., 2004). Triple helical collagen may also be cleaved by the cysteine protease cathepsin K, which is unique in its ability to cleave the triple helical region at multiple sites and is reportedly up-regulated in chondrocytes in a mouse osteoarthritis model and in the synovium of patients with rheumatoid arthritis (Garnero et al., 1998; Hou et al., 2001, 2002; Morko, Söderström, Säämänen, Salminen, & Vuorio, 2004). Cathepsin K has an acidic pH optimum although interaction with chondroitin sulphate has been shown to stabilise the protein at neutral pH and may promote accumulation of cathepsin K within cartilage (Li, Hou, & Brömme, 2000).

Additional proteases may also be involved in type II collagen degradation through the cleavage of the non-helical regions where the cross-links are located. These cleavages destabilise the collagen fibrillar structure, making the individual helices more susceptible to cleavage by collagenases and may result in the loss of tensile properties (Bader, Kempson, Barrett, & Webb, 1981; Poole et al., 1995). *Ex vivo* models of cartilage degradation have demonstrated that type II collagen becomes more extractable (Dodge & Poole, 1989), suggesting that the triple helix cross-links are cleaved, and have shown that this activity is inhibitable by metalloproteinase inhibitors (Mort et al., 1993). This implicates a MMP, possibly MMP-3 (stromelysin-1) which is highly expressed in cartilage (Kevorkian et al., 2004), albeit largely in an inactive proform (Martel-Pelletier et al., 1994; Mudgett et al., 1998). Other proteinases capable of cleaving in these non-helical regions include the neutrophil serine proteases cathepsin G and neutrophil elastase (Starkey, Barrett, & Burleigh, 1977) and the cysteine protease cathepsin B (Burleigh, Barrett, & Lazarus, 1974).

With a number of exceptions, most notably the membrane-type MMP (MT-MMP) proteases, the MMPs are synthesised as latent pro-enzymes with activity suppressed by the interaction of a pro-domain cysteine residue with the active site zinc atom (Cawston & Wilson, 2006; Nagase & Woessner, 1999). Physiological activation occurs through the cleavage and removal of the pro-domain and hence proteases are central to the regulation of downstream MMP activity and tissue degradation (Milner, Elliott, & Cawston, 2001; Nagase & Woessner, 1999). In contrast, the MT-MMP possess a furin recognition sequence at the end of the pro-domain and are constitutively active at the cell surface. A number of the MMP function as pro-MMP activating enzymes, notably MMP-3, which is capable of activating all three pro-collagenases and pro-MMP-9 (Brinckerhoff et al., 1990; Knäuper, López-Otin, Smith, Knight, & Murphy, 1996; Miyazaki et al., 1992; Murphy, Cockett, Stephens, Smith, & Docherty, 1987). Studies of MMP-3 knockout mice suggest that MMP-3 is an important activator of MMP activities in cartilage and that the absence of MMP-3 significantly reduces tissue degradation in arthritic disease models (van Meurs, van Lent, Holthuysen, Lambrou et al., 1999; van Meurs, van Lent, Stoop et al., 1999). Pro-MMP-3 can be activated by the serine protease plasmin (which must itself be activated by proteolysis of its pro-form, plasminogen, by proteases such as the plasminogen activators, which are themselves secreted in a latent pro-form; Cesarman-Maus & Hajjar, 2005) or the cysteine protease cathepsin B (Milner et al., 2001; Murphy, Ward, Gavrilovic, & Atkinson, 1992). Cathepsin B is also able to activate pro-urokinase plasminogen activator and may be an important initiator of the proteolytic cascade (Buttle et al., 1993; Kobayashi et al., 1991). The importance of pro-enzyme activation in cartilage pathology has been demonstrated in a murine antigen-induced arthritis model, where the sub-optimal stimulation of cartilage lead to a reversible loss of aggrecan degradation and the up-regulation and synthesis of pro-MMP3 and pro-collagenases (van Meurs, van Lent, van de Loo et al., 1999). Pro-MMP were retained in the cartilage matrix and subsequent stimulation of this 'loaded tissue' with IL-1α then led to a much more aggressive proteolysis of aggrecan compared to 'non-loaded' tissue (van Meurs, van Lent, van de Loo et al., 1999).

