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Proteases and Cartilage Degradation in Osteoarthritis

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1. Introduction

Osteoarthritis, the most common joint disease, affecting millions people world-wide, involves the degradation of the articular cartilage which provides frictionless contact between the bones in a joint during movement. To a first approximation, this tissue is composed of two components, a collagen framework and entrapped proteoglycans. The framework consists of type II collagen fibrils built on a type XI collagen core, and decorated with type IX collagen molecules and small proteoglycans. These composite fibrils give the tissue its integrity, tensile strength and ability to retain large proteoglycan aggregates. The extremely large size of the proteoglycan aggregates and their high negative charge endows them with an immense hydration capacity, giving cartilage the ability to absorb compressive loading by the slow displacement of bound water. Partial destruction or loss of the proteoglycans is the first step in the deterioration of cartilage as seen in arthritis. Subsequently, irreversible loss of collagen occurs leading to permanent cartilage degeneration. While glycosylhydrolases and free radicals could also participate, it is believed that proteolytic enzymes are the main agents responsible for the degradation of cartilage components in osteoarthritis. Currently two classes of proteases are thought to be the major mediators of collagen and proteoglycan cleavage. Collagen degradation was thought to be majorly due to the action of MMP (matrix metalloproteinase) collagenases while members of both MMP and ADAMTS (**a** **d**isintegrin **a**nd **m**etalloproteinase with **t**hrombo**s**pondin motifs) families are important mediators of the degradation of proteoglycans which due to their extended core protein conformation are susceptible to the action of many proteases (Mort and Billington, 2001). Recently however, there is increasing evidence for the role of the cysteine protease cathepsin K in collagen degradation in articular cartilage (Konttinen et al., 2002).

The cleavage of cartilage proteins often occurs at specific sites on these molecules depending on the particular protease mediating the event. This results in the generation of characteristic N- and C-terminal epitopes that can be used for the production of antibodies specific for these cleavage products (anti-neoepitope antibodies) (Mort et al., 2003). A series of such antibodies has been produced and their specificities validated. These allow evaluation of the roles of different proteases in the degradation of collagen and proteoglycans in mouse models of osteoarthritis and in human and equine osteoarthritic cartilage using immunohistochemical methods and immunoassays.

2. Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of functionally and structurally related zinc endopeptidases that cleave proteins of the extracellular matrix, including collagens, elastin, matrix glycoproteins and proteoglycans (Martel-Pelletier et al., 2001) and are considered to be responsible for much of the degeneration of articular cartilage.

Most MMPs are composed of three distinct domains: an amino-terminal propeptide involved in the maintenance of enzyme latency; a catalytic domain that binds zinc and calcium ions and a hemopexin-like domain that is located at the carboxy terminal zone of the protease and that plays a role in substrate binding (Nagase, 1997). All MMPs are synthesized as preproenzymes and most of them are either secreted from the cell or bound to the plasma membrane in an inactive or proenzyme state. Several proteolytic cleavages are required to activate them and are critical steps leading to extracellular matrix breakdown (Nagase, 1997). Most of the MMPs are optimally active at neutral pH (Martel-Pelletier et al., 2001).

The human genome codes for 24 MMPs which can be classified depending on which components of the cartilage matrix they degrade (Birkedal-Hansen et al., 1993; Lee and Murphy, 2004). The MMPs that are the most important in cartilage extracellular matrix degradation are the collagenases (MMP-1, -8 and -13), the stromelysins (MMP-3, -10 and -11) the gelatinases (MMP-2 and –9), matrilysin (MMP-7) and the membrane type MMPs, in particular MMP-14 which can also act as a collagenase (Nagase and Woessner, 1999).

2.1 Collagenases

Matrix metalloproteinases with collagenolytic abilities are termed collagenases. These proteases mediate the initial cleavage of the collagen triple helix, occurring at three quarters of the distance from the amino-terminal end of each chain, forming collagen fragments of three-quarter and one-quarter length (Harris and Krane, 1974) (Fig.1). This site is susceptible to cleavage due to a reduced proline and hydroxyproline content which results in lowering of the stability of the triple helix. The collagenases are able to unwind this region of the triple helix and cleave all three collagen strands (Chung et al., 2004). This initial cleavage allows other MMPs to further degrade these unwound collagen molecules (Burrage et al., 2006). There are 3 collagenases: collagenase-1 or interstitial collagenase (MMP-1); collagenase-2 or neutrophil collagenase (MMP-8); and collagenase-3 (MMP-13). In addition, MMP-2 and MMP-14 also have the ability to cleave triple helical collagen.

