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DRUG DISCOVERY

Can allosteric inhibitors of adamts4 and adamts5 prevent osteoarthritis disease progression?

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ABSTRACT

The success of targeted biologic therapy against rheumatoid arthritis and other inflammatory conditions has led to much interest in the pathophysiology of osteoarthritis, in the hope of defining novel therapeutic targets. One of the most important of these targets have been the ADAMTS4 and ADAMTS5 aggrecanases, enzymes that are essential for the early breakdown of aggrecan, a key step in the pathophysiology of osteoarthritis. This article wills overview recent research regarding ADAMTS activity, specificity and regulation, with particular regard to strategies for inhibiting these enzymes, and the clinical development of ADAMTS inhibitors as disease modifying compounds for osteoarthritis. Specific inhibition of either ADAMTS aggrecanase is an attractive option for OA disease modification. In a murine model of OA, ADAMTS5 is clearly the dominant aggrecanase, but in spontaneous human OA, the dominant aggrecanase is yet to be defined. The normal function of the ADAMTS aggrecanases is not fully known, but since ADAMTS5 plays an important role in versican turnover and myofibroblast differentiation, systemic inhibition of this enzyme is clearly not an option, due to unacceptable side effects. Various strategies for intraarticular ADAMTS inhibition are still realistic, although there may well be discrepancies between murine and human OA pathophysiology.

Keywords:
ADAMTS4; ADAMTS5; aggrecan; aggrecanase; cartilage; osteoarthritis; synovium

1. INTRODUCTION

The degradation of cartilage aggrecan was for many years believed to involve only matrix metalloproteinases (MMPs). The first report of a series of new N-terminal amino acid sequences of aggrecan catabolites released from cartilage explants exposed to interleukin (IL)-1 was published in 1991 (Manigila et al., 1991). The predominant aggrecan catabolite in the synovial fluid from patients with arthritis corresponded with the major aggrecan catabolite from cartilage explants treated with IL-1: a cleavage of the aggrecan core protein at the Glu373 – Ala374 peptide bond of human aggrecan, within the interglobular domain (IGD) of this molecule (Sandy et al., 1992; Lohmander et al., 1993). The enzymatic activity responsible for this novel form of cleavage of cartilage aggrecan was first referred to as 'aggrecanase' (Hardingham and Fosang, 1995). Aggrecanase activity was first shown to be soluble and diffusible in 1997 (Hughes et al., 1997).

The first member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family with aggrecanase activity was purified and cloned in 1999 (Tortorella et al., 1999). It had aggrecanase activity, as defined by its ability to cleave aggrecan within the IGD, at the Glu373 – Ala374 peptide bond. This protease was called aggrecanase-1, or ADAMTS4. Soon after, another aggrecanase, with a similar specificity for the Glu373 – Ala374 peptide bond, was identified and named aggrecanase-2 or ADAMTS5/11 (Abbaszade et al., 1999; Hurskainen et al., 1999). At least four other ADAMTS proteases (ADAMTS1, 8, 9 and 15) with aggrecanase activity have been described, but ADAMTS4 and ADAMTS5 remains the most important of them, and are prime candidates to being the major aggrecanase in human cartilage, and thus playing an important role in various forms of arthritis, osteoarthritis (OA) in particular (Lin and Liu, 2010; Fosang and Rogerson, 2010).

The notion that inhibiting aggrecanase activity might be beneficial in OA dates back at least ten years. In 2005, it was demonstrated that in a murine model of OA, deletion of active ADAMTS5 prevented cartilage degradation (Glasson et al., 2004; Glasson et al., 2005; Stanton et al., 2005). Although conclusive data is lacking that ADAMTS5 is the dominant aggrecanase also in human OA, this has been widely presumed to be the case. Work on developing small molecule ADAMTS4 and ADAMTS5 inhibitors was ongoing for some time (Gilbert et al., 2011; Deng et al., 2012), but met with problems with regard to their systemic effects on the metabolism of aggrecan and other matrix macromolecules in healthy tissues. Instead, there have two studies of recombinant monoclonal antibodies to ADAMTS5. Intra-articular administration of one of these [CRB0017, Rottapharm] was capable of reducing disease progression in a murine model of spontaneous OA (Chiusaroli et al., 2013). Systemic administration of the other [GSK2394002, GlaxoSmithKline] also inhibited progression of joint damage in a murine model of surgically induced OA, but it proved to have unacceptable cardiovascular side effects in monkeys (Miller et al., 2014; Larkin et al., 2014).

This article will summarize the available data on ADAMTS inhibition as a potential disease-modifying stratagem in OA, with particular regard to strategies involving allosteric inhibitors.

