Expression and activity of the aggrecanolytic ADAMTS in non-degenerate and degenerate human intervertebral disc

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Introduction: Degeneration of the intervertebral disc (IVD) has been implicated in the pathogenesis of low back pain. An early indicator for IVD degeneration is loss of the proteoglycan aggrecan predominantly from the nucleus pulposus (NP). Members of the ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) group of enzymes, namely 1, 4, 5, 8, 9 and 15, are aggrecanases which play a key role in degradation of proteoglycans especially aggrecan in articular cartilage. However little is known about their expression and activity in the IVD, and whether this is altered during disc degeneration. Here we investigate the gene expression and protein production of these aggrecanases and their natural inhibitor, TIMP 3, together with aggrecanase activity within non-degenerate and degenerate human IVDs.

Materials and Methods: Human normal cadaveric IVD tissue was obtained with informed consent from relatives and local ethical committee approval and degenerate human IVD tissue was obtained from patients undergoing spinal surgery with informed consent from the patients and local ethical committee approval. Fully quantitative real time RT-PCR using a genomic standard curve as previously described (1) was used to assess gene expression of the aggrecanases (ADAMTS 1,4,5,8,9 &15) and TIMP 3 in 24 non-degenerate and 28 degenerate disc samples, using the housekeeping gene 18s for normalization. Immunohistochemistry was also performed on 36 IVDs (9 non-degenerate & 27 degenerate discs) for ADAMTS 1,4,5,9 &15 and TIMP3. Additionally aggrecanase activity was investigated using Western blotting for the aggrecan fragments generated by aggrecanase (BC-3 neoepitope Ab) and MMP activity (BC-14 neoepitope Ab) in 4 non-degenerate and 4 degenerate NP samples.

Results: Gene expression of ADAMTSs 1,4,5,9 & 15 and TIMP 3 was seen in both the NP and AF of non-degenerate and degenerate human IVDs. No ADAMTS 8 gene expression was seen in any IVD samples investigated. A significant increase in ADAMTSs 1,4,5 &15 gene expression was seen in degenerate v/s non-degenerate discs, an increase was also observed in ADAMTS 9 although this did not reach significance (Fig. 1). TIMP 3 expression was observed in 100% of non-degenerate discs and 93% of degenerate discs with no change in the level of gene expression between disease states. Immunoreactivity for ADAMTS 1,4,5,9,15 and TIMP 3 was observed in cells of the non-degenerate discs of varying grade (Fig 2). Western blotting for aggrecanase degraded aggrecan fragments demonstrated increased aggrecanase activity within degenerate discs compared to non-degenerate discs (Fig. 3).

Discussion: This study, for the first time, has investigated the gene and protein expression of all the aggrecanolytic ADAMTSs in human IVD. We have demonstrated that ADAMTS 1,4,5,9 & 15 along with their inhibitor TIMP 3 are expressed by human IVDs with a significant increase in gene expression for ADAMTS 1,4,5 & 15 during disc degeneration without any concordant increase in the protein for ADAMTS 4,5 & 15 during disc degeneration. We also demonstrate that aggrecanase activity is increased in degenerate discs compared to non-degenerate discs. This study suggests that these ADAMTS are involved in the aggrecan turnover during normal homeostasis in the human IVD as their expression was observed in non-degenerate discs. The increased expression, production and activity of these enzymes during disc degeneration suggests that they play an important role in the excessive degradation of aggrecan observed during disc degeneration, and may be important targets for therapeutic inhibition.


Acknowledgements: The Authors thank the ARC for funding this work.