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The role of ADAMTS-7 and ADAMTS-12 in the pathogenesis of arthritis

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SUMMARY

Loss of articular cartilage caused by extracellular matrix breakdown is the hallmark of arthritis. Degradative fragments of cartilage oligomeric matrix protein (COMP) have been observed in arthritic patients. ADAMTS-7 and ADAMTS-12, two members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family, have been associated with COMP degradation *in vitro*, and are significantly overexpressed in the cartilage and synovium of patients with rheumatoid arthritis. Recent studies have demonstrated the importance of COMP degradation by ADAMTS-7 and ADAMTS-12. Specifically, the size of COMP fragments generated by ADAMTS-7 or ADAMTS-12 is similar to that of COMP-degradative fragments seen in arthritic patients. In addition, antibodies against ADAMTS-7 or ADAMTS-12 dramatically inhibit tumor necrosis factor-induced and interleukin-1β-induced COMP degradation in cartilage explants. Furthermore, suppression of ADAMTS-7 or ADAMTS-12 expression using the small interfering RNA silencing approach in human chondrocytes markedly prevents COMP degradation. COMP degradation mediated by ADAMTS-7 and ADAMTS-12 is inhibited by α_2-macroglobulin. More significantly, granulin-epithelin precursor, a newly characterized chondrogenic growth factor, disturbs the interaction between COMP and ADAMTS-7 and ADAMTS-12, preventing COMP degradation by these enzymes. This Review summarizes the evidence demonstrating that ADAMTS-7 and ADAMTS-12 are newly identified enzymes responsible for COMP degradation in arthritis, and that α_2-macroglobulin and granulin-epithelin precursor represent their endogenous inhibitors.

Keywords

ADAMTS; arthritis; COMP; degradation; GEP

INTRODUCTION

Arthritic conditions are characterized by the breakdown of extracellular matrix components and the subsequent loss of articular cartilage and bone. This process is thought to be

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Competing interests

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mediated by excessive proteolytic activity.[1] Cartilage oligomeric matrix protein (COMP), a prominent non-collagenous component of cartilage, accounts for approximately 1% of the wet weight of the tissue.[2] COMP is a 524 kDa disulfide-bonded, multi-domain glycoprotein composed of five 110 kDa subunits (Figure 1). Mutations in the type III or C-terminal globular domain of the human *COMP* gene have been linked to the development of pseudoachondroplasia and multiple epiphyseal dysplasia, which are autosomal-dominant forms of short-limb dwarfism.[3–6]

Fragments of COMP have been detected in the diseased cartilage, synovial fluid, and serum of patients with knee injuries, post-traumatic, primary osteoarthritis (OA) and rheumatoid arthritis (RA).[7,8] As the inhibition of cartilage degradative enzymes should slow or block disease progression, the isolation of these enzymes and their inhibitors is of great interest from both a pathophysiological and a therapeutic standpoint. Purified COMP has been reported to be digested by several matrix metalloproteinases (MMPs) *in vitro*, including MMP-1 (also known as interstitial collagenase-1), MMP-13 (also known as collagenase-3), MMP-3 (also known as stromelysin-1), MMP-9 (also known as gelatinase-B),[9] MMP-19 and MMP-20 (also known as enamelysin).[10] In addition, a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family, ADAMTS-4, was reported to cleave purified COMP *in vitro*.[11] All these assays, however, were performed using an *in vitro* digestion system with higher concentrations of enzymes and substrates than are found in physiological and pathological conditions. COMP degradation *in vivo* is probably mediated, at least in part, by these metalloproteinases, although no relationship between COMP degradation and ADAMTS levels has been found to date.