2.1.1 Collagenase-1 (MMP-1)

Collagenase-1, which is primarily produced by synoviocytes (Wassilew et al., 2010), has been found in increased concentration in synovial fluid of patients suffering from joint injuries and osteoarthritis (Tchetverikov et al., 2005). It can also degrade aggrecan and different types of collagen: type I, II, III, VII, X, IX and denatured type II (Martel-Pelletier et al., 2001; Poole et al., 2001). This collagenase preferentially degrades type III collagen and its expression is mainly found in the superficial zone of articular cartilage in well-established osteoarthritis (Freemont et al., 1997). Even though its affinity towards type II collagen is lower than for collagenase-3, it is found in higher concentration in osteoarthritic joints (Vincenti and Brinckerhoff, 2001). *In vitro* studies showed that human chondrocytes can produce significantly more collagenase-1 than collagenase-3 following stimulation with proinflammatory cytokines, namely TNF-α and IL-1 (Yoshida et al., 2005).

Fig. 1. Cleavage sites on type II collagen.
The type II collagen triple helix and non-helical telopeptides are indicated schematically. In reality there are many more turns in the triple helix. The ¾ / ¼ cleavage site for collagenases and the cleavage site for cathepsin K towards the N-terminus (Kafienah et al., 1998) are indicated along with the peptide sequences used to produce anti-neoepitope antibodies for the cleavage products. Asterisk indicates modification of proline to hydroxyproline.

2.1.2 Collagenase-2 (MMP-8)

Collagenase-2, which is mainly the product of neutrophils, degrades type I collagen with high specificity, but also cleaves collagen type II, III, VIII, X, aggrecan and link protein (Poole, 2001). It has been shown that collagenase-2 protein and mRNA are also produced by normal human chondrocytes (Cole et al., 1996), though recent data show that mRNA expression is very minor in normal and osteoarthritic chondrocytes (Stremme et al., 2003). Collagenase-2 is able to cleave the aggrecan molecule at the aggrecanase-site, between Glu^{373}-Ala^{374}, but cleaves preferentially between Asn^{341}-Phe^{342}, the MMP-site (Fosang et al., 1994) (Fig. 2).

2.1.3 Collagenase-3 (MMP-13)

Collagenase-3 was first cloned from human breast carcinoma in 1994 (Freije et al., 1994). It is predominantly a product of chondrocytes (Reboul et al., 1996) and has been shown to be expressed in human osteoarthritic cartilage (Mitchell et al., 1996), subchondral bone and hyperplasic synovial membrane in an osteoarthritis mouse model (Salminen et al., 2002). This collagenase is mostly expressed by chondrocytes surrounding osteoarthritic lesions (Shlopov et al., 1997) and can be found in superficial (Wu et al., 2002) and deep layers of osteoarthritic cartilage (Freemont et al., 1999; Moldovan et al., 1997). Matrix metalloproteinase-13 expression is strongly induced by interleukin-1 (IL-1), an important proinflammatory cytokine encountered in osteoarthritis (Gebauer et al., 2005; Vincenti and Brinckerhoff, 2001). Collagenase-3 degrades type II collagen preferentially, but also cleaves collagens type I, III, VII and X, aggrecan and gelatins (Poole et al., 2001). *In vitro* studies have shown that MMP-13 can cleave type II collagen about 5 times faster than type I collagen and about 6 times faster than type III collagen (Knäuper et al., 1996). Because type II collagen is its preferred substrate and because it can cleave type II collagen a least 5 to10 times faster than collagenase-1, collagenase-3 is considered to be one of the most important MMPs in osteoarthritis (Mitchell et al., 1996). It is also the collagenase with the most efficient gelatinolytic activity (Knäuper et al., 1996).
Many different *in vivo* studies have shown the importance of MMP-13 in osteoarthritis. Administration of specific MMP-13 inhibitors to animal models of osteoarthritis has shown a significant reduction in the severity of the pathology (Baragi et al., 2009; Johnson et al., 2007; Settle et al., 2010). Its importance in osteoarthritis was demonstrated, in a transgenic

mouse line expressing constitutively active human MMP-13 in hyaline cartilage where excessive MMP-13 expression resulted in articular cartilage degradation and joint pathology similar to osteoarthritis (Neuhold et al., 2001). Recently, MMP-13 knockout mice have been developed and surgical induction of osteoarthritis by destabilisation of the medial meniscus in these animals demonstrated that structural cartilage damage is dependent on MMP-13 activity (Little et al., 2009).