2. ACTIVITY AND SPECIFICITY OF ADAMTS4 AND ADAMTS5

ADAMTS4 and ADAMTS5 belong to the ADAMTS subgroup of the ADAM family of proteinases. Their protein domain structure (Figure 1) comprises a prepro domain, a catalytic domain, a disintegrin-like domain, a thrombospondin motif, a cysteine-rich domain, and a spacer domain without cysteine residues; ADAMTS5 has an additional C-terminal thrombospondin motif. Within the pro domain of ADAMTS4, there is a potential for a cysteine switch (Cys 194 in human, Cys 190 in mouse) to activate the enzyme; in ADAMTS5, the potential cysteine switch is at Cys209 both in human and in mouse. ADAMTS4 also contains a single cleavage site for furin, at residue 209-121 in human and at residue 205-208 in mouse, and the prodomain of human and murine ADAMTS5 also contains three potential furin recognition sites.

The crystal structures of ADAMTS4 and ADAMTS5 were recently published, either in complex with a bound inhibitor, or with the enzymes in apo form. The structure shows that the non-ligated and inhibitor-bound enzymes display different catalytic site configurations: a nonbinding, closed form and an open, binding form (Mosyak et al., 2008). It would appear as if ADAMTS4 and ADAMTS5 exist in at least two isoforms, of which only one is proteolytically active.

The activity of ADAMTS4 can be regulated through N-terminal processing, through C-terminal processing, or through glycosylation. It is synthesized as a latent proenzyme and retained in its inactive state by interactions between its pro and catalytic domains. Furin or furin-like propeptide convertases have major roles in activating ADAMTS4 (Wang et al., 2004; Tortorella et al., 2005). The activity of ADAMTS4 can also be modulated through C-terminal modification, in particular the removal of its spacer domain. Its aggrecanase activity can be affected either through a direct effect on enzyme activity, or through disrupting its binding to the extracellular matrix (Gao et al., 2004; Kashiwagi et al., 2004; Hashimoto et al., 2004). In IL-1-stimulated osteoarthritic chondrocytes, ADAMTS4 activity is inhibited by calcium pentosan polysulphate (Takizawa et al., 2008). There is also evidence that

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mannosylation on its first thrombospondin motif might affect the activity or secretion of ADAMTS proteases, possibly also ADAMTS4 (Wang et al, 2009).

The only known ADAMTS4 splice variant was described in human OA synovium, using an oligonucleotide primer pair designed to amplify across the exon 8/9 junction of human ADAMTS4 (Wainwright et al., 2006). This alternatively spliced transcript of ADAMTS4 is missing 161 base pairs from the 5' end of exon 9. The splice-variant protein produced would thus lack the spacer domain and have different C-terminus lacking homologies with the normal human ADAMTS4 C-terminal spacer domain (Figure 1). This protein would lose functions dependent on its spacer domain, like substrate and matrix binding and inhibition through fibronectin. The ADAMTS4 splice variant has been demonstrated to function as an aggrecanase, and to cleave other proteoglycan substrates. It is expressed *in vivo* in human OA synovium, and may well be a major contributor to the loss of aggrecan from the superficial zone of OA cartilage (Wainwright et al., 2013).

Although originally characterized as the proteases that cleaves the Glu373-Ala374 bond of the aggrecan core protein, ADAMTS4 and ADAMTS5 are capable of cleaving a number of other proteoglycans, including biglycan, brevican, versican, decorin, keratocan and fibromodulin, as well as glycoproteins like fibronectin and carboxymethylated transferrin (Gendron et al., 2007). Both ADAMTS4 and ADAMTS5 have a broad optimal activity at pH 7.0-9.5, and require the presence of calcium for their activity. The aggrecanase activity of ADAMTS4 is highly dependent on the NaCl concentration, with an optimum between 12.5 and 50 mM NaCl, and a rapid drop above 50 mM. At 150 mM NaCl, only 20% of maximal activity could be detected, and there was none at all at or above 200 mM. ADAMTS4 activity against non-aggrecan substrates, was only marginally affected by the NaCl concentration. In contrast, ADAMTS5 has a broad optimum between 150 and 250 mM NaCl, and thus it appears likely that under normal human physiological conditions, ADAMTS5 would have higher aggrecanase activity than ADAMTS4 (Gendron et al., 2007).