A functional genomic study that was performed to identify the physiological enzymes responsible for COMP degradation led to the isolation of ADAMTS-7 and ADAMTS-12 as COMP-binding partners.[12,13] Subsequent studies showed that both ADAMTS-7 and ADAMTS-12 were able to digest COMP *in vitro*, and were significantly upregulated in arthritic cartilage and synovium compared with normal control samples.[12,13]

ADAMTS-7 and ADAMTS-12 have a similar domain organization, and form a subgroup within the ADAMTS family (Figure 2). ADAMTS are secreted zinc metalloproteinases with a precisely ordered, modular organization that includes at least one thrombospondin type 1 repeat.[14] This family of enzymes has been shown to be proteolytically active; Table 1 summarizes the known substrates and inhibitors of these proteases. ADAMTS have been implicated in the pathogenesis of several diseases.[15–20] For instance, mutations in *ADAMTS-13* are associated with the development of thrombotic thrombocytopenic purpura, a disease characterized by decreased numbers of circulating platelets.[21] Mutations in the *ADAMTS-2* gene have been implicated in Ehlers–Danlos syndrome type 7C, a genetic condition characterized by defects in collagen synthesis.[22] These mutations have also been observed in bovine dermatopraxis.[22] Similar to ADAMTS-4,[11] ADAMTS-5 (also known as ADAMTS-11) has been associated with the breakdown of cartilage via aggrecan degradation.[23–27]

Several single nucleotide polymorphisms in the *ADAMTS-7* gene have been identified that suggest a strong association with the disease haplotype of keratoconus with cataract.[28]

ADAMTS-7 is expressed in bone, cartilage, synovium, tendon and ligament, all of which contain COMP.[2,29] ADAMTS-7 is also detectable, although at lower levels, in meniscus, skeletal muscle and fat.[12] Interestingly, ADAMTS-7 was identified as one of three high-molecular-weight gelatinase species in the urine of patients with a variety of cancers, including prostate and bladder carcinoma.[30] The observation that ADAMTS-7 has gelatinolytic activity in patients with prostate and bladder tumors suggests a functional role for this protease in tumor growth and invasion.[30]

ADAMTS-12 is one of three genes associated with bronchial hyper-responsiveness.[31] One study showed that the expression of ADAMTS-12 in Madin–Darby canine kidney cells prevented the tumorigenic effects of hepatocyte growth factor by blocking activation of the Ras–mitogen-activated protein kinase signaling pathway, and that this regulation involved the thrombospondin domains of the metalloproteinase.[32] Unlike ADAMTS-7, which does not cleave aggrecan or versican,[33] ADAMTS-12 is able to digest aggrecan *in vitro*.[32] Northern blot analysis of RNA from human adult and fetal tissues demonstrated that ADAMTS-12 transcripts are only detected at considerable levels in fetal lung.[34] However, a real-time polymerase chain reaction (PCR) assay revealed that ADAMTS-12 is detectable in cartilage, synovium, tendon, skeletal muscle and fat.[13] In addition, expression profiling analysis of the ADAMTS family revealed that ADAMTS-12 was significantly upregulated in cartilage from patients with OA compared with normal cartilage.[35]

This Review focuses on the roles of ADAMTS-7 and ADAMTS-12 in the pathogenesis of arthritic disease, including the association between these two metalloproteinases and COMP and their possible involvement in COMP degradation, as well as the inhibition of their activities as a potential therapeutic strategy for the treatment of arthritis.

THE INTERACTION OF ADAMTS-7 AND ADAMTS-12 WITH COMP

To identify the physiological enzymes required for COMP degradation, we performed a functional genetic assay based on the yeast two-hybrid system[36–38] and discovered an enzyme that potentially uses COMP as its substrate. Briefly, we linked the epidermal-growth-factor-like domain of COMP to the Gal4 DNA-binding construct and used this construct as bait to screen a cDNA expression library. We screened approximately 2.5 million clones and identified 21 that activated the three reporter genes. Four positive clones among them contained identical 1,613-bp inserts. Analysis using a basic local alignment search tool revealed that the 1,613-bp insert sequence encoded four thrombospondin type 1 repeats of ADAMTS-7.[12]

The interaction between COMP and ADAMTS-7 was confirmed using an *in vitro* glutathione *S*-transferase (GST) pulldown assay.[12] A purified recombinant ADAMTS-7 C-terminal GST fusion protein (GST7-CT) was immobilized and incubated with purified humanized COMP. GST7-CT, but not purified GST, was shown to bind efficiently to humanized COMP. The *in vivo* interaction between COMP and ADAMTS-7 was verified using a co-immunoprecipitation assay. A specific ADAMTS-7 protein was recognized by anti-COMP, but not control, IgG antibodies, demonstrating that ADAMTS-7 binds

specifically to COMP *in vivo*.[12] Similar protein–protein interactions were employed to demonstrate the binding of COMP to ADAMTS-12.[13]

