Introduction: Intervertebral disc (IVD) degeneration is a key cause of low back pain, which is a major problem to the western world affecting millions of people each year. However the pathogenesis of disc degeneration is poorly understood and a greater understanding is required to develop future therapies. A number of cytokines have been implicated in the pathogenesis of disc degeneration of which IL-1 and TNF have been shown to increase catabolic and decrease anabolic processes in articular chondrocytes and IVD cells. However, to date little information is available on the effect of these cytokines on the aggrecanases (ADAMTS 1,4,5,8,9,15) in human IVD cells. Here we investigate the effect of IL-1β and TNFα treatment on gene expression of all the aggrecanolytic ADAMTSs and their inhibitor TIMP 3 in human IVD cells.

Materials and Methods: Human normal cadaveric IVD tissue (n=3) was obtained with informed consent from relatives and local ethical committee approval and degenerate human IVD tissue (n=3) were obtained from patients undergoing spinal surgery with informed consent from the patients and local ethical committee approval. Nucleus pulposus (NP) tissue was separated and cells extracted using collagenase. Following initial expansion in monolayer culture, alginate bead cultures were established for cytokine treatments. Cells in alginate beads were cultured for 2 wks to allow cellular re-differentiation and then treated with 0, 10 or 100ng/ml IL-1β or TNFα for 48hrs. RNA was then extracted and real time RT-PCR performed for 18s, ADAMTS 1,4,5,9 & 15 and TIMP3. Real time PCR data was then analysed using the 2^ΔΔCt method, normalizing to the housekeeping gene (18s) and untreated controls.

Results: ADAMTS 1 gene expression was significantly decreased by IL-1β treatment within non-degenerate and degenerate NP cells. In contrast TNFα treatment resulted in a significant increase in ADAMTS 1 gene expression in non-degenerate and degenerate NP cells (Fig 1A). ADAMTS 4 gene expression was significantly increased in non-degenerate and degenerate NP cells following IL-1β or TNFα treatment. In addition, in degenerate NP cells TNFα stimulated ADAMTS 4 to a greater extent than IL-1β (Fig 1B). ADAMTS 5 gene expression was significantly increased by TNFα treatment in non-degenerate and degenerate NP cells but was unaffected by IL-1β treatment (Fig 1B). ADAMTS 9 gene expression was significantly increased in non-degenerate and degenerate NP cells following IL-1β or TNFα treatment. In contrast a significant decrease was observed for ADAMTS 15 and TIMP 3 gene expression following IL-1β or TNFα treatment in non-degenerate and degenerate NP cells (Fig 3).

Discussion: This study for the first time has investigated regulation of the aggrecanolytic ADAMTSs and their inhibitor TIMP 3 by IL-1β and TNFα in non-degenerate and degenerate human IVD cells. We have demonstrated that the cytokines IL-1β and TNFα, (both of which are implicated in IVD degeneration) resulted in stimulation of ADAMTSs gene expression, and that TNFα increased gene expression to a greater extent than IL-1β for some of the ADAMTSs. An inhibition of the natural inhibitor TIMP 3 was observed following both cytokine treatments suggesting IL-1β and TNFα treatment results in an imbalance between the aggrecanases and their inhibitor TIMP 3. Interestingly this study also demonstrated that ADAMTS 15 (like TIMP 3) gene expression was substantially down regulated following IL-1β or TNFα treatment suggesting ADAMTS 15 may be differentially regulated within the human IVD. Further studies are needed to ascertain the role of ADAMTS 15 in the IVD.

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