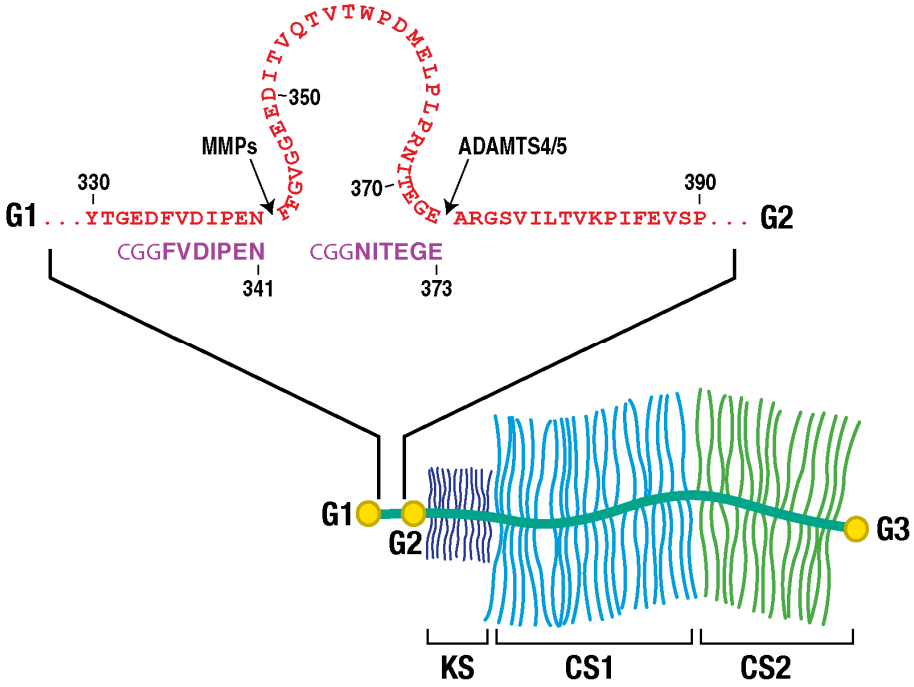

Fig. 2. Peptides used to generate anti-neoepitope antibodies to metalloproteinase cleavage products of aggrecan in the interglobular domain.
The domain structure of the aggrecan molecule is illustrated. The core protein (green) consists of two globular domains (G1 and G2) separated by an interglobular domain. A region rich in keratan sulfate (KS) follows along with two extended chondroitin sulfate rich regions (CS1 and CS2) which are substituted with glycosaminoglycan chains (blue). The CS1 region consists of a series of tandem repeats which can vary in number (Doege et al., 1997). The interglobular domain is susceptible to proteolytic attach. The sites of cleavage by MMPs and aggrecanases are indicated along with the sequences of peptides used to prepare anti-neoepitope antibodies which recognize the new C-termini of the G1-containing fragments that remain in the tissue following cleavage.

2.2 Gelatinases

Gelatinases are proteases that can further degrade denatured collagen, once the triple helix has been cleaved by collagenases. There are two gelatinases: gelatinase-A also termed 72 kDa or MMP-2 and gelatinase-B also termed 92 kDa or MMP-9.

2.2.1 Gelatinase-A (MMP-2)

Gelatinase-A degrades FACIT (fibril-associated collagens with interrupted triple helices) (Gordon and Hahn, 2010) collagens such as type IV collagen in the basement membrane and is a very efficient gelatinase degrading denatured fibrillar collagens and aggrecan (Poole, 2001). Gelatinase-A is mostly important in the completion of collagen degradation after specific cleavage of the triple helical region of fibrillar collagen molecules by collagenases (Nagase, 1997). This enzyme also cleaves the aggrecan molecule at the Asn^{341}-Phe^{342} site close to the G1 domain (Fosang et al., 1992) (Fig.2) and is mostly expressed in late stage osteoarthritis (Aigner et al., 2001).

It has been shown that in the horse, several joint cells, like chondrocytes and synovial fibroblasts, can produce gelatinase-A *in vitro* (Clegg et al., 1997a) and that the enzyme activity is increased in synovial fluid of joints of animals suffering from osteoarthritis (Clegg et al., 1997b). The activity of gelatinase-A was found to be increased in synovial fluid and synoviocytes of dogs with osteoarthritis, but was also detected in healthy joints (Volk et al., 2003). Recently, it has be shown that gelatinase-A deficiency in humans causes a disorder characterized by osteolysis and arthritis termed multicentric osteolysis with arthropathy, a disease that can be reproduced in gelatinase-A knockout mice (Mosig et al., 2007). Even if this enzyme seems to be implicated in the pathogenesis of osteoarthritis, it also plays a direct role in skeletal development.

