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Review

Identifying the human aggrecanase

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SUMMARY

It is clear that A Disintegrin And Metalloproteinase with ThromboSpondin motif (ADAMTS)-5 is the major aggrecanase in mouse cartilage, however it is not at all clear which enzyme is the major aggrecanase in human cartilage. Identifying the human aggrecanase is difficult because multiple, independent, molecular processes determine the final level of enzyme activity. As investigators, we have good methods for measuring changes in the expression of ADAMTS mRNA, and good methods for detecting aggrecanase *activity*, but no methods that distinguish the source of the activity. In between gene expression and enzyme action there are many processes that can potentially enhance or inhibit the final level of activity. In this editorial we discuss how each of these processes affects ADAMTS activity and argue that measuring any one process in isolation has little value in predicting overall ADAMTS activity *in vivo*.

Introduction

It is now over a decade since A Disintegrin And Metalloproteinase with ThromboSpondin motif (ADAMTS)-4[1], and ADAMTS-5[2] were identified as aggrecanases, based on their ability to cleave at the $Glu^{373} \downarrow {}^{374}Ala$ bond in the aggrecan interglobular domain (IGD). Since then ADAMTS-1, -8, -9, -15, -16 and -18 family members have also been identified as aggrecan-degrading enzymes *in vitro*, but none have seriously challenged ADAMTS-4 and ADAMTS-5 as the candidate human aggrecanases because these have substantially greater aggrecan-degrading activity *in vitro*. ADAMTS-4 and -5 are expressed in human cartilage and in many cases their expression levels are increased in joint disease, and at sites of localised aggrecan loss. The results of a recent genome-wide association study revealed eight polymorphisms in the ADAMTS-5 gene, two of which caused amino acid changes. However neither polymorphism was associated with difference in susceptibility to osteoarthritis (OA) in a large cohort of patients[3].

It is not clear whether ADAMTS-4 or ADAMTS-5 is the aggrecanase in human cartilage. Indeed there is evidence suggesting that both aggrecanases have key roles in degrading aggrecan *in vivo*[4]. Identifying the human aggrecanase is a major challenge because the final level of enzyme activity is determined by multiple, independent and interacting molecular processes, including promoter activity, epigenetic modifications, regulation by non-coding RNA (ncRNA) including microRNA (miRNA), and alternative splicing. At the protein level, ADAMTS activity is determined by the summed effect of prodomain processing, C-terminal processing, the availability and binding of endogenous enhancers or inhibitors, and the extent of glycosylation on both the aggrecan substrate and the enzymes themselves (Fig. 1). We herein discuss how each of these processes affects ADAMTS activity and argue that measuring any one parameter in isolation is an inadequate surrogate for determining the identity of the human aggrecanase.

Regulating ADAMTS-4 and -5 mRNA expression

Apart from activity studies that tell us a great deal about aggrecanases collectively, the most abundant studies on human ADAMTS-4 and -5 are those that have analysed mRNA expression either *in vitro* with and without catabolic stimuli, or immediately *ex vivo*, from normal or OA cartilage. Most[4–8] but not all[9,10] the *in vitro* studies in human cartilage or chondrocytes found that ADAMTS-4 mRNA was induced by catabolic cytokines such as interleukin-1β (IL-1β) or Tumor necrosis factor α (TNFα) and that ADAMTS-5 mRNA was not regulated by cytokines but expressed constitutively. These findings differ from most animal studies which show that ADAMTS-5 mRNA expression is upregulated by catabolic cytokines (reviewed in Ref. 11).

The absence of ADAMTS-4 and -5 in early OA gene profiling studies[12–14] suggests that on a global scale, these enzymes are lowly expressed. Other expression studies focussing specifically on

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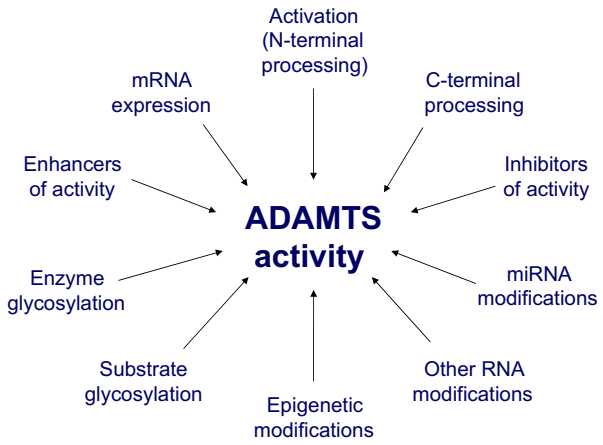

Fig. 1. Aggrecanase activity is determined by multiple molecular processes.

cartilage proteinases show that ADAMTS-5 is more highly expressed than ADAMTS-4 in both normal and OA cartilage[5,15]. Some studies report increased expression of both ADAMTS-4 and ADAMTS-5 in knee cartilage from patients with late, but not early stage OA[5,16]. Other studies report that only ADAMTS-4 mRNA is increased in knee cartilage[7,17] or hips[18] measured at the time of joint replacement, while others report decreases in both ADAMTS-4 and -5 mRNA in patients with late stage hip OA[15]. Given the differences between the aetiopathogenesis of hip and knee OA[19], it is not surprising that ADAMTS expression differs between knee and hip joints in humans[15] and presumably differs also with stage of disease, although this has not been systematically examined.