In order to identify the COMP-binding motif in ADAMTS-7 and ADAMTS-12, constructs were generated that expressed their various deletion mutations.[12,13] Results from filter-based β-galactosidase assays of these mutants showed that four C-terminal thrombospondin repeats of ADAMTS-7 or ADAMTS-12 were required for the interaction between these enzymes and COMP.

Immunostaining for COMP and ADAMTS-7 in human chondrocytes demonstrated that COMP co-localized with ADAMTS-7 predominantly in the cytoplasm and surface of chondrocytes. Co-localization of COMP and ADAMTS-12 in human chondrocytes was observed using the same approach. These findings are in agreement with the interactions detected in the yeast two-hybrid and co-immunoprecipitation assays, and indicate that the membrane binding of ADAMTS-7 and ADAMTS-12 in chondrocytes might be mediated, at least in part, by COMP.

Immunohistochemistry assays were performed on 17.5-day-old embryonic murine limbs to determine whether COMP, ADAMTS-7 and ADMATS-12 show overlapping expression patterns *in vivo*.[12,13] Consistent with previous findings, COMP was expressed both in chondrocytes and osteoblasts. Furthermore, ADAMTS-7 and ADAMTS-12 were highly expressed in the proliferative and pre-hypertrophic zones of growth plate chondrocytes, and were detectable in osteocytes, osteoblasts, periosteum and perichondrium (C-J Liu *et al.*, unpublished data).

COMP-DEGRADING ACTIVITIES OF ADAMTS-7 AND ADAMTS-12

It has been reported that the catalytic domain of ADAMTS-20 produced in transgenic bacteria can digest its substrates *in vitro*.[39] Using a similar method, we purified the catalytic domain of ADAMTS-7 as a GST fusion protein in bacteria. The recombinant catalytic domain of ADAMTS-7 was shown to digest COMP in a time-dependent manner.[12] The COMP-degrading activity of ADAMTS-7 was also demonstrated using the recombinant intact enzyme. Intriguingly, two 51–95 kDa fragments were seen in addition to the predominant 100 kDa fragment produced with the catalytic domain alone, suggesting that recombinant ADAMTS-7 might cleave COMP at more than one site.[12] Furthermore, co-transfection and *in vitro* digestion assays with conditioned medium or purified ADAMTS-12 indicated efficient COMP degradation by this enzyme.[13]

THE IMPORTANCE OF ADAMTS-7 AND ADAMTS-12 CLEAVAGE OF COMP

To elucidate the importance of ADAMTS-7-mediated or ADAMTS-12-mediated COMP degradation *in vivo*, it was necessary to determine whether cartilage from OA patients contained COMP fragments of a similar size to those seen in ADAMTS-7-mediated or ADAMTS-12-mediated COMP digestion *in vitro*. To this end, cartilage explants from six patients with OA were compared with COMP fragments produced by *in vitro* digestion with recombinant ADAMTS-7 or ADAMTS-12 using western blotting with anti-COMP

antibodies. All OA cartilage samples contained abundant fragments of a similar size to those produced by ADAMTS-12 and ADAMTS-7 degradation of COMP (110 kDa).[40]

We then investigated whether tumor necrosis factor (TNF) and interleukin (IL)-1β regulate the expression of ADAMTS-7 and ADAMTS-12. These inflammatory cytokines have previously been shown to induce the expression of a number of metalloproteinases involved in the development and progression of arthritis.[41–43] Human cartilage explants were cultured in the presence of TNF or IL-1β for 1 day, and real-time PCR was performed. Both TNF and IL-1β strongly induced mRNA expression of ADAMTS-7 and ADAMTS-12 compared with untreated tissues.[40] However, in human fetal fibroblasts, only TGF-β has been shown to significantly induce the expression of ADAMTS-12,[34] suggesting that cytokine-mediated induction of ADAMTS-12 expression might be cell-type specific.