2.2.2 Gelatinase-B (MMP-9)

Gelatinase-B has similar activities to MMP-2 but it can also act as an elastase. Though involved in collagen destruction, its collagenase action is at a very much lower level than that of gelatinase-A (Soder et al., 2006). Gelatinase-B can also cleave the aggrecan molecule at the same site as gelatinase-A, the Asn^{341}-Phe^{342} site (Fosang et al., 1992) (Fig. 2). This enzyme has been found in synovial fluid of humans (Koolwijk et al., 1995) and horses (Clegg et al., 1997b) with osteoarthritis, and its activity is increased in synovial fluid and synoviocytes of dogs (Volk et al., 2003) suffering from the same disease. Equine chondrocytes are also able of producing gelatinase-B *in vitro* (Clegg et al., 1997a).

2.3 Stromelysins

There are three stromelysins: stromelysin-1 or MMP-3, stromelysin-2 or MMP-10 and stromelysin-3 or MMP-11.

2.3.1 Stromelysin-1 (MMP-3)

Stromelysin-1 can degrade aggrecan, denatured collagens and interhelical collagen domains, as well as aggrecan and link protein. Importantly, stomelysin-1 can cleave the aggrecan molecule at the MMP site, at the Asn^{341}-Phe^{342} bond, to liberate the G1 domain from the remainder of the molecule (Flannery et al., 1992) (Fig.2). It has been shown that stromelysin-1 can activate the pro forms of collagenases and that this activation is a key step in cartilage degradation (Suzuki et al., 1990). In osteoarthritic cartilage, stromelysin-1 is localized in chondrocytes of the superficial and transition zone (Okada et al., 1992) and its strongest mRNA expression is found in early degenerative articular cartilage (Bau et al., 2002). In a rabbit model of surgically induced osteoarthritis, stromelysin-1 was found to be upregulated in the synovium initially, and in chondrocytes in the later phases of the disease (Mehraban et al., 1998), indicating that both cell types can produce stromelysin-1. It has been shown that in humans, the plasma level of stromelysin-1 was a significant predictor of joint space narrowing in knee osteoarthritis

(Lohmander et al., 2005). The concentration of this enzyme in human joint fluid can distinguish disease joints form healthy joints (Lohmander et al., 1993a). Another indication of the action of stromelysin-1 in the development of osteoarthritis is the significant decrease in severity of joint pathology in 2-year-old MMP-3 knockout mice (Blaney Davidson et al., 2007)

2.3.2 Stromelysins-2 and -3 (MMP-10 and MMP-11)

Stromelysin-2 has similar activities to MMP-3. This stromelysin can also activate procollagenases, and has been identified recently in synovial fluid and tissues from osteoarthritis patients, demonstrating the importance of this protease in articular cartilage degradation processes (Barksby et al., 2006).

Stromelysin-3 has been more implicated in general proteolysis, and shown to be up-regulated in osteoarthritic chondrocytes (Aigner et al., 2001). Unlike other MMPs, stromelysin-3 is activated intracellularly by the serine protease, furin, which processes many other proteins into their mature/active forms. MMP-11 is then secreted from cells in its active form (Pei and Weiss, 1995).

2.4 Other MMPs

Matrilysin (MMP-7), the smallest of the MMPs, lacking a hemopexin domain, is a protease that degrades aggrecan, gelatin, type IV collagen and link protein. Matrilysin cleaves the aggrecan molecule at the MMP-site (Fosang et al., 1992) and is mainly expressed in the superficial and transitional zones of osteoarthritic chondrocytes (Ohta et al., 1998). Matrilysin is the MMP with the highest specific activity against many extracellular matrix components (Murphy et al., 1991) and can also activate the zymogens of MMP-1 and MMP-9 (Imai et al., 1997).

There are six membrane-type matrix metalloproteinases (MT-MMPs) (Nagase and Woessner, 1999). Only MT1-MMP and MT3-MMP have been implicated in osteoarthritis (Burrage et al., 2006). The most important is MT1-MMP (MMP-14), expressed in human articular cartilage (Büttner et al., 1997) and synovial membrane. It degrades aggrecan, but also collagen type I, II, III and gelatin. It has been shown that MT1-MMP is highly expressed in osteoarthritic cartilage and could be responsible for the activation of progelatinase A in the extracellular matrix (Imai et al., 1995).