ADAMTS expression in cartilage represents only one part of the total joint picture, since ADAMTS enzymes are expressed in joint capsule and meniscus, as well as in synovium where ADAMTS expression is particularly well documented[20–28]. In distinct contrast to cartilage, both ADAMTS-4 and -5 are expressed constitutively in synovium and their expression by synovial fibroblasts is not regulated by IL-1 *in vitro*[20,21,29]. Expression profiling studies show that ADAMTS-4 and -5 are significantly decreased in synovium from patients with hip OA, compared with the levels in synovium from patients with neck of femur fractures[26]. A different study using synovium from patients with cruciate ligament injury as the non-arthritic comparator showed that ADAMTS-4, but not ADAMTS-5 was significantly increased in rheumatoid arthritis (RA) patients; however, in this study neither ADAMTS-4 nor ADAMTS-5 expression levels were changed in OA synovium[20]. Not surprisingly, synovial fibroblasts from mouse joints express a different repertoire of aggrecan-degrading activities compared with mouse chondrocytes, based on the pattern of neoepitopes detected by Western blotting[29]. During episodes of synovial hyperplasia it is entirely possible that synovial fibroblasts might be the major source of aggrecanases within the joint, and that synovial-derived enzymes mediate aggrecanolysis in cartilage[29]. There is less literature available on aggrecanase activity in the meniscus, however one study has shown that mechanical compression of meniscal tissue *in vitro* up-regulates ADAMTS-5 mRNA and down-regulates ADAMTS-4 mRNA expression[23]. The effect of theses changes on aggrecanase activity in the meniscus is not known.

ADAMTS mRNA expression and activity can be directly regulated by transforming growth factor-β (TGF-β)[6,20] and indirectly modulated by fibroblast growth factor-2 (FGF-2), S100A8 and high molecular weight hyaluronan (HA). TGF-β is well known for its anabolic role in matrix metabolism, but TGF-β can also promote joint destruction[30]. TGF-β strongly up-regulates ADAMTS-4 mRNA and protein expression in human synovial fibroblasts, whereas ADAMTS-5 is not regulated by this growth factor[20]. Another growth factor, FGF-2, inhibits IL-1-induced expression of ADAMTS-4 and -5 in primary human chondrocytes without inhibiting IL-1 actions on other target molecules[10]. Conversely, the stress-induced calcium-binding protein, S100A8, enhances IL-1-induced ADAMTS-5 expression in mouse cartilage, resulting in increased NITEGE^{373} immunoreactivity and aggrecan loss[31]. In other studies, high molecular weight HA (Mr~2700 kDa) can decrease IL-1α-induced expression of ADAMTS-4 mRNA and protein, without inhibiting ADAMTS-4 expression directly[8]; the mechanism is unclear. In summary, ADAMTS-4 and -5 mRNA can be up- or down-regulated by a variety of modulators typically found in arthritic joints. However we emphasise that to date, there are no corresponding studies linking increased mRNA expression with increased ADAMTS-4 or ADAMTS-5 activity.

In addition to modulators that are known, or thought, to act *via* intracellular signalling pathways, at least two transcription factors can potentially modify aggrecanase gene expression by direct binding to the ADAMTS-4 or -5 gene promoters. The human ADAMTS-4 promoter has two putative binding sites for the transcription factor nuclear factor of activated T cells-p (NFATp) and eight putative binding sites for the Runx family of transcription factors[32]. Over-expression of either NFATp or Runx2 in cells expressing 4.5 kb of the ADAMTS-4 promoter resulted in transactivation of the ADAMTS-4 promoter[32]. Similarly, analysis of 2.6 kb of the human ADAMTS-5 promoter revealed four putative binding sites for the family of Runx transcriptions factors and Runx2 transactivated the ADAMTS-5 promoter *in vitro*[33]. The *in vivo* influence of Runx2 and NFATp on aggrecanase activity *via* the ADAMTS-4 or ADAMTS-5 promoter is unknown.

ADAMTS-4 and -5 regulation by alternative splicing, epigenetics and ncRNA

There are other pre-translational mechanisms for modulating aggrecanase activity independently of mRNA expression; these include alternative splicing of the ADAMTS transcripts, regulation by ncRNAs including miRNA and gene silencing (or unsilencing) by epigenetic modifications to DNA and histones.

An alternatively spliced transcript of ADAMTS-4 has been identified in synovium from OA patients[27]. The splice variation occurs between exons 8 and 9 to create a longer protein which diverges from wildtype ADAMTS-4 near the start of the spacer region, after Arg^{696}. There is no information on the abundance of this transcript in synovium, its expression in cartilage and other joint tissues, or its expression in other patient groups. It will be fascinating to determine the efficacy of the ADAMTS-4 splice variant as an aggrecan-degrading enzyme. The splice variant is likely to have altered enzyme activity, given the importance of the spacer region in conferring maximum aggrecanolytic activity on ADAMTS-4[34]. There is no evidence for alternatively spliced transcripts of ADAMTS-5.

The genomes of eukaryotes studied to date are almost entirely transcribed, leading to vast numbers of ncRNAs with unknown functions[35]. These ncRNAs and miRNAs represent a new frontier in the molecular biology of human disease. miRNAs primarily affect gene expression by inhibiting protein translation, without affecting transcription. Much less is known about ncRNAs in terms of actions *in vivo* but they are thought to have important roles in epigenetic, transcriptional and post-transcriptional regulation of gene expression (reviewed in Ref. 35).

It is clear that miRNAs provide important regulatory checks in the immune system and inflammatory reactions, and a 16-miRNA gene signature that is differentially expressed in OA has recently been described[36]. Two independent studies have now reported the