Recent assays of metalloprotease mRNA expression in normal and arthritic cartilage from the femoral head has revealed a number of differences including a decrease in MMP-1 and -3 in pathology compared to normal and increase in MMP-2, -9 and -13 (Kevorkian et al., 2004). In addition a number of novel differences were identified, most notably an increase in MMP-28 and ADAMTS-16 in pathology. The roles of these two proteases in either normal or pathological cartilage are not known, but are under investigation (Table 2).

Table 2
Summary of proteases with pathological roles in cartilage

Enzyme	Class	Pathological role	Reference
Chondrocyte apoptosis			
Caspase cascade (caspase-2, -3, -6, -7, -8, -9, -10)	Cys	Induction of chondrocyte apoptosis	Fan et al. (2005), Hashimoto et al. (1998a), Kim and Song (1999), Lotz et al. (1999)
Degradation of cartilage proteoglycans			
ADAMTS-1, -4, -5 and MMP	Metallo	Aggrecan cleavage	Abbaszade et al. (1999), Jones and Riley (2005), Kuno et al. (2000), Tortorella et al. (1999)
Cathepsin D	Asp	Aggrecan cleavage	Handley et al. (2001)
Cathepsin B, L, K, S	Cys	Aggrecan cleavage	Hou et al. (2001), Nguyen et al. (1990)
ADAMTS-5	Metallo	Pathological cleavage of aggrecan IGD	Glasson et al. (2004, 2005), Stanton et al. (2005)
ADAMTS-1, -4	Metallo	Versican cleavage	Sandy et al. (2001)
Degradation of triple helical collagen			
MMP-1, -2, -8, -13, -14 (MT1-MMP)	Metallo	Cleavage of triple helical type II collagen	Aimes and Quigley (1995), Dioszegi et al. (1995), Freije et al. (1994), Ohuchi et al. (1997)
Cathepsin B, K	Cys	Cleavage of triple helical type II collagen	Burleigh et al. (1974), Garnero et al. (1998), Hou et al. (2001)
MMP	Metallo	Cleavage of triple helical collagen cross-links	Mort et al. (1993)
Cathepsin G, neutrophil elastase	Ser	Cleavage of triple helical collagen cross-links	Starkey et al. (1977)
Proteinase activation			
MMP-17 (MT4-MMP)	Metallo	Processing of ADAMTS-4	Gao et al. (2004)
MMP-3	Metallo	Processing of pro-collagenases	Brinckerhoff et al. (1990), Knäuper et al. (1996), Murphy et al. (1987)
Plasmin	Ser	Processing of pro-MMP-3	Milner et al. (2001)
Cathepsin B	Cys	Processing of pro-MMP-3, activation of uPA and tPA	Buttle et al. (1993), Kobayashi et al. (1991), Murphy et al. (1992)
uPA, tPA	Ser	Processing of plasminogen to plasmin	Cesarman-Maus and Hajjar (2005)

2.1.3. Synovium: inflammation, hyperplasia, pannus formation and protease up-regulation

The synovial tissue is the primary source of nutrients and signalling factors for cartilage and is therefore important in maintaining the health of the joint. The tissue is organised so that sub-lining cells support a layer of specialised lining cells, termed synoviocytes, which face and maintain the synovial fluid. Two distinct cell types, fibroblast-like synoviocytes and macrophage-like cells, comprise this synovial membrane.

Inflammation of the synovium (synovitis) is a feature of both rheumatoid and osteoarthritis and also animal models of these diseases and is associated with an increase in vascularisation and an increase in the number of mast cells within the synovial sub-lining (Benito, Veale, FitzGerald, van Den Berg, & Bresnihan, 2005; Lee & Weinblatt, 2001; Mapp et al., 2008; Myers et al., 1990; Nigrovic & Lee, 2007; Walsh et al., 2007). In rheumatoid arthritis synovitis is associated with hyperplasia of the synoviocytes, an infiltration of mononuclear cells including B cells, T cells and macrophages into the synovial sublining, and the formation of a locally invasive pannus (Lee & Weinblatt, 2001). A number of studies have shown that in arthritic pathologies the

synovial expression of a number of proteases, including MMP collagenases, ADAMTS aggrecanases and MMP-3, is increased as are the levels of these proteases in the synovial fluid (Hulejová et al., 2007; Smeets et al., 2003; Tchetverikov et al., 2004; Vankemmelbeke et al., 2001; Vankemmelbeke, Ilic, Handley, Knight, & Buttle, 1999). *In vitro* studies have implicated synovium-derived factors, in particular proinflammatory cytokines (Fell & Jubb, 1977; Saklatvala, Pilsworth, Sarsfield, Gavrilovic, & Heath, 1984) and proteases (Vankemmelbeke et al., 1999, 2001) as mediators of cartilage destruction. Synovium-derived proteases may contribute directly to the degradation of cartilage and bone in these pathologies as discussed elsewhere in this review.