3. Aggrecanases

Aggrecanases are members of the '**A** **D**isintegrin **A**nd **M**etalloproteinase with **T**hrombo**s**pondin motifs' (ADAMTS) family of proteins. Synthesized as inactive pre-proenzymes, the ADAMTSs have a catalytic domain containing a zinc binding motif with 3 histidine residues, HEXXHXXGX-XH, and a critical methionine residue located in a 'Met-turn' downstream of the third zinc-binding histidine (Kuno et al., 1997). The propeptide is removed by the action of the proprotein convertase proteases furin (Koo et al., 2007) or PACE-4 (Malfait et al., 2008). Currently there are 19 ADAMTS genes known in humans, numbered ADAMTS-1 to ADAMTS-20, the same gene product being described as ADAMTS-5 and ADAMTS-11 (Porter et al., 2005).

The degradation of aggrecan leads to articular cartilage softening and loss of fixed charges (Maroudas, 1976). Two major cleavage sites of the aggrecan molecule are situated in the IGD region of the core protein, allowing aggrecan molecules lacking the G1 domain to freely exit the cartilage matrix and so to no longer contribute to cartilage function (Sandy et al., 1991). The first cleavage site at the Asn^{341}-Phe^{342} bond, creating the neoepitope VDIPEN, was found to be

generated by MMPs (Flannery et al., 1992; Fosang et al., 1991; Fosang et al., 1992). The second site at the Glu^{373}-Ala^{374} bond, creating the NITEGE neoepitope, was found to result from aggrecan cleavage by enzymes that were called aggrecanases (Sandy et al., 1991). There are 4 other aggrecanase cleavage sites situated in the GAG rich region (CS2) of aggrecan molecules between the globular domains G2 and G3 (Glu^{1545}-Gly^{1546}, Glu^{1714}-Gly^{1715}, Glu^{1819}-Ala^{1820}, and Glu^{1919}-Leu^{1920}, human sequences) (Tortorella et al., 2000) and a fifth cleavage site closer to the G3 domain that has been identified recently in bovine cartilage (Durigova et al., 2008). It was shown that aggrecan cleavage at the aggrecanase sites is responsible for cartilage degradation, *in vitro*, (Malfait et al., 2002; Tortorella et al., 2001) and, *in vivo*, ((Janusz et al., 2004), and that aggrecan neoepitopes generated by aggrecanases are found in synovial fluids of patients suffering from osteoarthritis (Lohmander et al., 1993b; Sandy et al., 1992). Moreover, it was also shown that contrary to MMP-inhibitors, aggrecanase inhibitors can block aggrecan degradation in human osteoarthritic cartilage (Malfait et al., 2002), demonstrating the importance of aggrecanases in cartilage matrix destruction.

The ongoing search for activities responsible for cartilage matrix degradation indicates that the ADAMTS family members are the most important aggrecanases. Of all of the ADAMTS enzymes, the phylogenetically closely related ADAMTS-1, -4, -5, -8, -9, -15 and -20 (Collins-Racie et al., 2004) are considered to be potential aggrecanases. All of the ADAMTS messenger RNAs except ADAMTS-7 were found to be present normal and/or osteoarthritic cartilage from hip or knee joints (Collins-Racie et al., 2004; Kevorkian et al., 2004; Naito et al., 2007). They have been shown to be able to cleave the aggrecan molecule at the Glu^{373}-Ala^{374} bond, except for ADAMTS-20 for which this cleavage site has not been tested to date (Collins-Racie et al., 2004; Rodríguez-Manzaneque et al., 2002; Somerville et al., 2003; Tortorella et al., 2000; Tortorella et al., 2002). The only 3 ADAMTSs that have been shown to be able to cleave aggrecan at the 4 aggrecanase sites located in the GAG rich region are ADAMTS-1, -4 and -5 (Rodríguez-Manzaneque et al., 2002; Tortorella et al., 2002), making them potent aggrecanases.

