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Proteases involved in cartilage matrix degradation in osteoarthritis

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Abstract

Osteoarthritis is a common joint disease for which there are currently no disease-modifying drugs available. Degradation of the cartilage extracellular matrix is a central feature of the disease and is widely though to be mediated by proteinases that degrade structural components of the matrix, primarily aggrecan and collagen. Studies on transgenic mice have confirmed the central role of Adamalysin with Thrombospondin Motifs 5 (ADAMTS-5) in aggrecan degradation, and the collagenolytic matrix metalloproteinase MMP-13 in collagen degradation. This review discusses recent advances in current understanding of the mechanisms regulating expression of these key enzymes, as well as reviewing the roles of other proteinases in cartilage destruction.

Keywords

osteoarthritis; proteinase; cartilage; aggrecanase; collagenase

2. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease affecting millions of people worldwide [1]. The disease is a leading cause of disability in the elderly, causing pain, stiffness and loss of function in articulating joints. OA is characterised by changes in the anatomy of load-bearing joints that lead to degradation of articular cartilage, inflammation of the synovium (synovitis), changes to subchondral bone and growth of new bone and cartilage (osteophytes) at the joint edge (see Figure 1)[2, 3]. The causes of OA are not fully understood, but mechanical factors such as joint injury and obesity are thought to be primary initiators of disease, with other risk factors such as age, gender and genetics contributing to disease development and progression [3, 4]. There are currently no disease-modifying OA drugs available, and treatment is limited to symptomatic relief or surgical replacement of affected joints. There is thus considerable interest in developing effective treatments that can halt or reverse the progression of the disease.

Loss of cartilage is central to the aetiology of OA. Cartilage is composed of one cell type, the chondrocytes, which are surrounded by a large volume of extracellular matrix (ECM). The matrix can be divided into zones based on their distance from the chondrocyte and matrix composition (see [4] for review). The pericellular matrix is localized immediately

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adjacent to the cell and is enriched with perlecan, type VI collagen and various regulatory molecules and growth factors that modulate chondrocyte function. The zone next to the pericellular matrix is the territorial matrix and further removed is the interterritorial matrix whose major components are collagen II and aggrecan. Collagen provides the tissue with tensile strength, while aggrecan is the major cartilage proteoglycan, drawing water into the matrix and allowing it to resist compression. Degradation of collagen and aggrecan is central to OA pathology, although degradation of less abundant molecules that participate in matrix organisation is also likely to contribute to disease progression [4]. This review describes the current understanding of which proteinases are responsible for aggrecan and collagen degradation in OA, and discusses recent advances in understanding the factors regulating their expression and activity. Other proteinases with potential roles in OA pathology are also highlighted.

3. Aggrecan-degrading enzymes

Aggrecan is a large proteoglycan containing numerous chondroitin sulfate and keratan sulfate glycosaminoglycan moieties, which are central to the function of the molecule as they draw water into the cartilage matrix, giving it the ability to withstand compression. Aggrecan is sensitive to proteolysis at numerous sites along its length. Cleavage of aggrecan in the interglobular domain (IGD) between the N-terminal G1 and G2 globular domains is thought to be of greatest pathological importance, as this releases the glycosaminoglycan-bearing region of aggrecan from the cartilage matrix and so abrogates the function of the molecule.

Degradation of aggrecan is an early event in the development of OA and a considerable amount of research has been done to identify the enzyme(s) responsible. Early work of Thomas [5] showed that rabbit ears collapsed after intravenous injection of papain, with the ear cartilage reversibly losing its metachromatic staining. This demonstrated that cartilage proteoglycans, of which aggrecan is now known to be the most abundant, are susceptible to proteolytic degradation. The same effect was observed upon injection of rabbits with large doses of vitamin A [6], which was thought to cause release of endogenous cartilage-degrading acidic proteinases from lysosomes [7]. Lysosomal cathepsins were demonstrated to be present in cartilage and to be able to degrade cartilage proteoglycans at acidic pHs [8–10]. Cathepsin D was considered to be the major cathepsin in cartilage, as cathepsin D-like activity increased 3-fold in OA cartilage [10] and antibodies against cathepsin D inhibited proteoglycan and cartilage degradation at pH 5.0 [11]. However, OA cartilage has a neutral pH [10] and Woessner [9] showed that while pepstatin and chloroquine inhibited proteoglycan degradation at pH 5, they had no effect on degradation at pH 7.2. This important observation indicated that degradation of cartilage proteoglycans at physiological pH was unlikely to be mediated by cathepsins, but rather by an unidentified neutral proteinase.

Metalloproteinases found in articular cartilage and bone were subsequently shown to be capable of degrading proteoglycans at neutral pH [12, 13]. Matrix metalloproteinase 3 (MMP-3) was isolated from human articular cartilage [14] and found to cleave the Asn^{341}~Phe^{342} bond (where ~ indicates the cleavage site) in the aggrecan IGD [15]. Several other MMPs, including MMP-1, -2, -7, -8, -9 and -13, were later found to be able to cleave the same site, as well as other sites towards the C-terminus of the molecule [16–18]. MMPs were thus thought to be the primary aggrecan-degrading enzymes in OA until a landmark study by Sandy and colleagues [19] revealed that the majority of aggrecan fragments present in the synovial fluid of OA patients were cleaved not at the MMP-sensitive Asn^{341}~Phe^{342} bond, but at the Glu^{373}~Ala^{374} bond in the IGD. This novel cleavage site was also shown to be the primary site of aggrecan fragmentation in cytokine-stimulated chondrocyte and

cartilage explant cultures [20, 21]. Hydrolysis at this site in chondrocyte and cartilage explant cultures was not blocked by TIMP-1, TIMP-2 or synthetic MMP inhibitors [22, 23], indicating that an MMP could not be responsible for the 'aggrecanase' activity.

The first 'aggrecanase' was purified from IL-1-stimulated bovine nasal cartilage by researchers at DuPont Pharmaceuticals in 1999 [24]. The enzyme was named aggrecanase 1, or A Disintegrin And Metalloproteinase with Thrombospondin motifs 4 (ADAMTS-4) based on its homology to the previously identified enzyme ADAMTS-1 [25]. Shortly thereafter, a homologous enzyme was cloned from murine and bovine cartilage and named aggrecanase 2 or ADAMTS-5 (initially coined ADAMTS-11)[26, 27]. The ADAMTSs are zinc-dependent metalloproteinases of the metzincin family [28](Figure 2). They have numerous ancillary domains that modulate their substrate specificity and activity [29, 30]. ADAMTS-1, -8, -9, -15, -16 and -18, can also degrade aggrecan *in vitro* [31–35], but ADAMTS-5 is the most active 'aggrecanase' *in vitro*, followed by ADAMTS-4 [30]. ADAMTS-4 and ADAMTS-5 are thus considered to be the major enzymes responsible for pathological cleavage of aggrecan at the Glu^{373}-Ala^{374} bond in the IGD [23, 36–38].