To determine whether ADAMTS-7 and ADAMTS-12 are directly involved in COMP degradation induced by TNF and IL-1β, COMP degradation was compared in the absence and presence of antibodies that block ADAMTS-12 and ADAMTS-7 activity.[12] Both cytokines resulted in the expression of 110 kDa COMP fragments, which was dramatically reduced in the presence of anti-ADAMTS-12 or anti-ADAMTS-7 antibodies. In addition, COMP degradation was blocked completely by a combination of these two antibodies, and intact COMP (524 kDa) was observed, indicating that ADAMTS-12 and ADAMTS-7 have important roles in TNF-induced and IL-1β-induced COMP degradation. The importance of these two enzymes in COMP degradation *in vivo* was further verified via small interfering RNA-mediated silencing of ADAMTS-7 and ADAMTS-12 in human chondrocytes.[40]

Of course, a direct correlation between COMP cleavage sites *in vitro* and *in vivo* remains to be determined. Specifically, the importance of ADAMTS-7 and ADAMTS-12 in COMP degradation and arthritis could be more substantially established by the generation of mutant mice lacking ADAMTS-7 or ADAMTS-12 in order to observe their phenotype when challenged in a model of arthritis. Indeed, this approach has previously been used to reveal a primary role for ADAMTS-5 in aggrecan degradation, both in surgically induced OA and in antigen-induced inflammatory arthritis.[18,44]

INCREASED EXPRESSION OF ADAMTS-7 AND ADAMTS-12 IN RA

A quantitative real-time PCR assay was performed using sequence-specific probes and primers for ADAMTS-7 and ADAMTS-12 to determine whether the expression of these enzymes differs in OA and RA cartilage. ADAMTS-7 mRNA was found to be significantly upregulated in RA cartilage (P <0.001), and only slightly upregulated in OA cartilage, whereas ADAMTS-12 mRNA was significantly upregulated in both OA and RA cartilage (P <0.05 and P <0.001, respectively) compared with normal cartilage. These results confirm the findings of a real-time PCR assay that showed no significant differences in ADAMTS-7 expression between normal and OA cartilage (I Clark, personal communication), as well as significant upregulation of ADAMTS-12 in OA cartilage.[35] In addition, altered expression of ADAMTS-7 and ADAMTS-12 both in OA and RA synovium was observed.[12,13]

INHIBITION OF ADAMTS-7 AND ADAMTS-12

α_2-Macroglobulin (α_2M) is a member of the α-macroglobulin family of proteins found in the circulation of a broad range of species.[45] Human α_2M is found at relatively high levels (2–4 mg/ml) in plasma, and is a tetramer of four identical 185 kDa subunits, each of which has an exposed 39-amino-acid 'bait region' that contains cleavage sites for a variety of proteinases.[46,47] Previous reports have shown an association between α_2M and ADAMTS-7,[33] and it is known that α_2M inhibits ADAMTS-4 and ADAMTS-5 by competitive inhibition of cleavage activity by the bait region of ADAMTS-4 and ADAMTS-5.[48] *In vitro* digestion assays demonstrated that α_2M acts as a substrate for ADAMTS-7 and ADAMTS-12, and efficiently protects COMP degradation by these enzymes.[40]

One study reported that granulin-epithelin precursor (GEP; also known as PC-cell derived growth factor, progranulin, proepithelin and acrogranin) is highly expressed in chondrocytes of the musculoskeletal system.[49] Furthermore, COMP directly bound GEP and was shown to enhance GEP-mediated chondrocyte proliferation. GEP was first purified as a growth factor from conditioned tissue culture media in the early 1990s.[50,51] GEP is a 593-aminoacid secreted glycoprotein with an apparent molecular weight of 80 kDa,[52,53] and acts as an autocrine growth factor. GEP contains seven-and-a-half repeats of a cysteine-rich motif. Notably, GEP undergoes proteolytic processing with the liberation of small (~6 kDa) repeat units known as granulins (or epithelins), which retain biologic activity.[54]

The findings that COMP associates with ADAMTS-7 and ADAMTS-12[12,13] and interacts with GEP[49] led to studies to determine whether GEP binds to ADAMTS-7 and ADAMTS-12. Unpublished data revealed that this was the case (Luan Y *et al.*, personal communication). In addition, ADAMTS-7 and ADAMTS-12 are novel GEP-convertases and are involved in the proteolytic processing of GEP, with the liberation of smaller fragments.