3. REGULATION OF ADAMTS4 AND ADAMTS5

Results from human cartilage, chondrocytes and synovium agree that ADAMTS4 mRNA can be upregulated by interleukin (IL)-1 or tumour necrosis factor (TNF)α (Bau et al., 2002; Song et al., 2007; Bondeson et al., 2006). In contrast, ADAMTS5 appears to be constitutive in these cells (Bau et al., 2002; Bondeson et al., 2006; Bondeson et al., 2007; Bondeson et al., 2008). The most important endogenous inhibitor of ADAMTS4 and ADAMTS5 is TIMP-3, which potently inhibits these enzymes at biological concentrations, whereas the other TIMPs do not (Hashimoto et al., 2001; Kashiwagi et al., 2001). This may well be modulated by interactions between aggrecan and the C-terminal domain of the ADAMTSs (Wayne et al., 2007). Reactive-site mutants of the N-terminal inhibitory domain of TIMP-3, also inhibit ADAMTS4 and ADAMTS5 (Lim et al., 2010). ADAMTS4 activity is blocked when the C-terminal domain of fibronectin binds to the ADAMTS4 spacer domain, but enzyme inhibition can be relieved by C-terminal processing of ADAMTS4 to displace fibronectin or fibronectin fragments (Wang et al., 2004).

In models of cultured bovine and porcine chondrocytes or cartilage explants, ADAMTS4 is induced following stimulation with IL-1, tumour necrosis factor (TNF)α, oncostatin M or transforming growth factor β (Bau et al., 2002; Tortorella et al., 2001; Pratta et al., 2003; Yamanishi et al., 2002). In another study, ADAMTS4 gene expression could be upregulated through treatment with either IL-1β, TNFα or oncostatin M. There was an additive effect of combination treatment with oncostatin M and either IL-1β or TNFα in these systems, leading to marked induction of ADAMTS4 gene expression and also some induction of ADAMTS5 (Song et al., 2007). In OA synovium or cartilage, aggrecanase activity and ADAMTS4 expression is present constitutively, without any requirement for any catabolic stimulation (Ilic et al., 2000; Song et al., 2007). In tendon, aggrecanase activity also appears to be constitutive (Rees et al., 2007). In RA synovial fibroblasts, ADAMTS4, but not ADAMTS5, is upregulated by IL-6 (Mimata et al., 2012).

In another study, cultures of synovial cells from digested OA synovium were either left untreated, incubated with the p75 TNF soluble receptor Ig fusion protein etanercept (Enbrel), incubated with a neutralizing anti-IL-1β antibody, or incubated with a combination of Enbrel and anti-IL-1β. ADAMTS4 mRNA was significantly ($p<0.05$) inhibited by Enbrel, and more potently ($p<0.01$) inhibited by a combination of Enbrel and the neutralizing anti-IL-1β antibody. This would indicate that in the human OA synovium, the upregulation of ADAMTS4 is dependent on TNFα and IL-1 produced by the synovial macrophages. In contrast, ADAMTS5 mRNA was not affected by neutralization of either of these cytokines (Bondeson et al., 2006).

In contrast to this data from human, porcine and bovine models indicating that ADAMTS4 mRNA responds to IL-1, there are two papers indicating that this is not the case in murine cells. In mouse femoral head explant cultures, ADAMTS4 mRNA levels were unaffected by IL-1 (East et al., 2007). Monocytes from wild-type mice, but not monocytes from IL-1 deficient mice, did induce ADAMTS5 mRNA in chondrocytes without affecting ADAMTS4, again suggesting that murine ADAMTS4 is unresponsive to IL-1 (Zwerina et al., 2007).

Three papers agree that the upregulation of ADAMTS4 induced by IL-1 or TNFα is NFκB dependent (Bondeson et al., 2007; Bondeson et al., 2008; Seguin et al., 2006). ADAMTS4 has three NFκB binding sites on its 5' flanking region

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that are conserved between species. Mutation of either of these resulted in the loss of the IL-1 response to an ADAMTS4 gene luciferase reporter vector, indicating that the IL-1-induced upregulation of ADAMTS4 gene transcription depends on two or more NFκB binding sites in the 5' flanking region of this gene (Bondeson et al., 2008). In bovine nucleus pulposus tissue, TNFα treatment induced upregulation of aggrecanase activity, ADAMTS4 in particular, in a NFκB dependent manner (Seguin et al., 2006). In human OA synovial fibroblasts, treatment with IL-1 or TNFα resulted in upregulation of ADAMTS4 expression. In this model, adenoviral gene transfer of the endogenous inhibitor IκBα was used to specifically inhibit NFκB without affecting other signalling pathways or causing apoptosis. The ADAMTS4 induction by IL-1 or TNFα was potently inhibited by NFκB downregulation (Bondeson et al., 2007). These three papers (Bondeson et al., 2007; Bondeson et al., 2008; Seguin et al., 2006) suggest that, in various relevant *in vitro* systems, the upregulation of ADAMTS4, induced by either IL-1 or TNFα, is NFκB dependent.