The pathological importance of ADAMTSs to the development of OA was demonstrated by the finding that *Adamts5*$^{-/-}$ mice develop less severe cartilage damage in a murine surgical model of OA and in an antigen-induced arthritis model [39, 40]. Similarly, transgenic mice with a knock-in mutation of aggrecan preventing 'aggrecanase' cleavage of the Glu^{373}-Ala^{374} bond also develop less severe OA in the surgical OA and antigen-induced arthritis models [38]. *Adamts1*$^{-/-}$ and *Adamts4*$^{-/-}$ mice are not similarly protected [41, 42], indicating that ADAMTS-5 is the primary aggrecanase in mice. There is some evidence that ADAMTS-4 may contribute to cartilage degradation in other species, including humans [43–45]. ADAMTS-4 and ADAMTS-5 are thus attractive targets for the development of novel OA therapies, and several synthetic ADAMTS-4 and ADAMTS-5 inhibitors are in the early stages of development [46–49]. One of these inhibitors has recently been shown to block aggrecan degradation in a rat surgical OA model [48].

Cleavage of aggrecan at the Glu^{373}~Ala^{374} bond is thus a signature of pathological aggrecan loss in OA cartilage. Aggrecan cleavage at the MMP-sensitive Asn^{341}~Phe^{342} bond is also detectable in OA cartilage [50], and may occur later in the progression of disease. MMPs are also thought to contribute to C-terminal 'trimming' of aggrecan, which is considered non-pathological as it does not cause release of the majority of the glycosaminoglycan region of the molecule from the cartilage matrix [23, 36, 37].

4. Collagenases

The primary collagen found in the cartilage ECM is type II collagen, which forms a fibrillar network and provides the cartilage matrix with tensile strength. Along with aggrecan breakdown, degradation of collagen is a central feature of OA [51, 52]. The exact order in which cartilage matrix components are degraded during the development of OA is difficult to ascertain, but a number of *in vitro* studies on cartilage explants suggest that collagen degradation occurs only after aggrecan is lost from the tissue, and that the presence of aggrecan protects the collagen from degradation [53–56]. Furthermore, while aggrecan loss can be reversed, collagen degradation is irreversible, and cartilage cannot be repaired once collagen is lost [53, 55].

Ehrlich *et al.* [57] first demonstrated the presence of a collagen-degrading enzyme in OA cartilage in 1977. Fibrillar collagens are highly stable molecules that can be degraded by only a few mammalian enzymes, namely cathepsin K and the collagenolytic MMPs: MMP-1, -8, -13 and -14. MMP-13 is thought to be the primary collagenase in OA, with its expression increased in OA cartilage [51, 58–62] and in rodent surgical OA models [63].

Conditional expression of MMP-13 in murine cartilage induces spontaneous cartilage degradation [64], while *Mmp13*$^{-/-}$ mice are protected in a surgical OA model [65]. MMP-1 also efficiently cleaves type II collagen (R. Visse, Y. Tominaga, M. Wang, H. Nagase, personal communication), but its role in OA cannot be studied using murine models as murine MMP-1 differs considerably from the human enzyme [66].

The catalytic sites of the MMPs are highly homologous, and historically it has been difficult to generate sufficiently selective synthetic inhibitors to target individual MMPs. Previous attempts to treat cancer with MMP inhibitors failed due to lack of specificity of the inhibitors, which gave rise to toxicity and musculoskeletal side-effects [67]. MMP-13 is unusual among the MMPs, in that it has a very deep S_1' subsite. This feature has been exploited to generate highly selective MMP-13 inhibitors able to block collagen degradation in cartilage explants [51, 68, 69] as well as animal OA models [68, 70] without musculoskeletal side effects [68]. Further evaluation of the therapeutic efficacy of these inhibitors is eagerly awaited.

5. Other MMPs and ADAMs

In addition to *MMP13*, *ADAMTS4* and *ADAMTS5*, mRNA expression of various other MMPs (*e.g. MMP28*), adamalysins (e.g *ADAM12*, *ADAM15*) and ADAMTSs (*e.g. ADAMTS16*, *ADAMTS17*) is reportedly increased in OA [61, 71, 72]. ADAM-8 has been suggested to contribute to OA pathogenesis by cleaving fibronectin, generating fragments that stimulate further cartilage catabolism [73]. Single nucleotide polymorphisms in *ADAM12* [72] and *ADAMTS14* [74] have reported associations with knee OA. The effects of numerous gene mutations and ablations on murine OA have been reviewed by Little and Fosang [75].

MMP3 is the most strongly expressed MMP in OA cartilage, although its expression decreases in late OA [60, 76]. The enzyme is known to participate in the activation of other MMPs, such as MMP-1 and MMP-13 [77, 78], raising the possibility that it may contribute to OA by activating latent collagenases. The susceptibility of *Mmp3*$^{-/-}$ mice to OA is, however, unclear. Van Meurs *et al*. [79] showed that *Mmp3*$^{-/-}$ mice are protected against collagen loss and aggrecan cleavage at Asn^{341}~Phe^{342}, suggesting that MMP-3 promotes collagenase activation and either direct or indirect MMP-mediated aggrecan cleavage. However, Clements *et al*. [80] found that *Mmp3*$^{-/-}$ mice develop more severe surgically induced OA, suggesting that MMP-3 can also serve to protect cartilage in some circumstances.

Mmp9$^{-/-}$ mice are protected in an infectious arthritis model [81] but develop more severe OA in a surgically induced OA model [82]. This difference most likely reflects differences in disease etiology in the two models, as well as differences between mice strains.

Expression profiling studies suggest that MT1-MMP is similarly expressed in normal and OA cartilage [60, 61], although studies on isolated bovine chondrocytes suggest that MT1-MMP expression can be transiently increased by cyclic compression [83]. MT1-MMP is highly expressed in rheumatoid synovial fibroblasts and has been shown to promote invasion of these cells into cartilage [84]. The role of MT1-MMP in OA has not been studied in murine surgical models as MT1-MMP null mice exhibit severe skeletal abnormalities [85].

6. Transcriptional regulation of MMPs and ADAMTSs in OA

Studies on transgenic mice have confirmed the importance of MMP-13 and ADAMTS-5 in the development of OA. As described above, inhibitors targeting these enzymes are in

development as potential OA therapies. Additionally, there is considerable interest in understanding the factors that lead to increased activity of these enzymes in OA, with the hope of uncovering therapeutic targets upstream of the effector proteinases. Some of these newly described networks and regulatory mechanisms are discussed below.

6.1. RUNX2

Runt-related transcription factor 2 (RUNX2, also known as core-binding factor 1, or Cbfa1) is a central transcription factor regulating skeleton formation by stimulating osteoblast differentiation [86, 87] and directing the process of endochondral ossification [88] by stimulating expression of genes required for chondrocyte maturation and hypertrophy (*e. g. COL10A1*, collagen X)[89], degradation of the cartilage matrix (*e. g. MMP13*) [90, 91] and vascularization of the tissue (*e. g. VEGFA*)[92]. Chondrocyte hypertrophy, matrix degradation and vascular invasion are also characteristic of OA, leading to the theory that OA may involve aberrant recapitulation of this developmental programme in adult cartilage [93–95].

RUNX2 is not expressed in normal adult cartilage, but its expression and that of several of its target genes increases in early OA [96]. *Runx2*$^{+/-}$ mice develop less severe cartilage degradation and osteophyte formation in a surgical OA model [97]. *Mmp13* is a known RUNX2 target gene [90, 91] and *Runx2*$^{+/-}$ mice exhibit decreased *Mmp13* expression [97]. *ADAMTS4* and *ADAMTS5* are also thought to be RUNX2 target genes [91, 98, 99], although their expression in *Runx2*$^{+/-}$ mice has not been reported.

