Alpha-2-Macroglobulin is a Novel Substrate for ADAMTS-7 and Inhibits the Degradation of Cartilage Oligomeric Matrix Protein by this Enzyme

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Introduction: As we previously reported, ADAMTS-7 and ADAMTS-12, two members of ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family, degrade COMP in vitro and are significantly induced in the cartilage and synovium of arthritic patients (1, 2). The purpose of this study was to determine 1) whether alpha-2-macroglobulin (a2M) is a novel substrate for ADAMTS-7; and 2) whether a2M inhibits ADAMTS-7 cleavage of COMP.

Materials and Methods: Digestion of COMP by ADAMTS-7, digestion of a2M by ADAMTS-7 and the inhibition of ADAMTS-7-mediated digestion of COMP by a2M were analyzed.

Results: a2M is a novel substrate for ADAMTS-7: A previous report showing the a2M associates with ADAMTS-7, together with the fact that a2M inhibits ADAMTS-4 and ADAMTS-5 by competitive inhibition upon cleavage activity by the bait region of ADAMTS-4/-5, led us to determine whether a2M is a substrate of ADAMTS-7. Incubation of 140nM of a2M with various concentrations of purified recombinant ADAMTS-7 and SDS-PAGE showed Coomassie blue-stained products. Intact a2M in its tetramer form was detected at a molecular mass of 700 kDa (Fig. 1A, lane 1). One major a2M cleavage product with the apparent molecular weight of approximately 180 kDa was observed when 10nM of ADAMTS-7 was applied and its intensity strengthened gradually and reached maximum with increasing concentrations of ADAMTS-7 (Fig. 1A); A faint degenerative fragment with the molecular weight of 105 kDa was observed using ADAMTS-7 at 430 nM or higher (Fig. 1B).

a2M inhibits ADAMTS-7-mediated COMP degradation: Since a2M can be digested by ADAMTS-4 and ADAMTS-5 and inhibits the cleavage of aggrecan by these enzymes, we next examined whether a2M, as a substrate of ADAMTS-7, also acts as a competitive inhibitor of the degradation of COMP by ADAMTS-7. ADAMTS-7 at a concentration of 330 nM were preincubated with various amounts of a2M for 2h at 37 °C. After the preincubation COMP was added and allowed to incubate for an additional 2 h at 37 °C. The products were first analyzed on a non-reduced SDS-PAGE gel and visualized by staining (Fig. 2A). Accompanying the increase of a2M, the intensities of the 180 kDa (arrow 2) and 105 kDa (arrow 4) fragments of a2M became stronger, whereas the 110 kDa COMP degradative fragment became weaker and finally invisible (arrow 3) and the intact COMP (arrow 1) band appeared (Fig. 2A). Since both intact a2M (700 kDa) and COMP (550 kDa) were retained at the very top of the gel, we next performed Western blotting with anti-COMP antibody to determine whether the top band (arrow 1) was COMP rather than a2M (Fig. 2B).

Discussion: Our observations demonstrate that a2M is a novel substrate for ADAMTS-7, and more significantly, a2M represents the first endogenous inhibitor of ADAMTS-7.

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Digestion assay of a2M by lower (A) or higher (B) concentrations of ADAMTS-7. 0.14 μM a2M was incubated in the absence or presence of increasing concentrations of ADAMTS-7, as indicated, for 2h at 37 °C. The products were then separated by 8% non-reduced SDS-PAGE and visualized by Coomassie blue staining. Arrows 1, 2, 3 and 4 in (A) indicate the intact a2M, the 180-kDa fragment of a2M, the 110-kDa fragment of COMP and the 105-kDa fragment of COMP, respectively.

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