ADAMTS5 appears to be the antithesis of ADAMTS4 with regard to its regulation. ADAMTS5 activity is reduced by C-terminal processing, whereas ADAMTS4 activity is maintained or enhanced (Fosang et al., 2008). In human cartilage and synovium, ADAMTS5 is constitutive, leaving ADAMTS4 as the inducible aggrecanase, responding to IL-1 and TNFα in a NFκB dependent manner (Bondeson et al., 2008; Fosang et al., 2008). Data from murine chondrocytes indicated that in these cells, there may well be profound differences with regard to the regulation of ADAMTS4 and ADAMTS5, the latter being the inducible aggrecanase (Fosang et al., 2008; Fosang and Rogerson, 2010). This suggests that data obtained from murine models of OA should be carefully interpreted when inflammation and ADAMTS4/5 are implicated.

4. ADAMTS4 AND ADAMTS5 IN DISEASE AND HEALTH

The proteolytic degradation of aggrecan, a major macromolecular component of articular cartilage, is a key pathophysiological event in OA. It has long been debated which of the two classical aggrecanases ADAMTS4 and ADAMTS5 is responsible for OA pathology. It has been demonstrated that in knockout mice, ADAMTS5 is critical for the development of surgically induced OA, whereas ADAMTS4 is not. In fact, mice lacking ADAMTS4 develop normally and are affected by surgically induced OA in a similar manner as wild-type mice. Mice lacking ADAMTS5 are protected from cartilage breakdown in this model of surgically induced OA (Glasson et al., 2004; Glasson et al., 2005; Stanton et al., 2005).

Thus, it has been clearly demonstrated that in a murine model of OA, ADAMTS5 is the dominant aggrecanase, whereas ADAMTS4 appears to be relatively unimportant. Some degree of caution is called for when extrapolating these results to human beings, however. The murine proteome contains 641 proteases, the human one only 565, perhaps indicating an increased level of redundancy in protease function in the mouse. There are also differences in the regulation of ADAMTS4; the human, but not the murine, ADAMTS4 gene responds to IL-1 stimulation (Bau et al., 2002; Song et al., 2007; East et al., 2007; Zwerina et al., 2007). Studies in human OA synovium and cartilage have indicated that ADAMTS4 is the inducible aggrecanase, whereas ADAMTS5 is constitutive (Bondeson et al., 2008); in mice, the opposite may well be true (Fosang et al., 2008).

Disappointingly little is known about the relative role of ADAMTS4 and ADAMTS5 in idiopathic human OA. ADAMTS5 mRNA is more abundant than ADAMTS4 mRNA in both normal and OA cartilage (Bau et al., 2002; Kevorkian et al., 2004). ADAMTS5 mRNA levels are not upregulated in OA cartilage, as compared to normal human cartilage, however (Bau et al., 2002; Kevorkian et al., 2004; Naito et al., 2007), whereas ADAMTS4 mRNA levels have been reported to correlate with the progression of OA in humans (Naito et al., 2007). A possible explanation for this might be that the levels of low-density lipoprotein receptor-regulated protein-1, the main receptor that regulates ADAMTS5 endocytosis and degradation, are reduced in OA cartilage (Yamamoto et al., 2013). A study using a small interfering RNA approach could demonstrate that both ADAMTS4 and ADAMTS5 contribute to the aggrecanase activity in human cartilage explants (Song et al., 2007). Immunohistochemistry in human RA synovium has shown that ADAMTS4 and ADAMTS5 are localized mainly in the sublining layer of the RA synovium (Mimata et al., 2012).

Apart from their involvement in arthritis, ADAMTS4 and ADAMTS5 are likely to play a role in several other diseases. The expression and degradation of versican may well play a role in atherosclerosis. ADAMTS4 can be produced by a macrophage-like cell line, and it is expressed in macrophage-rich areas of human atherosclerotic plaques. In a mouse model, ADAMTS4 was overexpressed during the development of atherosclerosis (Wågsäter et al., 2008). ADAMTS4 has also been implicated in the development of neurodegenerative diseases, including Alzheimer's disease, and ADAMTS4 protein levels is overexpressed in multiple sclerosis lesions (Haddock et al., 2006). There has also been speculation concerning the potential role of ADAMTS4 and ADAMTS5 in malignant disease (Rocks et al., 2008). In malignant gliomas, there is interest in ADAMTS4 as a therapeutic target, since inhibition of this enzyme may well result in reduced invasiveness of this tumour (Held-Feindt et al., 2005).