RUNX2 expression can be increased by factors known to promote the development of OA, such as mechanical stimuli [91, 100], hypoxia-inducible factor 2α (HIF-2α) [101] and Indian hedgehog [102]. RUNX-2 may thus be a central transcription factor increasing expression of several OA-promoting genes.

6.2. Inflammation

The role of inflammatory pathways in the aetiology of rheumatoid arthritis is well documented [103]. It is increasingly accepted that inflammation also plays a role in the development of OA [4, 104]. For example, IL-1 is well known to stimulate the expression of MMPs such as *MMP1* and *MMP13* in OA cartilage [59, 60, 105]. Inflammatory cytokines can also increase chondrocyte expression of *ADAMTS4* and *ADAMTS5* [106] although some reports indicate that *ADAMTS5* expression is largely constitutive (see [28] for review). The central role of IL-1β in murine OA pathology has been demonstrated in numerous studies (see Glasson *et al*. [82] for review). For example, mice treated with an inhibitor of IL-1β converting enzyme developed less severe joint damage in two arthritis models [107], and ablation of IL-1β has been reported to decrease surgically induced OA [82].

6.2.1. Hypoxia-inducible factor 2α (HIF-2α)—Two recent studies have demonstrated that inflammatory cytokines can stimulate OA cartilage catabolism by inducing nuclear factor- κB (NF-κB)-dependent expression of the transcription factor HIF-2α [94, 108]. IL-1β-induced expression of *ADAMTS4*, *MMP1*, *MMP3*, *MMP9*, *MMP12* and *MMP13* in rabbit articular chondrocytes was increased by over-expression of HIF-2α and decreased by HIF-2α siRNA [108]. Ectopic expression of HIF-2α in murine cartilage induces spontaneous cartilage destruction [108], while HIF-2α-deficient mice are resistant to cartilage degradation and osteophyte development in a surgical OA model [94, 108]. HIF-2α has also been shown to regulate developmental endochondral ossification, by inducing expression of genes mediating chondrocyte hypertrophy (*e. g. Col10a1*), degradation of the cartilage matrix (*e.g. Mmp13*) and vascular invasion (*e. g. Vegfa*) [94]. These are known RUNX2 target genes (section 6.1) and HIF-2α has been shown to increase RUNX2 expression [101,

109], suggesting that HIF- 2α may stimulate expression of these genes via RUNX2. However, HIF-2α-dependent expression of *Mmp13*, *Col10a1* and *Vegfa* is not affected by expression of a dominant negative form of RUNX-2 [101], suggesting that HIF- 2α may also act independently of RUNX-2. HIF- 2α also increased expression of the transcription factor Indian hedgehog [101] that stimulates RUNX-2 expression [102, 110].

The role of HIF-2α in the development of human OA is unclear as both increased [94, 108] and decreased [111] HIF-2α expression has been reported in human OA cartilage. Saito *et al*. [94] reported that a single nucleotide polymorphism that increases HIF-2α expression is associated with knee OA in a Japanese cohort, although Nakajima *et al*. [112] were unable to replicate this association in a larger patient group. Additionally, In contrast to its hydroxylation-independent catabolic effects in a mouse chondrogenic cell line [94], HIF-2α has been shown to have hypoxia-dependent anabolic effects on human chondrocytes [113].

6.2.2. Histone deacetylases—Histone deacetylases (HDAC) modulate gene expression by increasing histone association with DNA, thus mediating chromatin condensation and inhibiting transcription factor binding [114]. HDACs thus play central roles in numerous physiological and pathological conditions [114]. HDAC can be divided into 3 classes on the basis of sequence homology: 2 classes of classical HDAC and the class III NAD^+-dependent sirtuin family.

HDAC inhibitors have been shown to block inflammatory cytokine-induced expression of *MMP1*, *MMP13*, *ADAMTS4* and *ADAMTS* 4 and *ADAMTS5* in human chondrocytes [115–117]. The protective effect of HDAC inhibitors is further illustrated by the demonstration that they can block inflammatory cytokine-stimulated degradation of both proteoglycans and collagen in bovine cartilage explants [115]. The effect of HDAC inhibitors on surgically induced murine OA has not been reported, but HDAC inhibitors can block collagen-induced arthritis in mice [118]. HDAC1, HDAC2 and HDAC7 are all expressed at elevated levels in human OA cartilage [117, 119].

HDAC inhibitors block both HIF-1α and HIF-2α transcriptional activity [120]. HDAC4, HDAC6 and HDAC7 have been shown to stimulate HIF-1α activity [121, 122], while the HDAC Sirtuin 1 (SIRT1) stimulates HIF-2α transcriptional activity [123]. HDACs may thus promote cartilage catabolism by stimulating HIF-2α activity.

Some studies suggest that HDACs can also serve to protect cartilage. SIRT1 contributes to cartilage homeostasis by inhibiting chondrocyte apoptosis [124] and stimulating expression of cartilage-specific genes [125]. HDAC4 has been shown to interact with and inhibit the activity of RUNX2 [126]. *Hdac4*$^{-/-}$ mice exhibit a similar cartilage hypertrophy phenotype to RUNX2-over-expressing chondrocytes [88, 126] and over-expression of HDAC4 inhibits chondrocyte hypertrophy [126]. Conversely, HDAC4 expression is reduced by the chondroprotective miR140 [127]. Further studies are required to delineate the roles of individual HDAC in cartilage homeostasis and OA development.

6.3. miR140

MicroRNAs (miRNAs) are non-coding RNA sequences that post-transcriptionally down-regulate gene expression by interacting with the 3′ untranslated region of target mRNAs, leading either to degradation of the mRNA or repression of its expression. miRNAs were first identified in 1993 as regulators of *C. elegans* larval development [128], and have since been shown to regulate expression of numerous genes, often in a tissue-specific or developmental-stage specific manner.

miR-140 is abundantly expressed in normal cartilage under the control of the master cartilage transcription factor Sox9 [129]. miR-140 expression is reduced in OA cartilage [62, 127, 130] and *miR-140*$^{-/-}$ mice develop accelerated spontaneous and surgically induced OA, while mice over-expressing miR-140 are resistant to antigen-induced arthritis [129]. *Adamts5* has been shown to be a target for miR140, with *Adamts5* expression increased in *miR-140*$^{-/-}$ chondrocytes and decreased in miR-140 over-expressing mice [129].

Iliopoulos *et al*. [62] identified 16 microRNAs with altered expression in OA cartilage. miR-22 is thought to increase expression of *MMP13* [62], while miR-9, miR-27a and miR-27b have been reported to inhibit *MMP13* expression in human OA chondrocytes [130–132].

6.4. Mechanical stimuli

Mechanical loading is an important factor in cartilage homeostasis. Both disuse and excessive use of joints can initiate cartilage degradation [133, 134], with mechanical injury shown to increase expression of *RUNX2* [91, 100], *MMP1*, *MMP3* [135], *MMP13* [136] and *ADAMTS5* [136]. Several research groups are investigating the molecular mechanisms by which moderate mechanical load maintains joint function and protects cartilage by suppressing expression of catabolic proteinases.

