

Is ADAMTS-5 the only aggrecanase in mouse cartilage?

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Introduction: Aggrecan provides cartilage with its weight-bearing properties. In arthritic cartilage, aggrecan is degraded by one or more "aggrecanases" that are members of the ADAMTS (A Disintegrin And Metalloproteinase with Thrombo-Spondin motifs) family of zinc-dependent enzymes. ADAMTS-4 and -5 are thought to be the primary aggrecanases, although ADAMTS-1, -8 and -9 have weak aggrecan degrading activity *in vitro*. Using targeted gene mutations we identified ADAMTS-5 as the major aggrecanase in mouse articular cartilage. The ADAMTS-5 deficient mouse is protected against aggrecan loss and cartilage erosion in models of inflammatory arthritis [1] and osteoarthritis [2], whereas the ADAMTS-4 deficient mouse is not [1,3]. In this study we examine the expression and modulation of aggrecanase activity by IL-1 α and retinoic acid in ADAMTS-4 and ADAMTS-5 deficient mice.

Materials and Methods: Catalytically-deficient mice were created by targeted exon deletions in either the *adamts-4* (TS-4 Δ -cat mice) or *adamts-5* (TS-5 Δ -cat mice) genes. Aggrecan catabolism was examined in explant cultures of femoral head cartilage harvested from 3 week old mice and stimulated with either IL-1 α (10 ng/mL) or retinoic acid (10 μ M) for 3 days. Aggrecan release into the medium or remaining in the cartilage was measured on each of days 1, 2, and 3 by the dimethylmethylene blue dye binding assay. Aggrecanase activity was examined by Western blot analysis of both medium and cartilage extracts, using neopeptide antibodies that recognise the EGE^{373,374}ARG, SELE¹²⁷⁹ and FREEE¹⁴⁶⁷ neopeptides in mouse aggrecan.

Results: The TS-4- and TS-5 Δ -cat mice are viable and fertile, with no obvious abnormalities in any tissues, including the

joints and skeleton. Treatment of cultured femoral head cartilage with IL-1 α or retinoic acid promoted loss of aggrecan from wildtype cartilage. Loss of aggrecan from TS-4 Δ -cat cartilage was slightly reduced, and aggrecan loss from TS-5 Δ -cat was markedly reduced. Western blot analysis showed that proteolysis at the E³⁷³ \downarrow ³⁷⁴A site in the aggrecan interglobular domain was blocked in the TS-5 Δ -cat mouse. However, ADAMTS-5 deficiency was not sufficient to block cleavage in the CS-2 domain at either the E¹²⁷⁹ \downarrow ¹²⁸⁰G site or the E¹⁴⁶⁷ \downarrow ¹⁴⁶⁸G site, since SELE¹²⁷⁹ and FREEE¹⁴⁶⁷ epitopes were generated in TS-5 Δ -cat cartilage following stimulation with IL-1 α or with retinoic acid. The SELE¹²⁷⁹ and FREEE¹⁴⁶⁷ fragments were detected as single bands in cartilage extracts and as doublets in the culture medium.

Discussion: These results show that aggrecan loss stimulated by either IL-1 α or retinoic acid, is driven predominantly by ADAMTS-5 in mouse cartilage. The data suggest that whereas ADAMTS-5 is responsible for cleavage in the interglobular domain, ADAMTS-5 is not essential for cleavage in the aggrecan CS-2 domain. ADAMTS-4 may cleave in the CS-2 domain and ADAMTS-5 may compensate for loss of catalytic activity in the TS-4 Δ -cat mouse. Alternatively, an aggrecanase other than ADAMTS-4 or -5 may be responsible for cleavage in the aggrecan CS-2 domain.

References:

- [1] Stanton H *et al* (2005) *Nature* **434**: 648-652. [2] Glasson S *et al* (2005) *Nature* **434**: 644-648. [3] Glasson S *et al* (2004) *Arthritis Rheum* **50**: 2547-2558.