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Aggrecan mutations alter cleavage patterns

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Keywords

Aggrecan, aggrecanase, cartilage, interglobular domain, MMPs

Context

The breakdown of aggrecan, a proteoglycan found in large amounts in articular cartilage, is a characteristic of many joint diseases. The protein core of aggrecan contains three globular domains (termed G1, G2, and G3). Many glycosaminoglycans (e.g. keratan sulphate and chondroitin sulphate) are bound to the aggrecan molecule in between the G2 and G3 domains. The charged matrix thus created draws in water, giving articular cartilage its resilience and load-bearing properties. Cleavage of the aggrecan molecule between the G1 and G2 domains (called the interglobular domain [IGD]) is especially harmful because this allows the entire glycosaminoglycan attachment region to escape from the cartilage matrix. Two types of protease are responsible for IGD cleavage: matrix metalloproteinases (MMPs) and aggrecanases. MMPs cleave aggrecan between residues Asn341 and Phe342 (N341-F342), while aggrecanases cleave between residues Glu373 and Ala374 (E373-A374). Aggrecan products resulting from cleavage at each of these sites have been found in vivo. The aim of this paper is to understand the mechanisms by which MMP and aggrecanase activities could be independent.

Significant findings

MMPs can cleave the aggrecan IGD at sites other than the major MMP site, N341-F342. However, aggrecanase activity in the IGD appears to be confined to E373-A374. Changing three amino acids at the MMP site of aggrecan cleavage does not appear to affect the activity of aggrecanase. Similarly, a short mutation introduced at the aggrecanase cleavage site does not appear to affect the activity of a wide range of MMPs. Sequential digests demonstrated that MMP13 is able to further degrade aggrecan after aggrecanase cleavage; however, aggrecanase can not further degrade aggrecan after MMP13 cleavage. Aggrecanase activity was inhibited using a 10-mer peptide spanning the N341-F342 MMP site of aggrecan cleavage, supporting the idea that aggrecanase activity may depend on residues in the MMP site of cleavage.

Comments

This study provides further evidence that MMP activity at the IGD of aggrecan occurs independently of aggrecanase activity. Therefore, therapeutic strategies to inhibit aggrecan loss need to consider the activities of both of these classes of enzymes. Although short mutations at the MMP site did not appear to affect aggrecanase activity, this study suggests that aggrecanase may still recognize some specific residues and/or structural elements around the MMP site of cleavage. To address this question, it would be informative to assay aggrecanase activity against an IGD mutant with mutations spanning the same 10-mer sequence as was used in the inhibition studies.

Methods

Overlap extension PCR, cotransfection and expression of IGD mutants in insect cells, restriction enzyme analysis, affinity and gel filtration chromatography, cartilage culture, protease digestion assays, and western blots

Additional information

Other publications supporting the idea that MMP and aggrecanase activities are independent include:

Hughes CE, Little CB, Buttner FH, Bartnik E, Caterson B: **Differential expression of aggrecanase and matrix metalloproteinase activity in chondrocytes isolated from bovine and porcine articular cartilage.** *J Biol Chem* 1998, **273**:30576-30582 (PubMed abstract).

Caterson, B, Flannery CR, Hughes CE, Little CB: **Mechanisms involved in cartilage proteoglycan catabolism.** *Matrix Biol* 2000, **19**:333-344 (PubMed abstract).

References

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