Previous reports suggest that GEP is strongly upregulated in the synovium of both OA and RA.[55] In addition, GEP is a potent inhibitor of TNF-induced protease,[56] and a processed granulin (a product of GEP cleavage) functions as an inhibitor of the protease thrombin.[57] These facts, together with the finding that GEP disturbs the interactions between COMP and ADAMTS-7 and ADAMTS-12, led us to question whether GEP functions as a specific inhibitor of COMP degradation by ADAMTS-7 and ADAMTS-12. Co-expression of GEP in the ADAMTS-7-transfected COMP stable line resulted in dose-dependent blockade of COMP degradation by ADAMTS-7 (C-J Liu, unpublished data). Additionally, GEP was shown to prevent ADAMTS-12-associated COMP degradation in an *in vitro* digestion assay (C-J Liu, unpublished data).

These findings, together with the observations that TNF induces ADAMTS-7 and ADAMTS-12 expression[40] and that GEP blocks TNF-induced proteinase,[56] suggest that GEP regulates ADAMTS-7 at two levels: first, GEP inhibits TNF-induced ADAMTS-7 expression, and second, GEP disrupts the association of ADAMTS-7 enzyme and its COMP substrate via a direct protein–protein interaction (Figure 3). Thus, in contrast to α_2M, which

has been shown to inhibit the activity of several enzymes, including ADAMTS-4 and ADAMTS-5,[48,58] GEP may represent a specific natural inhibitor of COMP degradation by ADAMTS-7 and ADAMTS-12.

The inhibition of ADAMTS-7 and ADAMTS-12 or their analogous compounds represents a potential novel approach for the treatment of arthritic disorders. Furthermore, it is conceivable that ADAMTS-7 and ADAMTS-12 might be employed as novel biomarkers for arthritic disease activity.

CONCLUSIONS

In industrialized societies, arthritis is the leading cause of physical disability, increased healthcare usage and impaired quality of life. The impact of arthritic conditions is expected to grow as the population continues to age in the coming decades. The understanding of the precise etiology, pathogenesis and progression of arthritic diseases remains beyond our reach; however, accumulating evidence points to a significant role for proteases in the pathological processes of arthritis.[1] The destruction of the extracellular matrix of articular cartilage and bone in arthritic joints is thought to be mediated by excessive proteolytic activity.[1] Several recent studies suggest that monitoring of COMP levels (in both joint fluid and serum) can be used to assess the risk, presence and progression of arthritis.[61–64] Furthermore, altered COMP distribution patterns and degradation rates have been observed in human OA and RA cartilage.[7,8] Using a functional genomic approach combined with biochemistry and cellular and molecular biology techniques, two novel enzymes that belong to the ADAMTS metalloproteinase family, ADAMTS-7 and ADAMTS-12, have been shown to physically associate with and degrade COMP.[12,13] In addition, α_2M and GEP were identified as substrates of ADAMTS-7 and ADAMTS-12, and can inhibit the degradation of COMP by these enzymes (Figure 3).[40] These findings not only extend our understanding of the degradative events involved in arthritic disorders, but also promise to increase our ability to monitor the biological and physical properties of cartilage extracellular matrix that could be altered by a wide range of disease processes.

Acknowledgments

Owing to space constraints, some of the primary research contributions to the field have not been included.

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REVIEW CRITERIA

Full-text, English-language articles published between 1992 and 2008 were identified by a Medline search; the search was last updated on 23 October 2008. Search terms included “ADAMTS”, “COMP”, and “GEP” cross-referenced with “arthritis”. Emphasis was placed on ADAMTS-7-mediated and ADAMTS-12-mediated COMP degradation, as well as their regulation by their naturally-occurring inhibitors, including GEP.