A recent study of a mouse osteoarthritic model demonstrated that depletion of the macrophage-like synoviocytes prior to disease induction reduced cartilage damage, suggesting a role for synovial macrophage-like cells in cartilage pathology (Blom et al., 2007). In this study macrophage depletion also decreased the level of MMP-mediated aggrecan IGD cleavage products observed within the synovial fluid and inhibited the up-regulation of MMP-2, -3 and -9 mRNA in synovium, inferring that macrophage-like synoviocytes influence MMP-activity within pathological cartilage.

Mast cells are present in the sub-lining of normal synovium and increase markedly in the arthritides where they appear to become activated (Nigrovic & Lee, 2007). The activities of the mast cell serine proteases tryptase and chymase may directly contribute to matrix degradation: both proteases are reported to activate pro-MMP-3 and may therefore be important initiators of the MMP activation cascade leading to collagenase activation (Gruber et al., 1989; Lees, Taylor, & Woolley, 1994). Chymase is also reported to activate pro-MMP-1 directly (Lees et al., 1994; Saarinen, Kalkkinen, Welgus, & Kovanen, 1994). These proteases may also modulate the pathological process through cytokine activation or inactivation: chymase can degrade cytokines including IL-6, IL-13, IL-5 and TNF (Zhao, Oskeritzian, Pozez, & Schwartz, 2005). In contrast, chymase has been reported to activate IL-1β (Mizutani, Schechter, Lazarus, Black, & Kupper, 1991). Mast cells might therefore serve to regulate the inflammatory response. Mast cells have also been identified as a source of MMP-9 in rheumatoid arthritis, which is thought to be important for mast cell migration through the tissue (Di Girolamo et al., 2006).

The pannus is involved in the erosion of both cartilage and bone and contains both fibroblast-like synoviocytes and mononuclear cells (Abeles & Pillinger, 2006); Lee & Weinblatt, 2001). It has been proposed that within the pannus the synoviocyte-derived cells are involved in matrix invasion and degradation, with synovial fibroblast-like cells the primary mediators of marginal cartilage destruction (Abeles & Pillinger, 2006). Erosion of bone by pannus is believed to be mediated primarily by monocyte-derived osteoclasts which are attracted to the joint and stimulated to differentiate by pro-inflammatory cytokines (Abeles & Pillinger, 2006; Mor, Abramson, & Pillinger, 2005; Redlich et al., 2002). Comparison of active and end-stage rheumatoid arthritis has demonstrated an increased expression of MMP, including the collagenases MMP-1 and -13 and MMP-3, by fibroblast-like synoviocytes, the roles of which in cartilage degradation have already been discussed (see above) (Smeets et al., 2003). An up-regulation of cathepsin K in synovial fibroblast-like cells has also been reported in both rheumatoid and osteoarthritis (Hou et al., 2001, 2002; Hummel et al., 1998). In rheumatoid arthritis cathepsin K-positive cells were observed in areas of high proliferation within the synovium and at sites of cartilage and bone degradation (Hou et al., 2001). *Ex vivo* studies demonstrated engulfment and internalization of type II collagen by fibroblast-like synoviocytes at sites of cartilage erosion and its accumulation within lysosomes in the presence of a cathepsin K inhibitor (Hou et al., 2001). These studies suggest that fibroblast-like synoviocytes expressing cathepsin K are important in the clearance of endocytosed collagen at sites of cartilage erosion. The immune cells (B cells, T cells, dendritic cells and macrophages) of the rheumatoid pannus are believed to be involved in antigen presentation, antibody production and cytokine generation. An important role in antigen presentation has been identified for cathepsin S, which is involved in both the processing of endocytosed proteins to peptides suitable for presentation and the cleavage of the invariant chain to CLIP during MHC class II maturation (Riese et al., 1996; Shi et al., 1999; Villadangos et al., 1999). Studies of cathepsin S knockout mice revealed a decrease in their susceptibility to collagen-induced arthritis and impaired invariant chain processing in both dendritic cells and B cells (Nakagawa, Brissette et al., 1999). This suggests that antigen presentation, mediated by cathepsin S in dendritic cells and B cells is an important driving factor in the maintenance of inflammatory arthritis.

