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Review

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 thought 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. This article is part of a Special Issue entitled: Proteolysis 50 years after the discovery of lysosome.

1. 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 Fig. 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 localised immediately 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, whilst 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.

2. Aggrecan-degrading enzymes

Aggrecan is a large proteoglycan containing numerous chondroitin sulphate and keratan sulphate 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.

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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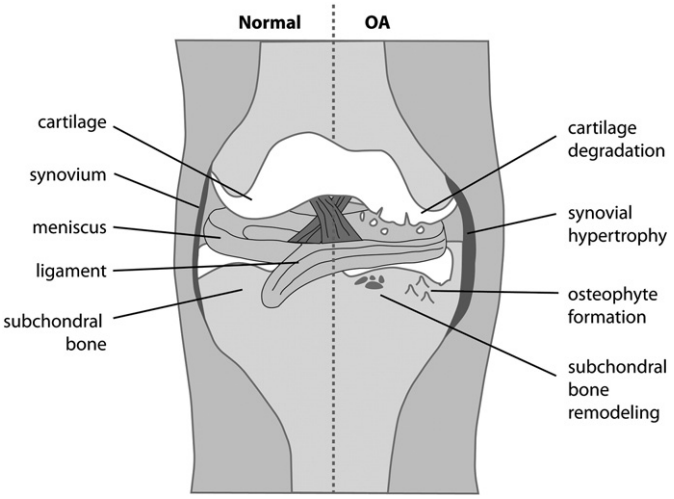

Fig. 1. Cartoon representation of normal and osteoarthritic joint. OA is characterised by changes to various tissues within synovial joints. The cartilage matrix is degraded by collagenases and aggrecanases, leading to fibrillation and subsequent loss of the articulating cartilage surface. Synovial fibroblasts 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.

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 whilst 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 et al. [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] (Fig. 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].

3. 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, whilst 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], whilst *Mmp13*$^{-/-}$ mice are protected in a surgical OA model [65]. MMP-1 also efficiently cleaves type II collagen