3.1 Aggrecanase-1 (ADAMTS-4)

Aggrecanase-1 has been well studied and evidence for its importance in aggrecan catabolism in cartilage is becoming stronger. ADAMTS-4 protein has been shown to be co-localized with aggrecan degradation products *in vitro* and *in vivo* (Naito et al., 2007). Selective inhibition of ADAMTS-4 and ADAMTS-5 has been shown to block the degradation of type II collagen by its protective effect on aggrecan molecules (Pratta et al., 2003). However, even if ADAMTS-4 has been shown to be able to cleave the aggrecan molecule *in vitro* (Tortorella et al., 2001), studies carried out with ADAMTS-4 knockout mice failed to show a protection against aggrecan loss after destabilizing knee surgery (Glasson et al., 2005). A similar study by Stanton et al. showed that, *in vitro*, ADAMTS-4 expression is not induced by IL-1α in mice suggesting that ADAMTS-4 may not be an important aggrecanase in osteoarthritis in mice (Stanton et al., 2005). However, in human osteoarthritis, ADAMTS-4 seems to play an important role in aggrecan degradation. In fact, this aggrecanase is induced in human cartilage, *in vitro*, by proinflammatory cytokines (Song et al., 2007), and is increased in osteoarthritic cartilage (Naito et al., 2007; Roach et al., 2005).

3.2 Aggrecanase-2 (ADAMTS-5)

ADAMTS-5 has also been well studied and its importance in aggrecan catabolism in cartilage has been shown. As mentioned for ADAMTS-4, selective inhibition of ADAMTS-

4 and ADAMTS-5 has been shown to have a protective effect on aggrecan molecules (Pratta et al., 2003). Studies carried out with ADAMTS-5 knockout and ADAMTS-4/-5 double knockout mice showed that these animals are more resistant to cartilage degradation after destabilizing knee surgery (Glasson et al., 2005; Majumdar et al., 2007; Stanton et al., 2005). In vitro, ADAMTS-5 expression is induced by IL-1α in mice, demonstrating its importance in osteoarthritis in that species (Stanton et al., 2005). ADAMTS-5 is also important in osteoarthritis in humans, its expression is high in human osteoarthritic cartilage and it is responsible for aggrecan degradation in normal and diseased cartilage (Bau et al., 2002; Plaas et al., 2007; Song et al., 2007). However, in the human, putative damaging polymorphisms in the ADAMTS-5 gene did not show any modification in susceptibility to osteoarthritis (Rodriguez-Lopez et al., 2008). The search for the most important aggrecanase in human osteoarthritis is still going strong (Fosang and Rogerson, 2010).

3.3 ADAMTS-1

ADAMTS-1 mRNA and protein are present in normal and OA cartilage (Kevorkian et al., 2004). This enzyme can cleave aggrecan at the Glu^{373}-Ala^{374} bond and at 4 additional aggrecanase sites between G2 and G3 (Rodríguez-Manzaneque et al., 2002). Concerning the expression of ADAMTS-1 in inflammatory conditions, ADAMTS-1 expression in articular chondrocytes is downregulated *in vitro* by human recombinant interleukin-1β (IL-1β) (Wachsmuth et al., 2004). An ADAMTS-1-KO mouse (Mittaz et al., 2004) showed that overall, ADAMTS-1 does not seem to be a key enzyme in normal and diseased cartilage, or in bone development and growth (Little et al., 2005).

4. Cathepsins

While the triple helical regions of the fibrillar collagens such as types I and II are resistant to the action of most proteases except the MMP collagenases (Nagase and Fushimi, 2008) which make an initial cleavage at the three quarter point, the cysteine protease, cathepsin K, is also able to degrade triple helical collagens (Garnero et al., 1998). Rather, this protease appears to erode the collagen fibrils from their termini, gradually reducing the chains to peptides with concomitant unwinding of the triple helix. Unlike the MMPs, cathepsins are single domain proteases which do not rely on additional modules to bind to their extracellular matrix substrates (Turk et al., 2001). However, the collagenolytic activity of cathepsin K is dependent on the presence of chondroitin 4-sulfate CS (Li et al., 2000) a major component of the aggrecan molecule which forms well-defined complexes with the enzyme (Cherney et al., 2011). While it was originally assumed that cathepsin K is unique to the osteoclast (and this cell does indeed contain huge amounts of the protease), many other cell types are now known to produce the enzyme (Anway et al., 2004; Sukhova et al., 1998). Its increasing abundance in chondrocytes close to the articular surface (Konttinen et al., 2002) suggests that its action may contribute to cartilage fibrillation seen with aging and joint disease.

5. Anti-neoepitope antibodies

The anti-cleavage site (anti-neoepitope) antibody approach has proven very productive as a means of detecting specific cleavage products in the extracellular matrix, thus demonstrating

the action of one or a particular group of proteases (Mort et al., 2003; Mort and Buttle, 1999). In addition, since these cleavage products can accumulate in body fluids – synovial fluid, blood or urine – their quantitation can represent a measure of disease activity.
Our work has centered on aggrecan fragments generated by the action of MMPs and aggrecanases (ADAMTS family members, particularly ADAMTS-4 and -5) (Hughes et al., 1995; Sztrolovics et al., 2002) (Fig. 2) and on collagen cleavage epitopes generated by the action of collagenases (Billinghurst et al., 1997; Lee et al., 2009; Song et al., 1999) as well as the degradation of collagen in cartilage by cathepsin K (Dejica et al., 2008; Vinardell et al., 2009) (Fig. 1).