Inflammation of the synovium occurs early in arthritic disease and prior to pannus formation in rheumatoid arthritis and is associated with fibrin deposition, an influx of mononuclear cells and an increase in vascularisation (Lee & Weinblatt, 2001). Cellular infiltration into the synovium is likely to involve degradation of the existing stromal matrix and extracellular matrix proteases such as the MMP are likely to be involved in

such processes. Furthermore, the increase in cathepsin K producing fibroblast-like cells at sites of synovial vascularisation and angiogenesis may indicate a role for this protease in the loosening of the matrix to facilitate angiogenic growth (Hou et al., 2001, 2002). It has been proposed that pannus formation in rheumatoid arthritis is initiated by the formation of fibrin clots within the synovial fluid at the synovial surface, which provide a scaffold into which synovial fibroblast-like cells migrate (Sánchez-Pernaute et al., 2003). This model proposes that, as is observed in other inflammatory conditions (Berckmans et al., 2002), inflammation induces an influx of plasma components into the synovial fluid including components of the coagulation cascade which become activated; but that in rheumatoid arthritis there is an imbalance between coagulation and fibrinolysis leading to the formation and persistence of clots (Andersen & Gormsen, 1970). The coagulation cascade comprises a proteolytic activation series of serine protease ending in thrombin which then cleaves fibrinogen to fibrin (Cirino, Napoli, Bucci, & Cicala, 2000), whereas fibrinolysis is mediated by plasmin, which is generated by the activa-

Table 3
Summary of proteases with pathological roles in synovium

Enzyme	Class	Pathological role	Reference
Synovium-derived proteases leading to degradation of cartilage and bone			
ADAMTS	Metallo	Proteolysis of cartilage aggrecan	Vankemmelbeke et al. (1999, 2001)
MMP	Metallo	Proteolysis of cartilage and bone fibrillar collagen	Smeets et al. (2003), Tchetverikov et al. (2004)
Cathepsin K	Cys		Hou et al. (2001)
Modulation of inflammatory cytokines			
Chymase	Ser	Cleavage and inactivation of IL-5, -6, -13 and TNF. Processing and activation of pro-IL-1β	Mizutani et al. (1991), Zhao et al. (2005)
Angiogenesis into synovium			
Cathepsin K	Cys	'Loosening' of matrix by proteolysis	Hou et al. (2001, 2002)
Pannus formation via fibrin clot			
Coagulation cascade (culminating in thrombin)	Ser	Processing of fibrinogen to fibrin	Andersen and Gormsen (1970), Berckmans et al. (2002), Cirino et al. (2000), Sánchez-Pernaute et al. (2003)
Plasmin	Ser	Fibrinolysis via fibrin cleavage	Cesarman-Maus and Hajjar (2005)
uPA, tPA	Ser	Processing of plasminogen to plasmin	Cesarman-Maus and Hajjar (2005)
Thrombin	Ser	Cleavage and inactivation of uPA	Braat et al. (2000), Ronday et al. (1996), Sánchez-Pernaute et al. (2003)
MMP	Metallo	Cleavage of fibrin generating unusual epitopes that contribute to autoimmunity	Bini et al. (1999), Sánchez-Pernaute et al. (2003)
Antigen presentation			
Cathepsin S	Cys	Processing of proteins to peptides for MHC class II presentation	Nakagawa, Brissette et al. (1999), Riese et al. (1996), Shi et al. (1999), Villadangos et al. (1999)
Mast cell migration			
MMP-9	Metallo	Cleavage of matrix components	Di Girolamo et al. (2006)
Activation of proteases			
Tryptase, chymase	Ser	Processing of pro-MMP-3	Gruber et al. (1989), Lees et al. (1994)
Chymase	Ser	Processing of pro-MMP-1	Lees et al. (1994), Saarinen et al. (1994)

tion of plasminogen by proteases such as urokinase-type plasminogen activator (uPA) and tissue plasminogan activator (Cesarman-Maus & Hajjar, 2005). The imbalance in these processes has been suggested to arise either due to the cleavage of uPA by thrombin which generates a proteolytically inactive form or through an increased expression of inhibitors of fibrinolysis (Braat, Jie, Ronday, Beekman, & Rijken, 2000; Ronday et al., 1996; Sánchez-Pernaute et al., 2003). It is also suggested that the persistence of fibrin in synovial fluids results in MMP-mediated cleavage of fibrin, which may generate unusual fibrin epitopes that contribute to autoimmunity (Bini, Wu, Schnuer, & Kudryk, 1999; Sánchez-Pernaute et al., 2003). The role of protease-activated receptors (PARs) such as the thrombin-activated receptors PAR-1 and -3, and the plasmin receptor PAR-2, in synovium also merits further investigation (Ferrell et al., 2003; Shin et al., 1999) (Table 3).