ADAMTS4 and ADAMTS5 gene expression has been detected in a vast array of tissues, including heart, lung, skeletal muscle, liver, brain, placenta, tendon, cartilage and synovium. Yet, surprisingly little is known about the normal function of these enzymes. ADAMTS4-null mice have been reported to be fully fertile and have a normal

phenotype, as have ADAMTS5-null mice, and even mice with double deletion of these aggrecanases. ADAMTS5 has been reported to play a role in chondrogenesis or osteogenesis, but ADAMTS5-null mice do not exhibit skeletal or growth plate abnormalities (Glasson et al., 2005; Stanton et al., 2005). Later studies have indicated that ADAMTS5-null mice have reduced apoptosis and decreased versican cleavage in the interdigital webs, however (McCulloch et al., 2009), and that ADAMTS5-null mice have impaired contraction and dermal collagen deposition in an excisional wound healing model (Velasco et al., 2011). ADAMTS5 is known to have versicanase activity, and is colocalized with versican in arterial muscle cells, meaning that inhibition of ADAMTS5 is likely to cause accumulation of versican in the cardiovascular system (Hattori et al., 2011). Since versican is an important proteoglycan that plays a role in cell adhesion and proliferation, and the assembly of the extracellular matrix, this finding has far-reaching implications. ADAMTS5 has also been reported to affect myoblast fusion, and thus skeletal muscle function (Stupka et al., 2013).

5. STRATEGIES OF INHIBITING ADAMTS4 AND ADAMTS5

Already in the 1990s, there was optimism with regard to the use of various broad-spectrum matrix metalloproteinase (MMP) inhibitors as disease-modifying agents in OA. They were effective in both mouse and guinea-pig models of the disease, but in humans, these non-specific MMP inhibitors caused what is known as the musculoskeletal syndrome, with painful joint stiffening and adhesive capsulitis (Hutchinson et al., 1998). Selective inhibitors of MMP-13, which may well be the dominant collagenase in OA, have been reported to be in early phase clinical development as disease-modifying drugs for OA (Baragi et al., 2009).

Several pharmaceutical companies (Wyeth/Pfizer, Schering-Plough, Rottapharm SpA, Alantos Pharm, Japan Tobacco) have patented small-molecule inhibitors of ADAMTS4 and ADAMTS5. Some of these compounds are claimed to be specific for either ADAMTS4 or ADAMTS5, whereas others have effect also against both these enzymes, other ADAMTS members, or even against MMPs (Gilbert et al., 2011; Deng et al., 2012). The Wyeth/Pfizer aggrecanase inhibitor AGG-523 was used in a phase I clinical trial in osteoarthritis (Gilbert et al., 2011), but has not been taken further. Nor have the other small-molecule ADAMTS inhibitors entered any further clinical development as potential disease-modifying anti-osteoarthritic drugs, due to the realization that the normal function of these enzymes in primates is too complex for systemic inhibition of their activity to be feasible.

Following the great success of targeted biologic therapy in RA, directed against TNFα and other targets, there was interest in developing similar therapeutic strategies for OA. A study of the monoclonal antibody CRB0017 [Rottapharm], directed against the spacer domain of ADAMTS5, showed that in a spontaneous murine OA model in STR/ort mice, intra-articular administration of this antibody significantly prevented disease progression in a dose-dependent manner (Chiusaroli et al., 2013). There was no comparison with systemic administration, nor was it assessed to what degree the antibody leaked from the synovial space.

Another study used systemic administration of the anti-ADAMTS5 antibody GSK2394002 in a model of surgically induced murine OA. Whereas an anti-ADAMTS4 antibody had no effect on disease progression, although it bound specifically to ADAMTS4 within cartilage, GSK2394002 demonstrated both structural disease modification and alleviation of pain-related behaviour (Miller et al., 2014). Due to the available data on the normal function of ADAMTS5 in vascular and fibroblast proteoglycan processing, the effect of GSK2394002 on cardiovascular physiology was carefully monitored in cynomolgus monkeys. A single administration of the antibody caused increased mean arterial pressure, and ST elevations on the ECG indicating cardiac ischemia, effects that were sustained for up to 8 months after administration of the single dose (Larkin et al., 2014). These side effects were considered too formidable to allow for further clinical development of GSK2394002.