Recent studies have identified the transcription co-regulator CITED2 (cAMP-responsive element-binding protein/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2) as a novel mediator of mechanical responses in cartilage [137, 138]. Experimental mechanical stimuli have been shown to increase levels of CITED2, which in turn suppresses *MMP1* and *MMP13* expression by decreasing interaction of the MMP transactivator Ets-1 with its co-activator p300 [137, 138]. Immobilization of rat hind limbs reduces CITED2 expression, leading to increased expression of *MMP1* and *MMP3*, and cartilage degradation [138, 139]. The effects of CITED2 on ADAMTS expression and its role in OA remain to be studied.

Fibroblast growth factor 2 (FGF-2) is thought to act as a transducer of protective mechanical signals from the pericellular matrix to chondrocytes [140]. *Fgf*$^{-/-}$ mice develop accelerated spontaneous and surgically induced OA, accompanied by an increase in *Adamts5* expression and aggrecanase activity [63]. Addition of exogenous FGF-2 to cytokine-stimulated normal human cartilage explants suppresses *ADAMTS4* and *ADAMTS5* expression and reduces cartilage degradation [106, 116]. The mechanism by which FGF-2 suppresses *ADAMTS* expression is not currently known [63]. In addition to these protective effects, FGF-2 has also been reported to have catabolic effects (reviewed in [141]), stimulating expression of *MMP1*, *MMP3* and *MMP13* in cartilage explants and cultured chondrocytes [116, 135, 142–144]. Factors such as the force of the mechanical load and the pattern of FGF receptor expression may determine whether FGF-2 transmits a protective or a catabolic signal to chondrocytes.

6.5. Extracellular sulfatases

Sulf-1 and Sulf-2 are recently described extracellular sulfatases that remove the 6-O-sulfate group from glucosamine residues of heparan sulfate [145]. The enzymes modulate the activity of various heparin-binding growth factors and chemokines by blocking their binding to heparan sulfate components of the ECM [146]. Cartilage expression of Sulf-1 and Sulf-2 increases with age and in OA [147, 148]. The enzymes appear to be chondroprotective, as *Sulf-1*$^{-/-}$ or *Sulf-2*$^{-/-}$ mice develop accelerated spontaneous OA upon aging, and also develop more severe cartilage damage in a surgical OA model [148]. *Sulf*-null chondrocytes showed a more catabolic phenotype than wild-type chondrocytes, with increased expression

of MMP-13 and ADAMTS-5, and decreased expression of aggrecan and type II collagen [148]. *Sulf-1*$^{-/-}$ or *Sulf-2*$^{-/-}$ mice showed an increase in FGF-2-dependent Erk1/2 phosphorylation, and a decrease in BMP-7-dependent Smad1/5 phosphorylation, indicating that the Sulfs inhibit FGF-2 activity and promote BMP-7 activity [148]. Similar effects were observed upon treating human chondrocytes with Sulf-1 and Sulf-2 siRNA [148]. BMP-7 is known to have anabolic effects on cartilage [149], while both anabolic and catabolic effects have been reported for FGF-2 [63, 144]. The Sulfs are likely to affect the activity of growth factors and cartilage proteins other than BMP-7 and FGF-2, with their overall effect on cartilage homeostasis determined by the balance of these changes. For example, both the ADAMTSs and their endogenous inhibitor Tissue Inhibitor of Metalloproteinases 3 (TIMP-3) can bind to heparin [35, 150, 151] and the effect of Sulf-1 and Sulf-2 on their activity remains to be determined.

6.6. Syndecan 4

Expression of the transmembrane heparan sulfate proteoglycan syndecan 4 is elevated in human OA cartilage and in rodent OA models [152, 153]. *Sdc4*$^{-/-}$ mice developed less severe cartilage damage in a surgical OA model, accompanied by a reduction in both proteoglycan loss and aggrecan cleavage at the Glu^{373}~Ala^{374} 'aggrecanase' bond [153]. Mice injected with syndecan 4 blocking antibodies were similarly protected against ADAMTS-5-mediated cartilage damage [153]. ADAMTS-5 interacts with the heparan sulfate chains of syndecan 4 [153], but the molecular mechanisms by which syndecan 4 promotes ADAMTS-5 activity are unclear. *Sdc4*$^{-/-}$ mice have reduced levels of *Mmp3* expression, and Echtermeyer *et al*. [153] argue that MMP-3 contributes to activation of ADAMTS-5. ADAMTSs are thought to be activated primarily by proprotein convertases [154, 155], and there is currently no evidence for direct activation of ADAMTS-5 by MMP-3 or by other MMPs, or for reduced ADAMTS-5 activation in the *Sdc4*$^{-/-}$ mice.

Syndecan 1 is also expressed at elevated levels in OA cartilage [152, 156], and has been shown to retain ADAMTS-4 on the surface of human chondrosarcoma cells [157]. Syndecan 4 may increase ADAMTS-5 activity by similarly modulating the localisation of the enzyme, or that of its physiological inhibitor, TIMP-3. It would be interesting to investigate the effect of the sulfatases Sulf-1 and Sulf-2 (section 6.5) on syndecan 4 binding to ADAMTS- 5.

6.7. DDR-2

The discoidin domain receptors DDR-1 and DDR-2 are receptor tyrosine kinases that bind to native collagen types I, II, III, IV and V [158–160]. Binding of collagen to the extracellular domains of the DDRs causes autophosphorylation of their cytoplasmic domains, initiating downstream signalling events including increased expression of the collagenases MMP-1 and MMP-13 [158, 161]. Expression of DDR-2 is increased in human OA [162, 163]. *Ddr2*$^{+/-}$ mice are protected against spontaneous and surgically induced OA and show reduced expression of MMP-13 [164].

The most abundant collagen in cartilage is type II collagen, which has been shown to phosphorylate DDR-2, albeit less strongly than collagen type I and III [158]. However, type II collagen is localised in the inter-territorial matrix and is not present in the pericellular matrix, so there is unlikely to be direct contact between chondrocytes and type II collagen in healthy cartilage. Xu *et al*. [164] suggest that damage to the pericellular matrix may occur early in the development of OA, leading to aberrant interaction of chondrocytes with type II collagen and initiation of catabolic signalling. The serine proteinase HtrA1 (section 8.3) is localised in the pericellular matrix [165, 166] and is able to degrade a variety of matrix components, leading Xu *et al*. to propose that it may play a role in degradation of the

pericellular matrix in early OA, initiating catabolic DDR-2 signalling and further MMP-13-mediated degradation of the collagen matrix.

6.8. PAR-2

Expression of protease-activated receptor 2 (PAR-2) is increased in human OA chondrocytes [167, 168] and subchondral bone osteoclasts [169]. The receptor appears to promote cartilage degradation, as *PAR-2*$^{-/-}$ mice are protected in surgical OA models [170, 171]. Similarly, treatment of wild-type mice with a PAR-2 antagonist or a PAR-2 blocking antibody protects against the development of OA [170].

PAR-2 is a member of a family of seven transmembrane G-protein-coupled receptors that are activated by cleavage of their extracellular domains by serine proteinases, generating a tethered ligand that stimulates receptor activation and downstream signaling. The mechanisms of PAR-2 activation in OA and the downstream signaling consequences remain to be elucidated. PAR-2 can be activated by matriptase 1 (section 8.1, [172]), but other cartilage serine proteinases are also likely to contribute to its activation. PAR-2 activation has been shown to increase expression of MMP-1 and MMP-13 in OA chondrocytes [168], but effects on ADAMTS expression have not been reported.