2.1.4. The joint capsule: protease and inflammatory mediator generation

The joint capsule is a collagen-rich structure populated primarily by fibroblasts that may be similar in phenotype to those found in the adjoining synovium (Kleftogiannis, Handley, & Campbell, 1994). A potential role for capsular tissue in the arthritides has remained largely unstudied, despite the possibility that it may provide a source of mediators of joint damage and inflammation. The joint capsule is known to be a potential source of aggrecanase activity (Ilic et al., 2000), and capsule has the capacity to increase breakdown of cartilage proteoglycan *in vitro*, even when the cartilage tissue has been killed. This suggests the action of a capsule-derived aggrecanase on cartilage (Vankemmelbeke et al., 1999) (Table 4).

2.1.5. Bone: altered metabolism and extracellular matrix proteolysis

Late-stage rheumatoid arthritis is associated with the development of bone erosions and osteoporosis, with multinucleated osteoclasts within the osteolytic lesions (Eggelmeijer et al., 1993; Ishikawa, Ohno, & Hirohata, 1984; Magaro et al., 1991). Osteoarthritis is associated with bone sclerosis and collagen type I synthesis and metabolism (Mansell & Bailey, 1998; Mansell, Tarlton, & Bailey, 1997; Seibel, Duncan, & Robins, 1989).

Bone is a predominantly extracellular tissue. The major organic component of bone is type I collagen, fibrils of which form the matrix scaffold (Yasuda, Kaleta, & Brömme, 2005). The major cell types present in bone are osteoblasts, osteocytes and osteoclasts, which are the cell types responsible for the synthetic and degradative tissue processes in response to systemic and local signals.

Osteoclast-mediated bone resorption requires the attachment of the osteoclast to the bone surface to form a sealed resorption lacuna followed by demineralization of the surface by secretion of protons into the lacuna and resorption of collagen (Yasuda et al., 2005). Disease, inhibitor and expression studies identified the cysteine protease cathepsin K as the critical enzyme for osteoclast-mediated matrix solubilisation (Drake et al., 1996; Everts et al., 1992; Hou et al., 1999; Kamiya et al., 1998). Cathepsin K is highly expressed by osteoclasts and, in contrast to other cathepsins, has a potent activity against helical collagen, possessing an apparently unique ability to cleave the triple helix at multiple sites (Drake et al., 1996; Garnero et al., 1998).

Inhibitor studies of bone resorption also suggested a distinct role for one or more MMPs which was dependent on bone type (Everts et al., 1999). Further studies have implied that MMP-mediated collagen proteolysis may be physiologically important in removing any remaining matrix from areas vacated by osteclasts at the end of the resorption cycle (Everts et al., 2002). This enzyme appears to be MMP-13 derived from cells other than osteoclasts (Delaissé et al., 2003; Fuller & Chambers, 1995). Furthermore, it has been proposed that MMP-13-generated collagen fragments are activating factors of osteoclasts and may influence osteoclast recruitment (Holliday et al., 1997; Parikka et al., 2001; Zhao, Byrne, Boyce, & Krane, 1999). A lysosomal cysteine protease related to the caspases, which is known as aspariginyl endopeptidase or legumain, has also been implicated in the control of bone resorption (Choi et al., 1999). The C-terminal region of this molecule appears to be the active component and is also known as osteoclast inhibitory peptide 2. There is no evidence that this activity is dependent on proteolysis. Rather, there may be a receptor for this peptide on osteoclast precursors (Choi, Kurihara, Oba, & Roodman, 2001). Proteolysis of collagen by cathepsin K and MMP collagenase generates distinct C-terminal fragments (CTx and ICTP, respectively) that are detectable in serum and urine. One report