Theoretically, there are four methods of inhibiting ADAMTS4 or ADAMTS5: to mutate aggrecan to render it non-degradable by the aggrecanase, to block the aggrecan cleavage site, to make use of an antibody or other inhibitor that binds to the aggrecanase, or to introduce a null-aggrecanase isoform that binds to aggrecan without cleaving it (Figure 2). In mice, introduction of an aggrecanase-resistant mutant aggrecan has been used in a model of OA, where it protected the animals from cartilage loss (Little et al., 2007), but the use of a similar strategy in humans would not be possible. Since aggrecan is of course much more abundant inside the joint than either of the main aggrecanases, strategies directed against the aggrecanase are more attractive. The results discussed above (Larkin et al., 2014) show that in primates, systemic administration of an ADAMTS5 inhibitor is not feasible, most probably due to the role of this enzyme in the normal turnover versican in the cardiovascular system. Local administration of the antibody or null-aggrecanase isoform would appear to be the more attractive option, but even so, care must be taken to minimize leakage of the inhibitor into the systemic circulation.

6. DISCUSSION

The introduction of targeted biologic therapy of inflammatory disease, i.e. rheumatoid arthritis (RA), other arthritides, inflammatory bowel disease and psoriasis, has been a major breakthrough in modern medicine. The development of the anti-TNFα strategies for rheumatic disease were reliant on *in vitro* data from human RA synovial cell cocultures,

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along with *in vivo* data from the murine collagen-induced arthritis model, indicating that these strategies had considerable efficacy against inflammatory arthritis in the mouse, and that they were safe to use (Williams et al., 1992; Williams, 2007). It is notable that some early papers using experimental arthritis in the rat indicated a proportionally greater importance of interleukin-1 in those animals (Bendele et al., 1999). Still, in human beings, anti-TNFα therapy proved a clinical revolution: it has been quoted as a triumph of modern drug development, from applied basic science via animal models and clinical trials into widespread and lucrative clinical use (Maini and Feldmann, 2007; Feldmann, 2009).

The revolution in drug discovery for RA, with not only anti-TNF biologics, but also effective anti-B cell, anti-IL-6 and anti-T cell co-stimulation drugs gaining prominence, has led to more energetic work in the struggle to identify therapeutic targets in other chronic diseases, OA not excluded. None of the older drug candidates, including diacerein, doxycycline, licifelone, risedronate and strontium ranelate, could be clearly demonstrated to have clinical benefit in OA (Hellio Le Graverand-Gastineau, 2010; Bondeson, 2011); nor have the widely available 'nutraceuticals' glucosamine and chondroitin sulphate been proven to affect either joint pain or joint space narrowing in human OA (Wandel et al., 2010). Since there is no doubt that macrophage-produced cytokines produced by the inflamed synovium play a role in driving inflammatory and destructive pathways in OA (Bondeson et al., 2010), there have been several clinical trials of anti-TNFα biologics in OA, but they do not appear to be efficacious in established hand or knee OA, particularly in patients without evidence of active synovitis (Bondeson, 2011). A number of other potential therapeutic targets were defined, one of them the inducible nitric oxide synthetase (iNOS). In murine joint instability-induced OA, iNOS-deficient mice developed significantly less OA lesions that wild-type mice, with 50% reduction of both osteophytes and cartilage lesions (van den Berg et al., 1999). In a canine model of OA induced by joint instability, treatment with the oral selective iNOS inhibitor cindunistat [SD-6010, Pfizer] led to significant reduction of OA lesions. Still, a recent clinical study of cindunistat in patients with symptomatic osteoarthritis of the knee showed no significant benefit (Hellio Le Graverand et al., 2013). Another potential therapeutic target in OA is fibroblast growth factor-18 [Sprifermin, Merck-Sarono], which promotes cartilage development and repair. In a rat model of OA, intra-articular injections of sprifermin induced a significant, dose-dependent reduction in cartilage degeneration. In a recent clinical study, sprifermin had no effect on medial femorotibial compartment cartilage volume, although it did significantly and dose-dependently reduce the loss of total and lateral femorotibial compartment cartilage volume (Lohmander et al., 2014).

Of the potential therapeutic targets in OA, the ADAMTS4 and ADAMTS5 aggrecanases have obvious theoretical appeal, since they are responsible for a key element in disease progression. The problem appears to be that exploitation of this state of affairs began too early. There is a growing amount of evidence that there are fundamental differences between rodents and primates with regard to the normal function of aggrecanases, their regulation and disease contribution. Although it is known that in crude murine models of OA, ADAMTS5 is the main aggrecanase driving cartilage loss, there is a lack of evidence that this is true in spontaneously occurring human OA; there is a need to identify the dominant aggrecanase. The relative contribution to OA pathology of aggrecanases produced in the synovium and aggrecanases produced in the cartilage also remains to be elucidated, as does the contribution from the newly described splice variant of ADAMTS4.

