Caveolin-1 Expression in Human Intervertebral Disc

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Introduction: Low back pain is a condition that affects 11 million people in the UK alone and is a huge economic burden. Many cases can be attributed to degeneration of the intervertebral disc (IVD), an age-related process that involves structural, mechanical, cellular and molecular changes in the disc. Importantly, changes in cellular activity such as decreased proteoglycan production and increased secretion of catabolic cytokines and matrix-degrading proteases contribute to the loss of structural integrity observed in disc degeneration. Although age-related, degeneration appears to occur at an accelerated rate in some individuals, suggesting that additional factors may play a part. We have previously demonstrated biomarkers of cellular senescence within the human IVD suggesting that cellular senescence could explain the altered cell function in disc degeneration (1), particularly as senescence occurs with aging, but also prematurely in response to stress (e.g. cytokines, load) in a number of tissues. Here, we hypothesised that stress-induced premature senescence (SIPS) occurs within the IVD and investigated the expression and production of caveolin-1, a protein that has previously shown to be upregulated in SIPS in both fibroblasts and articular chondrocytes.

Materials and Methods: Human IVD tissue was obtained either at surgery or post-mortem examination following local research ethics committee approval and informed consent from the patient/relatives. Tissue was fixed in 10% neutral buffered formalin and processed to paraffin wax. Sections were taken for H&E staining to score the degree of morphological degeneration and further sections taken for immunohistochemistry. A score of 0 to 3 indicates a histologically non-degenerate IVD, 4 to 7 indicates evidence of intermediate (moderate) degeneration, and 8 to 12 indicates severe degeneration. The presence of caveolin-1 protein within IVDs was examined by immunohistochemistry with mouse monoclonal anti caveolin-1 IgG1 antibody. Skin tissue was used as a positive control and IgG1 as a negative control.

Additionally, human IVD tissue was separated into nucleus pulposus (NP) and annulus fibrosus (AF), finely chopped and digested by treatment with pronase, collagenase, and hyaluronidase. Isolated cells were lysed in TRIzol reagent and RNA extracted. Following RT reaction, cDNA was analysed for levels of caveolin-1 by semi-quantitative PCR and agarose gel electrophoresis. Densitometry was conducted using Gene Snap and GeneTools.

Results: Caveolin-1 gene expression was identified in human IVD within both the NP and AF in samples derived from non-degenerate, degenerate and prolapsed discs. A positive correlation was found between increasing degenerative grade and caveolin-1 gene expression in NP cells from non-prolapsed IVD. A significant increase in caveolin-1 gene expression was observed in degenerate NP compared to non-degenerate NP; however no significant difference was observed between prolapsed and non-prolapsed NP (Figure 1). In contrast, caveolin-1 expression by AF cells did not significantly alter with disease state (Figure 1).

Discussion: This study has demonstrated for the first time that cells of human intervertebral discs express caveolin-1 and furthermore that caveolin-1 expression increases with grade of IVD degeneration, but that this increase does not correlate with age. This is consistent with a role for caveolin-1 in degenerative rather than age-induced changes in the NP. Previous data using these samples has described accelerated senescence (characterised by a variety of biomarkers including reduced cell replication potential, elevated levels of the cell cycle inhibitor p16INK4a and increased senescence-associated β-galactosidase activity) in degenerate human IVD compared to age-matched non-degenerate discs. Caveolin-1 is an integral membrane protein that is a principle component of the plasma membrane compartments termed caveolae where it has been co-localised with a variety of signal transduction molecules. Interestingly SIPS in other cells/tissues is upregulated by the expression of caveolin-1 suggesting that caveolin-1 plays a strong role in promoting SIPS. Its expression in IVD tissue where increased cellular senescence has been shown suggests that caveolin-1 may play a prominent role in IVD degeneration and the processes that contribute, in particular cellular senescence.


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Figure 1. Caveolin-1 gene expression normalised to 18S in NP and AF cells derived from non-degenerate, moderately degenerate, severely degenerate and prolapsed IVDs (* indicates p < 0.05 compared to non-degenerate discs).

Figure 2. Photomicrograph of caveolin-1 immunohistochemistry in non-degenerate (A) and degenerate (B) human NP. C. Immunohistochemical negative control processed with IgG1 in place of primary antibody.

No correlation was found between caveolin-1 mRNA or protein levels and age of the subjects.