Table 4
Summary of proteases with pathological roles in capsule

Capsule-derived proteases leading to degradation of cartilage and bone	
Enzyme	ADAMTS
Class	Metallo
Pathological roles	Proteolysis of cartilage aggrecan
Reference	Ilic et al. (2000), Vankemmelbeke et al. (1999)

suggests that the serum level of the ICTP collagenase fragment correlates with disease severity in rheumatoid arthritis (Hakala, Risteli, Manelius, Nieminen, & Risteli, 1993). This implicates MMP collagenase activity in the formation of bone erosions in rheumatoid arthritis and other arthritides. Inhibitor and gene knockout studies have identified roles for MT1-MMP (MMP-14) in osteoclast migration (Delaissé et al., 2003; Engsig et al., 2000; Sato, Foged, & Delaissé, 1998). MT1-MMP is located on the plasma membrane at the leading edge of migrating osteoclasts, suggesting that its collagenolytic activity may aid the movement of the osteoclast along bone surfaces (Irie, Tsuruga, Sakakura, Muto, & Yajima, 2001), potentially via a Brownian ratchet motor mechanism as suggested by studies of MMP-1-mediated collagen proteolysis (Saffarian, Collier, Marmer, Elson, & Goldberg, 2004). MT1-MMP-mediated processing of the α_v subunit of $\alpha_v\beta_3$ integrin and shedding of the adhesion molecule CD44 has also been implicated in cell migration (Delaissé et al., 2003; Deryugina, Ratnikov, Postnova, Rozanov, & Strongin, 2002; Kajita et al., 2001). The proteolytic activity of MMP may also influence osteoclast-mediated bone turnover by affecting cell signalling. For example, MMP-9 activity may release TGFβ from the matrix, resulting in the stimulation of osteoclast chemotaxis (Dallas, Rosser, Mundy, & Bonewald, 2002) (Table 5).

2.2. Tendinopathies

2.2.1. Tendon: tenocyte apoptosis and increased collagen turnover

The tendon is a predominantly extracellular tissue the main component of which is type I collagen, which comprises between 50 and 85% of the tissue dry weight (Elliott, 1965). The tissue is maintained by a resident population of fibroblast-like cells called tenocytes. Type I collagen fibrils are organised into fibres oriented along the long axis of the tissue, parallel to the predominant direction of strain, and are surrounded by a thin layer called the endotenon (Kastelic, Galeski, & Baer, 1978). Bundles of fibres form fascicles and the whole tendon is enveloped with a thin surface layer known as the epitenon (Józsa & Kannus, 1997). Tendons may be surrounded by sheaths, either: a synovial sheath, typically found surrounding tendons associated with pulleys across synovial joints or; paratenon (a condensation of connective tissue) at sites such as the Achilles tendon (Benjamin, 2004). Sheaths may become inflamed, often as a result of overuse injury in athletes, and are associated with tendon pain.

Tendon pathologies occur as either acute or chronic conditions and are the result of trauma, repeated microtrauma, or the insidious process of clinically silent (pain-free) degeneration (Riley, 2004). A variety of chronic tendinopathies have been described and encompass 'spontaneous' tendon rupture, painful tendinopathy and calcific tendinitis. Tenocyte apoptosis is a feature of tendon degeneration and may contribute to pathology by obstructing repair as described above in arthritic cartilage (Tuoheti et al., 2005; Yuan, Murrell, Wei, & Wang, 2002).

Tendon ruptures are often described as spontaneous as they occur without any preceeding clinical pain, though a small proportion may be associated with systemic diseases such as rheumatoid arthritis (Aström & Rausing, 1995; Kannus & Józsa, 1991). A number of degenerative changes have been reported including, hypoxia, loss

Table 5
Summary of proteases with pathological roles in bone

Enzyme	Class	Pathological role	Reference
Bone resorption			
Cathepsin K	Cys	Cleavage of helical type I collagen	Drake et al. (1996), Everts et al. (1992), Garnero et al. (1998), Hou et al. (1999), Kamiya et al. (1998)
MMP-13	Metallo	Cleavage of matrix from demineralised zones	Delaissé et al. (2003), Everts et al. (2002), Fuller and Chambers (1995)
Osteoclast activation and migration			
MMP-13	Metallo	Cleavage of type I collagen generating an activation factor for osteoclasts	Holliday et al. (1997), Parikka et al. (2001)
MMP-14 (MT1-MMP)	Metallo	Promotes osteoclast migration via processing of the integrin α_v subunit, shedding of CD44 and cleavage of type I collagen	Delaissé et al. (2003), Deryugina et al. (2002), Engsig et al. (2000), Irie et al. (2001), Kajita et al. (2001), Sato et al. (1998)
MMP-9	Metallo	Promotes osteoclast chemotaxis via release of TGFβ from matrix	Dallas et al. (2002)