6.9. Wnt signalling

Wnt signalling is critical in skeletal development, and there has been considerable interest in the role of Wnt signalling in the development of OA [173]. Wnt signalling affects multiple cellular pathways in chondrocytes, but recent studies have indicated that Wnt signalling promotes OA at least in part by increasing expression of cartilage-degrading proteinases. For example, siRNA against the Wnt co-receptor LRP-5 causes a decrease in *MMP13* expression [174]. Furthermore, Wnt-induced signalling protein 1 (WISP-1) has been shown to increase expression of *MMP3*, *MMP9* and *ADAMTS4*, but not *ADAMTS5* [175]. Adenoviral expression of WISP-1 in mouse knee joints induced cartilage degradation and aggrecan hydrolysis at both Asn^{341}~Phe^{342} and Glu^{373}~Ala^{374}, indicators of MMP and aggrecanase cleavage, respectively[175]. WISP-1 expression is increased in human and murine OA cartilage [175], and a single nucleotide polymorphism in WISP-1 is reportedly associated with spinal OA in Japanese women [176].

7. Inhibitors of MMPs and ADAMTSs

The tissue inhibitors of metalloproteinases (TIMPs) are the endogenous inhibitors of the MMPs and some members of the ADAM and ADAMTS families (see [177] for review). The MMPs are strongly inhibited by all four of the mammalian TIMPs (TIMP-1, -2, -3 and -4), with the exception of some of the membrane-type MMPs that are poorly inhibited by TIMP-1. Conversely, ADAMTS-4 and ADAMTS-5 are effectively inhibited only by TIMP-3 [178, 179]. As TIMP-3 can inhibit both MMPs and ADAMTSs, it is a central inhibitor of cartilage degradation. Addition of exogenous TIMP-3, but not TIMP-1 or TIMP-2 blocks cartilage degradation in explant cultures [180], and injection of TIMP-3 blocks cartilage breakdown in a rat surgical model of OA [181]. The chondroprotective role of TIMP-3 is confirmed by the finding that *Timp3*$^{-/-}$ mice develop increased cartilage degradation upon aging [182] and increased cartilage damage in an antigen-induced arthritis model [183]. While TIMP-3 mRNA levels are not significantly altered in OA [63, 184, 185], levels of TIMP-3 protein are reduced in human OA cartilage [185]. TIMP-3 can be endocytosed and degraded by chondrocytes [186], suggesting that its activity in cartilage may be regulated post-translationally rather than transcriptionally. Agents such as pentosan polysulfate that block TIMP-3 endocytosis are able to increase cartilage levels of TIMP-3

and to inhibit aggrecan degradation [186]. Pentosan further protects cartilage by increasing the affinity of TIMP-3 for ADAMTS-4 and ADAMTS-5 by more than 100-fold [186].

The susceptibility of other *Timp*-null mice to OA has not been reported. TIMP-2 has no effect on glycosaminoglycan release from bovine, porcine or human cartilage explants, while TIMP-1 has been shown to partially inhibit glycosaminoglycan release from human but not bovine or porcine cartilage [56, 106, 180]. Expression of TIMP-4 is decreased in OA cartilage [61], and a single nucleotide polymorphism in the 3′ untranslated region of TIMP-4 is reportedly associated with OA in a Korean cohort [187].

8. Serine proteinases in OA

8.1. Collagenase activators

The collagenases MMP-1 and MMP-13 are known to be activated by a number of other proteinases, including MMP-3 and the serine proteinase plasmin, which is in turn generated from plasminogen by urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) [77, 78]. Increased expression of MMP-3 [60, 76], tPA [188] and uPA [188] have all been reported in OA. As discussed above (section 4), degradation of aggrecan in cytokine-stimulated bovine and porcine cartilage explant model systems occurs within the first week, while collagen degradation occurs later [23, 53–56]. Collagenolysis can be initiated during the first week of culture by addition of proMMP activators such as MMP-3 or *p*-aminophenylmercuric acetate [189], indicating that collagenases are expressed during this time, but that they are largely present as inactive zymogen forms and that their activation is the rate-limiting step in cartilage collagenolysis. Collagenase activity can be inhibited by addition of serine proteinase inhibitors, indicating that serine proteinases are primarily responsible for activation of collagenases in cartilage [189].

Milner *et al*. [172] have recently reported that the type II transmembrane serine proteinase matriptase 1 (or membrane-type serine proteinase 1, MT-SP1, Figure 2)[190] is a novel activator of collagenase activity and is up-regulated in OA cartilage. In addition to activating proMMP-1 and proMMP-3 *in vitro* [172, 191], addition of recombinant matriptase 1 stimulated collagen breakdown in bovine and human OA cartilage explants [172]. The enhanced collagen degradation could be blocked by the metalloproteinase inhibitor GM6001, suggesting that matriptase 1 acted by increasing MMP-mediated collagenolysis [172]. Matriptase 1 was found to stimulate cartilage expression of *MMP1*, *MMP3* and *MMP13* [172], and to activate the collagenase activator uPA [192]. Matriptase 1 thus stimulates cartilage collagenolysis through multiple inter-related mechanisms, making it an attractive target for the development of chondroprotective therapies.

Activated protein C (APC) is known primarily as a serine proteinase of the coagulation cascade, but Jackson *et al*. [193] showed that APC is also expressed by OA chondrocytes in regions of cartilage fibrillation, although not in normal cartilage. Addition of exogenous APC increased cytokine-stimulated aggrecan and collagen degradation in ovine cartilage explants [193] and collagen degradation in equine explants [194]. The catabolic effects of APC could be partially inhibited by addition of a broad-spectrum MMP inhibitor [193, 194], indicating that APC acts by increasing MMP activity. APC had no effect on expression of a range of MMPs, ADAMTSs or TIMPs, but increased activation of proMMP-2 and proMMP-9, although not proMMP-13 [193, 194].

8.2. ADAMTS activators

The ADAMTSs are activated by members of the family of proprotein convertases (PC), including furin, Paired Basic Amino Acid Cleaving Enzyme 4 (PACE4), PC5/6 and PC7 (Figure 2)[154, 155]. Inhibition of furin-like enzymes inhibits aggrecan and collagen

degradation in cartilage explants [195]. PACE4 is thought to be the primary member of the family responsible for aggrecanase activation in cartilage, and its expression is increased in OA [76, 196]. Reducing PACE4 expression with siRNA significantly inhibited aggrecanase activity in cultured human chondrocytes and partially blocked aggrecan degradation in OA cartilage explants [196].

8.3. HtrA1

HtrA (high temperature requirement A) was originally identified as an *E. coli* heat shock protein and was then shown to be a trypsin-like serine proteinase involved in degradation of misfolded proteins [197, 198]. Homologues were subsequently identified in mammals, with 4 isotypes found in humans [199](Figure 2). Expression of HtrA1 is elevated in OA cartilage [76, 166, 172, 200, 201], and expression of *HtrA3* and *HtrA4* may also be increased in OA [76]. Few serine proteinases are thought to participate directly in degradation of the cartilage ECM, but HtrA1 degrades a variety of cartilage matrix proteins, including aggrecan, decorin, fibromodulin and fibronectin *in vitro* [165, 200]. The enzyme has been suggested to degrade the pericellular matrix, a factor proposed to increase catabolic DDR-2 signalling (section 6.7) [164, 166]. Type VI collagen is absent from the pericellular matrix surrounding chondrocytes expressing HtrA1, suggesting that this enzyme may contribute to type VI collagen degradation as well [166]. Type VI collagen is resistant to MMP-1, MMP-2, MMP-3 and MMP-9, but can be degraded *in vitro* by serine proteinases including elastase, trypsin and cathepsin G [202].