KEY POINTS

- ADAMTS-7 and ADAMTS-12, two members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family, associate with and degrade cartilage oligomeric matrix protein (COMP) in vitro, and are significantly induced in the cartilage and synovium of patients with rheumatoid arthritis
- The size of COMP fragments generated by ADAMTS-7 or ADAMTS-12 is similar to that of COMP-degradative fragments seen in arthritic patients
- Blockade of ADAMTS-7 and ADAMTS-12 activity by specific antibodies or suppression of ADAMTS-7 and ADAMTS-12 expression by the small interfering RNA silencing approach dramatically inhibits tumor necrosis factor-induced and interleukin-1β-induced COMP degradation
- ADAMTS-7-mediated and ADAMTS-12-mediated COMP degradation is inhibited by α2-macroglobulin
- Granulin-epithelin precursor, a newly identified chondrogenic growth factor, disturbs the interaction between COMP and ADAMTS-7 and ADAMTS-12, and prevents COMP degradation by these enzymes

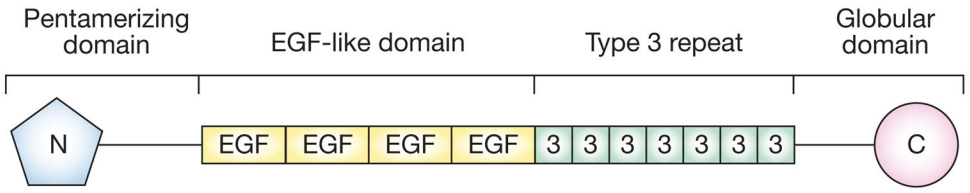

Figure 1.

Domain structure and organization of COMP. N-terminal pentamerizing, EGF-like, type 3 repeat and C-terminal globular domain are indicated. ADAMTS-7, ADAMTS-12 and GEP bind to the EGF domain of COMP. Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; COMP, cartilage oligomeric matrix protein; EGF, epidermal growth factor; GEP, granulin-epithelin precursor.

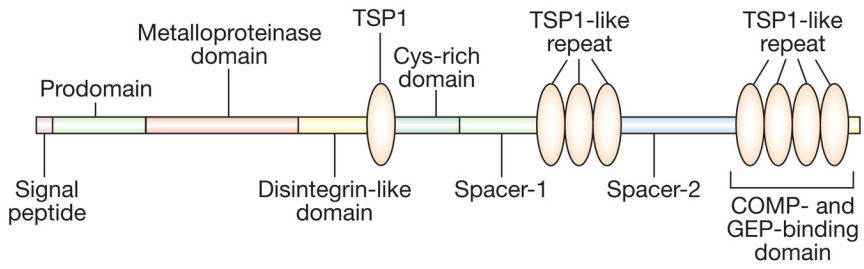

Figure 2.

Domain structure and organization of ADAMTS-7 and ADAMTS-12. The C-terminal COMP-binding and GEP-binding TSP1 motifs are indicated. Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; COMP, cartilage oligomeric matrix protein; GEP, granulin-epithelin precursor; TSP1: thrombospondin 1.