6. Immunohistochemical demonstration of protease action in cartilage

Anti-neoepitope antibodies can be used to demonstrate the effects of increased MMP activities in articular cartilage. This is illustrated in sections of joints of mice lacking the endogenous MMP inhibitor, tissue inhibitor of metalloproteinases-3 (TIMP-3). *Timp3*$^{-/-}$ mice are phenotypically normal, although old animals show some lung pathology (Leco et al., 2001) (Fig.3). However, detailed examination of the articular cartilage of adult animals demonstrates a decrease in glycosaminoglycan content (weaker Safranin O staining) and damage to the articular surface. Compared to wild type animals, there is a dramatic increase in the staining of the articular cartilage with an anti-VDIPEN antibody (Lee et al., 1998) which recognizes the G1 domain of aggrecan that remain located in the tissue following cleavage by MMPs. Although the aggrecanase cleavage site in mouse aggrecan generates the G1 terminating in the sequence …NVTEGE rather than …NITEGE, the antibody raised to the human epitope is fully functional with the mouse epitope and can be used to investigate the role of aggrecanases in cartilage degeneration in animal models of arthritis (van Lent et al., 2008).

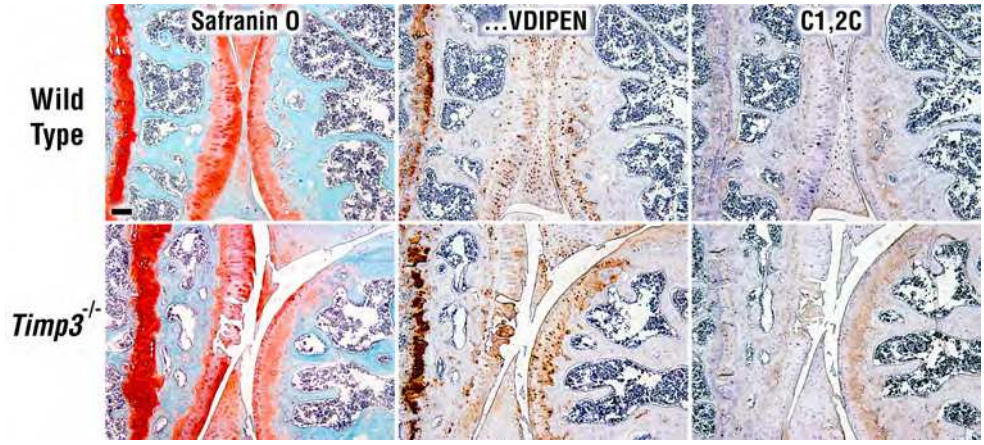

Fig. 3. Effect of increased MMP activity in mouse cartilage.
Hind joint sections of wild type and *Timp3*$^{-/-}$ 1-year-old FVB mice. Paraffin embedded samples were stained with Safranin O and Fast Green which identifies areas of fixed negative charge, or incubated with rabbit antibodies to either VDIPEN or the collagen epitope C1,2C, followed by a secondary horse radish peroxidase coupled system. Intense staining of the growth plate is visible on the left of the sections for glycosaminoglycans (Safranin O) and for the VDIPEN epitope indicating normal turnover of aggrecan. The magnification bar represents 100 µm.

Staining for the cleavage product for type II collagen by collagenases (the C1,2C epitope, Fig. 1) was also increased in the joints from *Timp3*$^{-/-}$ animals (Fig. 3) illustrating the broad inhibitory potential of TIMP-3.

Recently we have generated an antibody which is able to recognize and quantitate a cleavage product of type II collagen generated on the cleavage of the triple helical region by the action of cathepsin K (Dejica et al., 2008). Immunohistochemical studies demonstrated regions of cartilage reflecting cathepsin K activity (Fig. 4). Staining was dramatically increased in cartilage taken from osteoarthritis patients compared to that obtained from individuals with macroscopically normal tissue. The cleavage products are localized towards the articular surface in similar sites to those identified as due to the action of MMP collagenases as determined using the polyclonal antibody C1,2C which recognizes the C-terminal neoepitope of the 3/4 cleavage fragment (Wu et al., 2002). These areas of collagen degradation co-localize with the sites rich in cathepsin K (Konttinen et al., 2002; Vinardell et al., 2009).