HtrA1 can also degrade aggrecan, cleaving at the $VQTV^{356}$~^{357}TWPD bond in the IGD, between the MMP and ADAMTS cleavage sites [201]. Aggrecan fragments bearing the $VQTV^{356}$ neo-epitope are detectable in OA but not in normal cartilage [201]. HtrA1 is unlikely to contribute greatly to pathological aggrecan cleavage however, as $VQTV^{356}$ fragments are present at 20-fold lower levels in OA cartilage extracts than ADAMTS-generated $NITEGE^{373}$ fragments [201].

8.4. Other serine proteinases

Fibroblast activation protein α (FAPα) is a type II transmembrane serine proteinase with increased expression in OA [203]. However, the substrates and function of the enzyme in cartilage are unknown.

Complement 1s is able to degrade insulin-like growth factor binding protein 5 (IGFBP5) *in vitro* [204]. Complement 1s inhibitors have been shown to reduce proteolysis of IGFBP5 in a canine OA model, leading to an increase in concentrations of insulin-like growth factor 1 (IGF1) and reduced cartilage damage [205].

9. Cysteine proteinases

9.1. Cathepsins

The papain-like cysteine proteinase cathepsin K is the only enzyme other than the collagenolytic MMPs that can hydrolyse native triple helical type I and type II collagen [206]. Chondrocyte expression of cathepsin K is increased in OA [207, 208] and the enzyme has been proposed to play a role in degradation of collagen in the cartilage matrix and in subchondral bone [209, 210].

Cathepsin K is highly expressed in osteoclasts, and studies on null mice and patients with genetic mutations indicate that cathepsin K is important for physiological bone development and remodelling [211, 212]. Such a developmental bone phenotype prevents use of the null mice to determine the role of cathepsin K in OA development. No conditional cartilage

knockout of cathepsin K has been reported to date. Mice over-expressing cathepsin K develop increased spontaneous cartilage damage upon aging [209], although these mice also have a developmental bone phenotype that may hamper interpretation of the results [213]. Cathepsin K inhibitors reduce collagen breakdown in OA cartilage explants [214] and animal OA models [215]. Taken together, these studies suggest that cathepsin K contributes to the development of OA. However, Takahashi *et al*. [208] suggest that synovial cathepsin K can also protect cartilage, as siRNA down-regulation of the proteinase in synovium increased expression of *MMP13* and accelerated cartilage degradation in a rabbit surgical OA model.

Cathepsin K is primarily localized intracellularly within lysosomes, but can also be secreted from synovial fibroblasts [210]. *In vitro*, the enzyme is active against collagen between pH 4.0 and 6.5 [206, 216]. pH values as low as pH 5.5 have been reported for OA cartilage [207], suggesting that the pH within arthritic joints may permit extracellular cathepsin K to retain collagenolytic activity. Additionally, cathepsin K retains some collagenolytic activity at neutral pH. *In vitro*, cathepsin K has been shown to form an oligomeric complex with chondroitin-4-sulfate, increasing the stability and collagenolytic activity of the enzyme [217, 218]. Cathepsin K can also cleave aggrecan at multiple sites in the G1 domain and CS2 region, as well as at one site in the IGD region [216], generating chondroitin sulfate-containing fragments that can interact with cathepsin K and stimulate its collagenolytic activity [216].

Expression of other cathepsins, including cathepsin B, D and S, is increased in OA cartilage, synovium and synovial fluid [10, 210, 219]. Cathepsins B and D can also cleave aggrecan *in vitro* [9, 16] but aggrecan degradation at neutral pH cannot be blocked by cathepsin inhibitors [220], suggesting that these enzymes do not contribute to pathological aggrecan degradation *in vivo*. Cathepsin B has recently been shown to degrade the HDAC Sirt1 (section 6.2.2) [221].

9.2. Calpains

Expression of the calcium-dependent cysteine proteinases μ-calpain (calpain 1) and m-calpain (calpain 2) is increased in OA cartilage [76]. The enzymes are expressed by chondrocytes and synovial fibroblasts [222, 223], and while they are intracellular enzymes, they have been detected in synovial fluid [224, 225]. They have a neutral pH optimum, so may be active extracellularly [226].

m-Calpain has been shown to cleave aggrecan *in vitro* at a number of sites in the IGD, KS and CS1 regions [222, 227, 228]. Cleavage in the KS region has been studied in the greatest detail, using an antibody recognising the C-terminal neoepitope $PGVA^{709}$ [227, 228]. Cleavage at this site would cause release of the majority of the GAG-bearing region of aggrecan from the cartilage matrix, as is the case with MMP and ADAMTS cleavage in the IGD. This neoepitope has been detected in bovine and human cartilage [222, 227], but G1-$PGVA^{709}$ fragments are present in OA cartilage at levels 18-fold lower than MMP-generated G1-$DIPEN^{341}$ fragments and 63-fold lower than ADAMTS-generated G1-$NITEGE^{373}$ fragments [228]. This suggests that a calpains play a minor role in pathological aggrecan cleavage *in vivo*. Fragments corresponding to m-calpain cleavage in the CS1 region have been found in both normal and OA cartilage [228], suggesting that calpains may be involved in normal aggrecan turnover.

9.3. Caspases

Chondrocyte death is a central feature of OA, and is thought to occur through a combination of autophagy and apoptosis [229, 230]. Expression of caspase 3 is increased in OA cartilage

[230] and intra-articular injection of caspase inhibitors has been shown to reduce cartilage degradation in a rabbit surgical OA model [231].

10. Conclusions and future prospects

OA remains a disease with insufficient disease-modifying treatments. With an increasing number of people suffering from the disease, the identification of novel therapeutic targets is a priority. The central role of aggrecanases and collagenases in cartilage degradation has been verified in recent years by studies on transgenic mice. While these enzymes are also thought to play pivotal roles in human OA, there are likely to be some differences in the roles of individual enzymes between the two species. For example, murine MMP-1 differs considerably from the mammalian enzyme, so its role in OA cannot be studied in transgenic mice. Also, ADAMTS-4 plays little role in murine OA, but may contribute to human cartilage degradation. Further research is needed to fully delineate the role of individual proteinases in human OA.

Studies on mice with specific gene ablations have also identified a network of factors that regulate *MMP13* and *ADAMTS* expression in chondrocytes (Figure 3). Mechanical damage is a primary risk factor for OA, and is now understood that one of the ways in such stimuli can act on chondrocytes is by stimulating proteinase expression via RUNX2 and Indian hedgehog. Conversely, protective mechanical stimuli can inhibit proteinase expression through CITED2 and FGF-2. Obesity may act as a risk factor not only through increasing mechanical strain but also through the pro-inflammatory properties of adipokines [232]. The mechanisms by which other OA risk factors such as gender and age increase proteinase expression remain unknown. Mechanisms regulating cartilage expression of enzymes such as HtrA1, matriptase and cathepsin K require further study. Similarly, while TIMP-3 is known to be able to protect cartilage by inhibiting MMPs and ADAMTSs, the role of endogenous serine and cysteine proteinase inhibitors in OA remains poorly studied. Given that many pathways can stimulate an increase in proteinase expression, development of inhibitors targeting the effector proteinases and using them in combination may block cartilage damage more effectively than therapies aimed at only one activating factor.