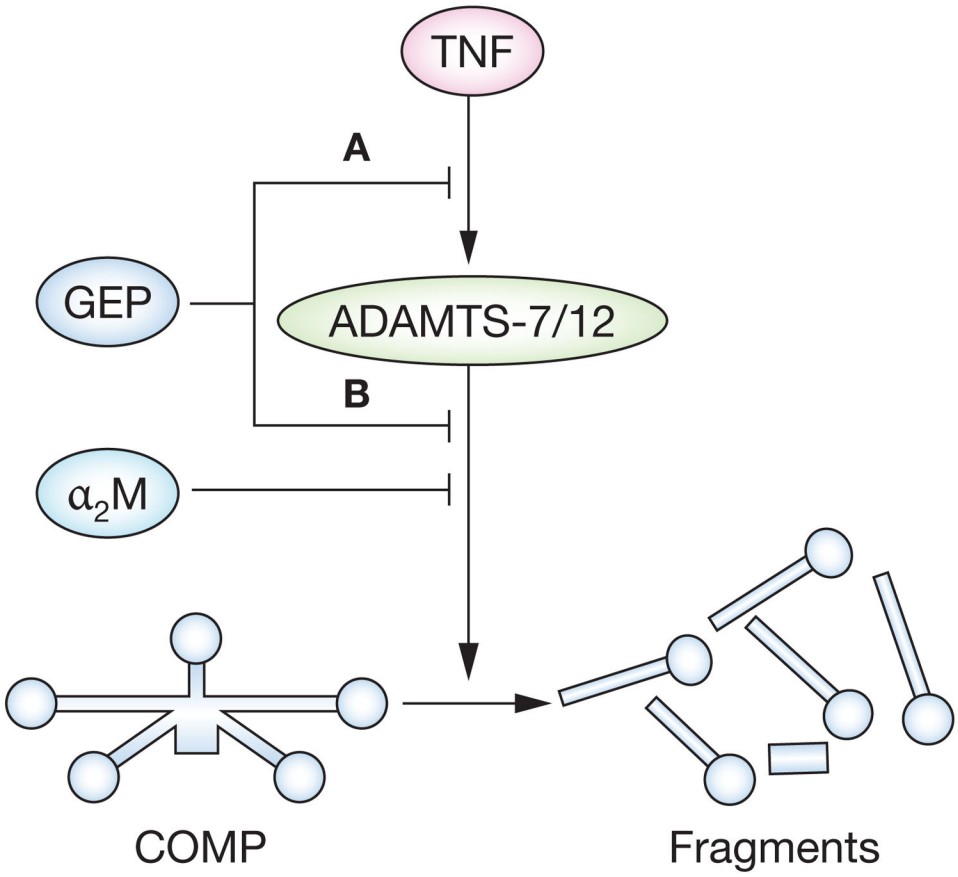

Figure 3.

A diagram of ADAMTS-7 and ADAMTS-12 cleavage of COMP and inhibition of ADAMTS-7 and ADAMTS-12 by α_2M and GEP. GEP regulates the ADAMTS enzymes at two levels as follows: (**A**) GEP inhibits TNF-induced ADAMTS-7 and ADAMTS-12 expression, and (**B**) GEP disrupts the association and cleavage of COMP by ADAMTS-7 via direct protein–protein interaction. Abbreviations: α_2M, α_2-macroglobulin; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; COMP, cartilage oligomeric matrix protein; GEP, granulin-epithelin precursor; TNF, tumor necrosis factor.

Table 1

The known substrates and inhibitors of the ADAMTS family of enzymes.

Enzyme (ADAMTS-)	Alternative name	Known substrate(s)	Known inhibitor(s)
1	METH-1	Aggrecan, versican	NA
2	PCINP	Collagen I, II and III N-propeptides	Papilin, chordin-derived peptides
3	KIAA0366	Procollagen II N-propeptide	NA
4	Aggrecanase-1	Aggrecan, brevican, COMP, decorin, fibromodulin, versican	TIMP-3, TIMP-1, TIMP-2, α_2M, C-terminal portion of fibronectin
5	Aggrecanase-2, ADAMTS-11	Aggrecan	TIMP-3, α_2M
6	NA	NA	NA
7	ADAMTS-7B	COMP, α_2M	α_2M, GEP
8	METH-2	Aggrecan	NA
9	KIAA1312	Aggrecan, versican	NA
10	NA	NA	NA
12	NA	Aggrecan, COMP, α_2M	α_2M, GEP
13	vWFCP	von Willebrand factor	Thrombin
14	NA	Procollagen I N-propeptide	NA
15	NA	Aggrecan	NA
16	NA	α_2M	NA
17	NA	NA	NA
18	NA	NA	NA
19	NA	Aggrecan	NA
20	NA	Aggrecan	NA

Abbreviations: α_2M, α_2-macroglobulin; ADAMTS, disintegrin and metalloproteinase with thrombospondin motifs; COMP, cartilage oligomeric matrix protein; GEP, granulin-epithelin precursor; NA, not applicable; TIMP, tissue inhibitor of metalloproteinases; vWFCP, von Willebrand factor cleaving protease.