The Involvement of RGD Integrins in the Response of Human Annulus Fibrosus Cells to Cyclic Tensile Strain: An Altered Mechanotransduction Pathway with Intervertebral Disc Degeneration

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Introduction: Mechanical stimuli are important for intervertebral disc (IVD) matrix homeostasis, with physiological force leading to maintenance of disc tissue integrity. It has been shown that the mechano-response of human annulus fibrosus (AF) cells to cyclic tensile strain (CTS) is altered in cells derived from degenerate tissue, from reduced matrix catabolism to reduced matrix anabolism [1]. Furthermore, the aberrant response occurs through an altered mechanotransduction pathway, with interleukin (IL) -1 and -4 involved in the mechano-response of AF cells derived from non-degenerate, but not degenerate IVDs [2]. Further elucidation of the altered mechanotransduction pathway operating in IVD cells derived from degenerate tissue is important and could lead to the discovery of novel therapeutic targets for IVD degeneration. The aim here was to investigate the role of the cell surface receptors integrins, specifically RGD-recognizing integrins, as mechano-receptors in the response of human AF cells to CTS and to ascertain whether their involvement also differed with degeneration.

Methods: AF cells derived from 3 non-degenerate and 3 degenerate human IVDs (obtained at time of surgery or at postmortem examination with full patient or family consent and with the approval of Central Manchester, Bury, Rochdale, Salford, and Trafford Research Ethics Committees) were extracted, expanded in monolayer and cyclically strained, in the presence and absence of RGD or RAD peptides, with 10% strain at 1.0 Hz frequency for 20 minutes using a Flexercell® strain device. Total RNA was extracted from the cells at time points of baseline control, 1 and 24 hours post CTS. Quantitative RealTime PCR was used to analyze the gene expression of the matrix protein, type I collagen and matrix degrading enzyme, ADAMTS4. In addition, the phosphorylation of focal adhesion kinase (FAK) was analyzed by densitometry of western blots following CTS treatment of non-degenerate AF cells. Statistical significance was ensured using the Mann Whitney U test, with p values of less than 0.05 classed as significant.

Results: CTS treated AF cells derived from non-degenerate IVDs decreased ADAMTS4 gene expression and increased tyrosine phosphorylation of FAK. Pre-treatment of non-degenerate AF cells with RGD, but not RAD peptides resulted in the inhibition of the CTS-induced reduction in ADAMTS4 gene expression (Figure 1).

Discussion: The mechano-response of non-degenerate AF cells exposed to 1.0Hz CTS (decreased ADAMTS4 gene expression) was RGD-integrin -dependent, suggesting a role for these integrins as mechano-receptors in non-degenerate AF cells. In addition, FAK became phosphorylated following CTS treatment of non-degenerate AF cells, providing further insight into the mechanotransduction pathways operating in these cells. Interestingly, the mechano-response of degenerate AF cells treated with 1.0Hz CTS (decreased type I collagen gene expression and no change in ADAMTS4 expression) was RGD-integrin –independent, suggesting an altered mechanotransduction pathway with IVD degeneration.

Figure 1 Effect of CTS on ADAM-TS -4 gene expression of AF cells from non-degenerate IVDs +/- RAD or RGD peptides.

In contrast, CTS treatment of AF cells derived from degenerate IVDs led to a decrease in type I collagen gene expression; however, treatment with either RGD or RAD peptides prior to mechanical stimulation had no effect on the observed mechano-response (Figure 2).

Figure 2 Effect of CTS on type I collagen gene expression of AF cells from degenerate IVDs +/- RAD or RGD peptides.

The use of in vitro mechanical loading systems enable quantified and controlled amounts of mechanical stimuli to be administered to cells in culture. The advantages of these systems are that specific mechanical stimuli can be considered in isolation (e.g. CTS, as reported in this study) through the removal of uncharacterized intrinsic factors. However, removal of cells from their native environment can potentially lead to differential mechano-responses when compared to cells stimulated in situ. The use of both in vitro and in vivo mechanical loading systems are therefore required for future studies of IVD cell mechanotransduction, with advantages offered by both systems.

Unfortunately, FAK phosphorylation could not be investigated in degenerate AF cells due to limited availability of human samples. Although the use of human tissue limits the number of samples available for analysis, the benefits obtained (cells acquired from IVDs of correct size, exposed to physiologically relevant mechanical loads and gait) means that human studies remain a necessity.

In conclusion, we report that RGD-recognizing integrins act as mechano-receptors in the response of human AF cells derived from non-degenerate IVDs to 1.0Hz CTS. The aberrant mechano-response observed with degenerate AF cells occurs independently of these integrins, suggesting an altered mechanotransduction pathway operating with IVD degeneration, which warrants further investigation.

Significance: Elucidating the altered mechanotransduction pathway of cells derived from degenerate IVDs will help enable the discovery of novel therapeutic targets aimed at preventing / treating mechanically-induced degenerative changes. These future therapies could help reduce the social and economic burden caused by back pain.

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References

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