OA is a disease involving the whole joint. To date, the role of proteinases in cartilage structural changes has been studied extensively, but the role of proteinases and proteinase inhibitors in synovial hypertrophy, osteophyte formation and subchondral bone remodelling is less well understood. Interestingly, *Mmp13*$^{-/-}$ mice develop osteophytes more rapidly than wild-type animals after surgical induction of OA [65]. The role of proteinases in joint components other than cartilage is important to understand if proteinase inhibitors are to be developed as OA therapeutics.

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Abbreviations

ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
APC	activated protein C

CITED2	cAMP-responsive element-binding protein/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2
ECM	extracellular matrix
ERK	extracellularly-regulated kinase
FAPα	fibroblast activation protein α
FGF-2	fibroblast growth factor 2
Gla	γ-carboxyglutamate
HDAC	histone deacetylase
HIF-2α	hypoxia-inducible factor 2α
IGD	interglobular domain
IGF	insulin-like growth factor
IGFBP	IGF binding protein
MMP	matrix metalloproteinase
OA	osteoarthritis
PACE4	paired basic amino acid cleaving enzyme 4
PAR	protease-activated receptor
PC	proprotein convertase
RUNX2	runt-related transcription factor 2
SIRT1	Sirtuin 1
TIMP	tissue inhibitor of metalloproteinases
tPA	tissue-type plasminogen activator
uPA	urokinase-type plasminogen activator
WISP-1	Wnt-induced signalling protein 1

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Highlights

1. Osteoarthritis is a characterised by degradation of the cartilage extracellular matrix
2. Collegen is degraded by matrix metalloproteinases such as MMP-13
3. Aggrecan is degraded by related ADAMTS metalloproteinases
4. Less abundant cartilage components are degraded by a variety of proteinases
5. Factors such as inflammation and mechanical damage stimulate enzyme expression

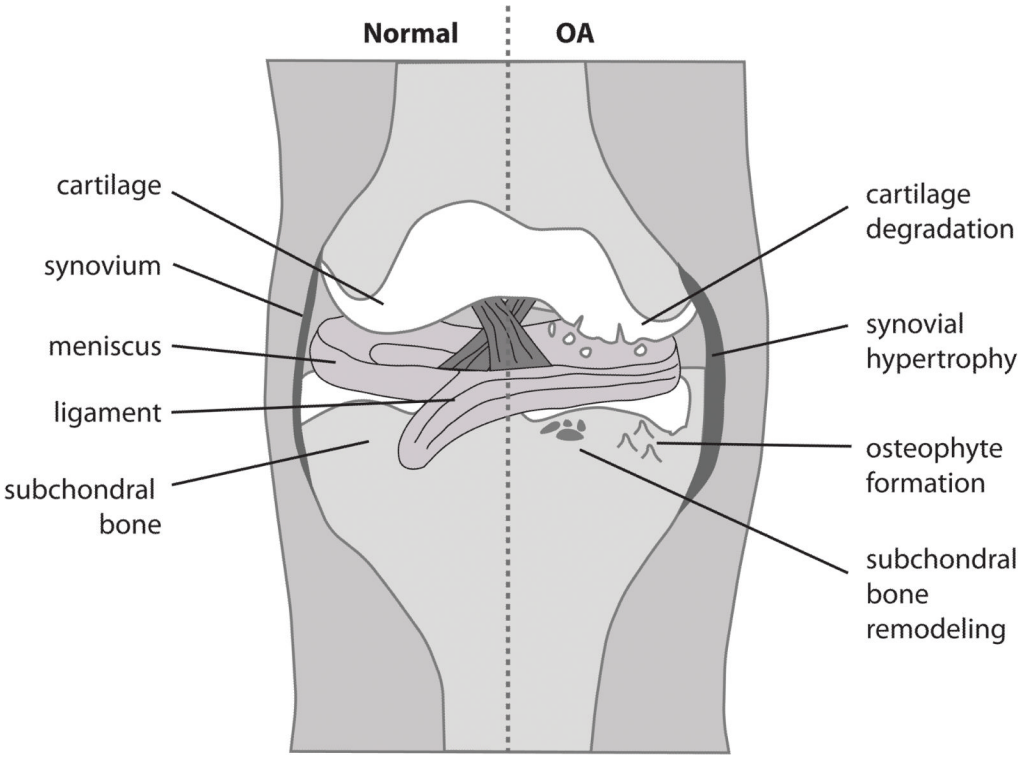

Figure 1. Cartoon representation of normal and osteoarthritic joint

OA is characterised by changes to various tissues within synovial joints. The cartilage matrix is degraded by collagenases and aggrecanases, leading to fibrillation and subsequent loss of the articulating cartilage surface. Synovial fibroblasts undergo hypertrophy and inflammatory cells infiltrate the synovium. Bone remodelling leads to the formation of osteophytes at the cartilage/bone interface and subchondral bone sclerosis.

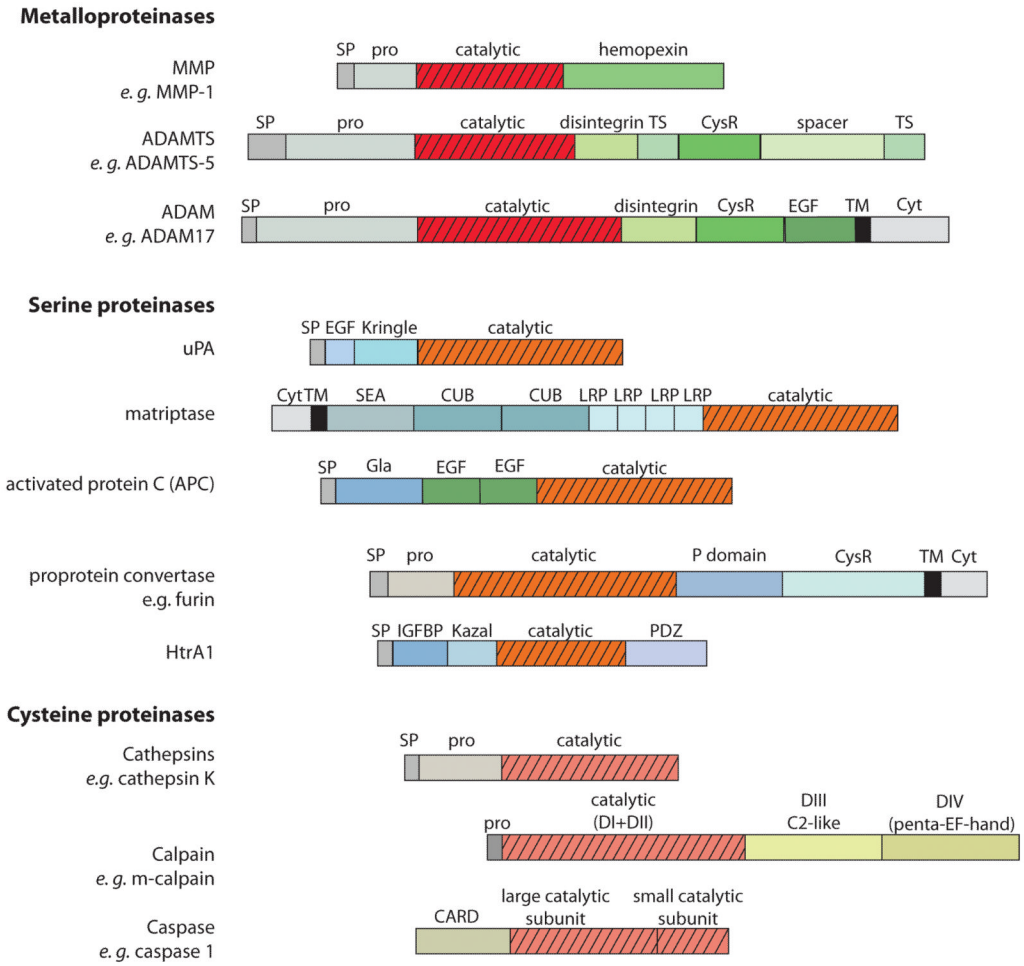

Figure 2. Schematic representation of domain structure of proteinases involved in OA cartilage destruction