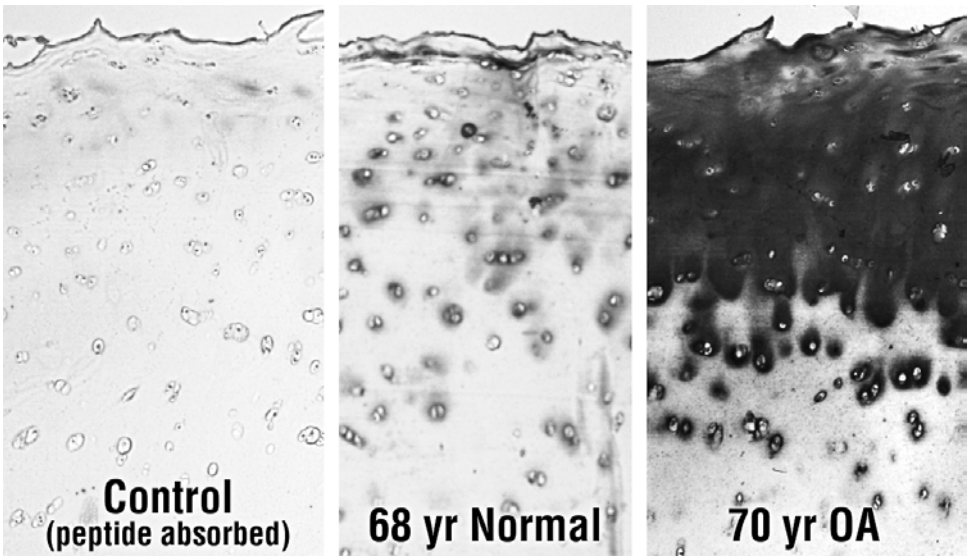

Fig. 4. Localization of cathepsin K generated type II cleavage products in cartilage from normal individuals and osteoarthritis (OA) patients.
Frozen sections were treated with chondroitinase ABC to remove glycosaminoglycans and stained using a rabbit antibody raised against the C2K epitope and a horse radish peroxidase labeled second step system. The reaction product was silver enhanced (Gallyas and Merchenthaler, 1988). A control section where the first step antibody was absorbed with the immunizing peptide is included.

The C2K epitope can be released from the tissue by digestion with chymotrypsin and quantitated using a competitive ELISA. Using this approach we demonstrated increased levels of cathepsin K-generated type II collagen fragments in cartilage from osteoarthritis patients relative to normal individuals. In addition, when cartilage was maintained in organ culture for two weeks in the presence of a specific cathepsin K inhibitor, a

reduction in the levels of this epitope was observed, indicating that relatively short periods of cathepsin K activity produce detectable levels of this epitope (Dejica et al., 2008).

Together these results indicate that in addition to its critical role in bone resorption (Brömme and Lecaille, 2009; Tezuka et al., 1994), cathepsin K acts along with the MMPs and ADAMTS family members in the destruction of cartilage in osteoarthritis.

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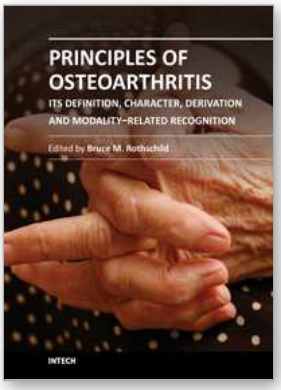

Principles of Osteoarthritis- Its Definition, Character, Derivation and Modality-Related Recognition

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This volume addresses the nature of the most common form of arthritis in humans. If osteoarthritis is inevitable (only premature death prevents all of us from being afflicted), it seems essential to facilitate its recognition, prevention, options, and indications for treatment. Progress in understanding this disease has occurred with recognition that it is not simply a degenerative joint disease. Causative factors, such as joint malalignment, ligamentous abnormalities, overuse, and biomechanical and metabolic factors have been recognized as amenable to intervention; genetic factors, less so; with metabolic diseases, intermediate. Its diagnosis is based on recognition of overgrowth of bone at joint margins. This contrasts with overgrowth of bone at vertebral margins, which is not a symptomatic phenomenon and has been renamed spondylosis deformans. Osteoarthritis describes an abnormality of joints, but the severity does not necessarily produce pain. The patient and his/her symptoms need to be treated, not the x-ray.

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