Recent papers demonstrating that ADAMTS5 plays an important role in myofibroblast differentiation, and in versican turnover within the cardiovascular system, demonstrate that the normal function of this enzyme is of considerable importance (Hattori et al., 2011; Stupka et al., 2013). The full details about the normal function of both ADAMTS4 and ADAMTS5 is yet to be revealed, but it may well be that both these enzymes are important players in the turnover of not only aggrecan, but also various small proteoglycans, in a variety of tissues. In particular, ADAMTS5 plays an important role in versican turnover, and a recent study has undermined the credibility of systemic ADAMTS5 inhibitors as potential disease modifying agents in OA (Larkin et al., 2014). Systemic inhibition of ADAMTS4 has not been investigated, but is likely to have similar safety concerns with regard to long-term treatment. Intra-articular administration of allosteric ADAMTS inhibitors remains an intriguing possibility, however. There is a need to further assess the relative contributions of ADAMTS4 and ADAMTS5 in human OA, and animal studies may well provide serious problems here, due to the suspicion of differential roles for aggrecanases in rodents and primates. Of the different strategies of ADAMTS inhibition, the intra-articular administration of a null-aggrecanase has obvious appeal, since here we have a mode of blocking the cleavage of aggrecan at the IGD, and thus preventing a key early pathophysiological step in OA. The obvious safety concern for such a strategy would be that the null-aggrecanase would also affect the intra-articular metabolism of various small proteoglycans, and also the risk that small amounts of the null-aggrecanase would enter the systemic circulation. The use of an allosteric inhibitor to block the IGD cleavage site of aggrecan would have several advantages, firstly that the controversy with regard to the relative role of ADAMTS4 and ADAMTS5 in human OA would no longer have relevance, secondly that this strategy would not affect the aggrecan-mediated cleavage of small proteoglycans. The relative abundance of aggrecan and aggrecanases would be a potential problem, however. Still, in the hitherto futile search for a disease-modifying drug in OA, specific intra-articular inhibition of aggrecan cleavage remains an intriguing option.

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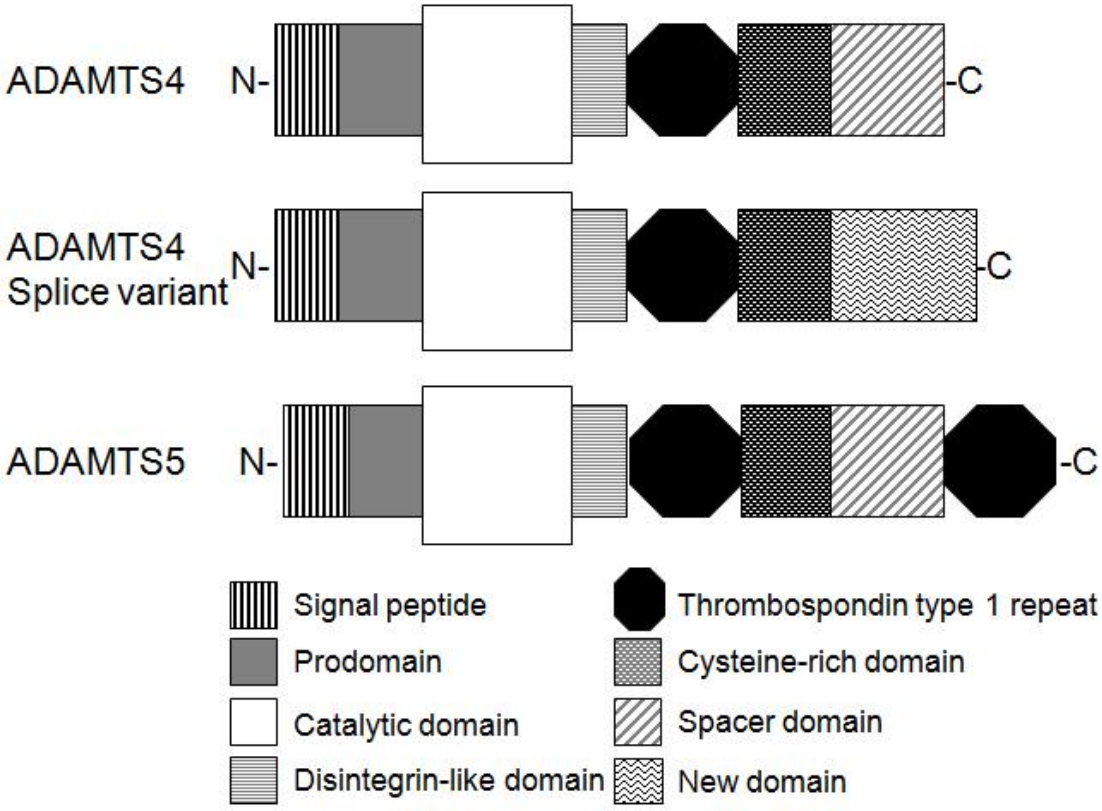