Table 6
Summary of proteases with pathological roles in tendon

Enzyme	Class	Pathological role	Reference
Tenocyte apoptosis			
Caspase cascade (caspase -2, -3, -6, -7, -8, -9, -10)	Cys	Induction of tenocyte apoptosis	Fan et al. (2005), Tuoheti et al. (2005), Yuan et al. (2002)
Inappropriate processing of tendon extracellular matrix			
MMP	Metallo	Extracellular matrix cleavage	Millar et al. (1998), Riley et al. (2002)

of fibrillar collagen structure, glycosaminoglycan accumulation between the collagen fibres and cell rounding together with an absence of inflammatory cells (Kannus & Józsa, 1991). Similar changes have been reported in chronic tendinopathy, including an abnormal fibre structure and arrangement, focal changes in cellularity, rounded cells and an increase in proteoglycan content (Movin, Gad, Reinholt, & Rolf, 1997).

Chronic tendon pathologies are associated with a small but significant decrease in collagen content, with an increase in the proportion of type III collagen, relative to type I (Riley et al., 1994). Analyses of the type III collagen content and of slow-forming collagen cross-links have indicated that collagen turnover is increased in chronic pathologies (Bank, TeKoppele, Oostingh, Hazleman, & Riley, 1999). MMP proteases have been implicated in this collagen turnover and comparisons of normal and ruptured tendon have demonstrated an increase in the amount of active MMP-1, and a decrease in the amounts of MMP-2 and -3 together with increased collagen denaturation and turnover (Riley, 2005; Riley et al., 2002). A key role for the inhibition of metalloprotease activity in the development of tendinopathy is inferred from the effects of the broad-spectrum metalloprotease inhibitor Marimastat, which induces tendinopathy by an unknown mechanism (Millar et al., 1998). Other pharmacologicals, the fluoroquinolones, have been associated with tendinopathy in some patients and have also been shown to modulate MMP activity *in vitro* (Corps et al., 2002; Williams, Attia, Wickiewicz, & Hannafin, 2000).

A recent assay of metalloprotease mRNA expression in normal and chronic pathological Achilles tendons has revealed a number of differences including a decrease in MMP-3 in both chronic and ruptured pathologies compared to normal and an increase in MMP-1 in ruptured pathology (Jones et al., 2006). In addition a number of novel differences were identified, most notably an increase in ADAM-12 in both chronic and ruptured pathology and an increase in MMP-23 in chronic painful pathology. The roles of these proteases in either normal or pathological tendon are not known, but are under investigation (Table 6).

3. Summary

Pathologies of the synovial joint affect all the constituent tissues and protease-mediated degeneration of these tissues is a common feature. Most tissues of the joint are composed primarily of ECM and key roles in the degeneration of this are performed by proteases active at neutral pH (serine and metallo proteases). However some proteases with an acidic pH optimum have been shown to be active at neutral pH under some circumstances (Almeida et al., 2001; Berardi et al., 2001; Brix, Lemansky, & Herzog, 1996; Buck, Karustis, Day, Honn, & Sloane, 1992; Buttle et al., 1991; Dehrmann, Coetzer, Pike, & Dennison, 1995) and there is no doubt that the activity of cathepsin K in the acidic lacunae of osteoclasts is crucial to the turnover of the organic matrix of long bone. MMP collagenases are likely to play a prominent role in the cleavage of cartilage collagens. It appears that the turnover of cartilage proteoglycans is mediated by the ADAMTS aggrecanases, with ADAMTS-4 and -5 being prominent here. Current evidence suggests that ADAMTS-5 is the key protease in aggrecan breakdown in animal models of arthritis.

In addition to the cleavage of structural proteins, proteolytic activities are also employed to regulate the activities of other proteases. Proteases combine to form complex regulatory networks, the correct function of which is required for tissue homeostasis and the imbalance of which may be an underlying feature of pathology. There is little doubt that proenzyme activation and protease inhibition by protease inhibitors are also key to the successful control of homeostasis. A precise understanding of the proteases within these networks would aid both a true understanding of pathology and the identification of possible therapeutic interventions.

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