The MMPs, ADAMTSs and ADAMs all contain an N-terminal signal peptide (SP), followed by a pro-domain (pro) and a metalloproteinase catalytic domain. The MMPs then contain a hemopexin ancillary domain [233], while the ADAMTSs contain disintegrin, thrombospondin (TS), cysteine-rich (CysR) and spacer ancillary domains [234]. The ADAMs contain C-terminal disintegrin, CysR, epidermal growth factor-like (EGF-like), transmembrane (TM) and cytoplasmic (Cyt) domains [235].

The serine proteinases involved in OA cartilage destruction are more structurally diverse than the metalloproteinases. uPA consists of an N-terminal SP, followed by an EGF-like domain, a Kringle domain and a C-terminal catalytic domain [236]. Matriptase 1 is a type II transmembrane protein, with an N-terminal cytoplasmic domain followed by a TM region [190]. This is followed by a sea urchin sperm protein/enteropeptidase/agrin (SEA) domain, 2 complement C1r/C1s, Uegf, Bmp1 (CUB) domains, 4 low-density lipoprotein receptor-related protein (LRP) domains and a C-terminal catalytic domain [190]. APC consists of a γ-carboxyglutamate (Gla) domain, followed by 2 EGF-domains and a trypsin-like serine proteinase domain [237]. Proprotein convertases such as furin are subtilisin-like serine proteinases consisting of an N-terminal SP and pro-domain, followed by a serine proteinase catalytic domain, a conserved regulatory P domain and a CysR domain [238]. Some of the proprotein convertases (*e. g.* furin) are type I transmembrane proteins and contain a C-terminal TM and cytoplasmic domain, while others (*e. g.* PACE4) lack these domains and are soluble [238]. HtrA1 consists of an N-terminal SP, followed by an insulin growth factor

binding protein (IGFBP) domain, Kazal proteinase inhibitor (KI) domain, a trypsin-like serine proteinase domain and a C-terminal PDZ domain [199].

The cathepsins have comparatively simple structures, consisting of an SP, pro-domain and catalytic domain with no additional ancillary domains [239]. Classical calpains such as m-calpain consist of 4 domains, with domain I (D1) and domain II (DII) forming the catalytic domain, and domains III and IV (DIII and DIV) regulating catalytic activity and stability [240]. Domain III is C2-like, and domain IV contains 5 EF-hand repeats. Caspases have all contain a large and a small catalytic subunit. N-terminal to this is either an N-terminal caspase recruitment domain (CARD) domain (*e. g.* in caspase 1, 2, 4 and 5) or an N-terminal death effector domain (DED) (*e. g.* in caspase 8, 10) [241].

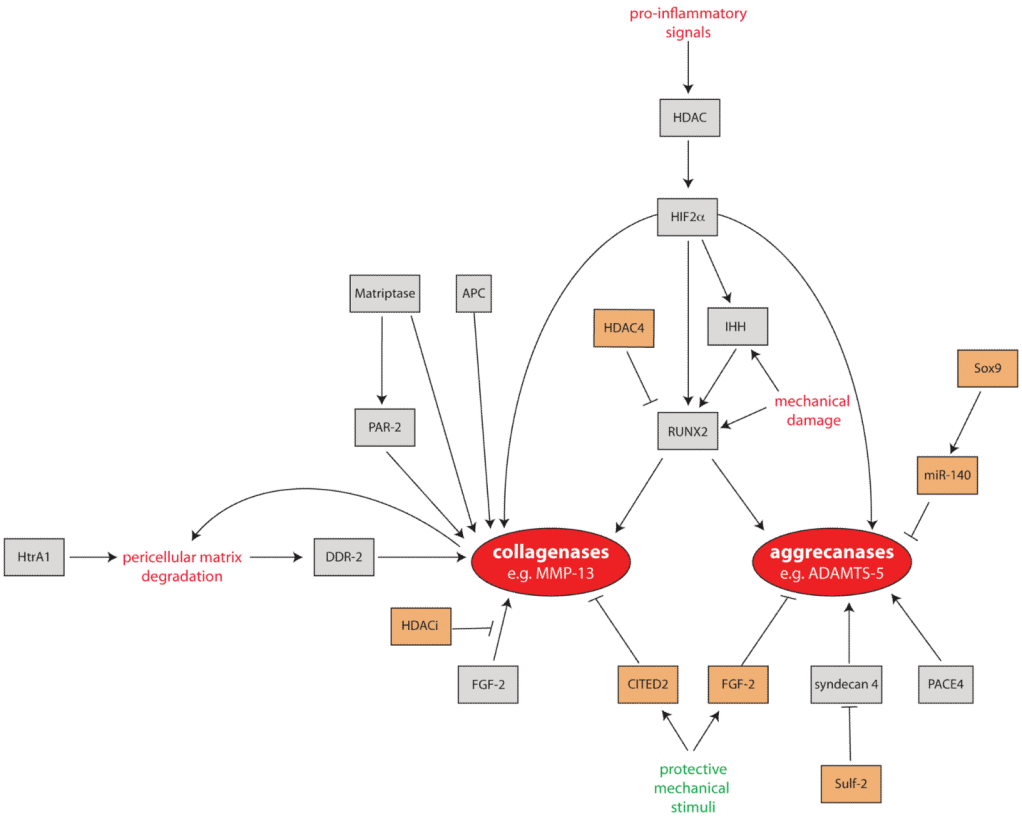

Figure 3. Factors regulating expression and activity of collagenases and aggrecanases in OA

The expression and activity of collagenases (*e. g.* MMP-13 and MMP-1) and aggrecanases (*e. g.* ADAMTS-4 and ADAMTS-5) can be stimulated (orange boxes) or inhibited (grey boxes) by a number of inter-related mechanisms. Expression of the central transcription factor RUNX2 is increased by mechanical and pro-inflammatory stimuli, which act via HIF-2α, HDACs and Indian hedgehog. Expression of collagenolytic MMPs can also be increased in response to FGF-2 and DDR-2 signalling, and collagenase activity increased by matriptase activation of proMMP-1 and proMMP-3 zymogens and PAR-2 signalling. These catabolic stimuli can be counteracted by a variety of chondroprotective signals. For example, *MMP13* expression can be reduced by the mechano-sensitive transcription factor CITED, and *ADAMTS5* expression can be reduced by miR-140 and the mechano-responsive growth factor FGF-2.