Figure 1

The structure of ADAMTS4, compared with its only known splice variant, and with ADAMTS5

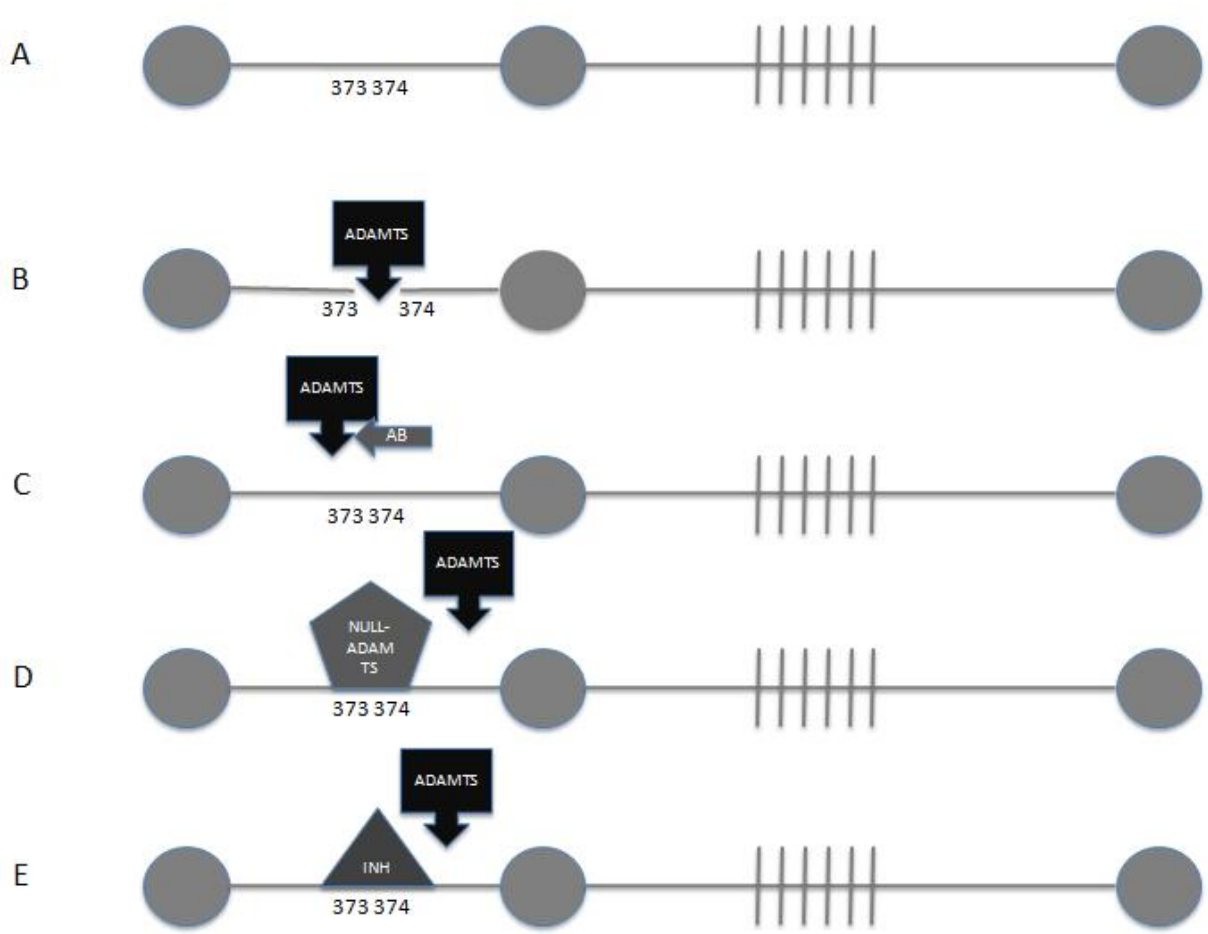

Figure 2

Strategies of aggrecanase inhibition. Aggrecan (A) is cleaved by an ADAMTS aggrecanace (B), and this can be abrogated via an anti-ADAMTS antibody (C), a null-aggrecanase (D) or an inhibitor binding to the IGD cleavage site in aggrecan (E)

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