Notochordal Cell Marker Expression in the Adult Human Nucleus Pulposus

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INTRODUCTION:
Low back pain (LBP) affects 60-80% of individuals in developed societies, and is thus a major cause of morbidity. Degeneration of the intervertebral disc (IVD) has been implicated in the pathophysiology of LBP, but current therapeutic interventions are largely conservative, involving pain management and mobility-reducing surgery. Novel cell-based regenerative therapies are being developed, but in order for these to be successful it is necessary to fully understand the developmental origins of the NP so that the tissue is restored to that of a healthy structure and correct functionality.

The anatomical origin of the nucleus pulposus (NP) has been subject of much debate, with one school of thought suggesting that NP cells derive from cells of the surrounding AF which is mesenchymal in origin, whilst other often compelling evidence suggests the NP cells are notochordally-derived. Morphologically identifiable notochordal cells have been reported to disappear from the NP after the age of 10 years. However, as morphological changes in such cells are known to occur, other methods need to be employed to identify whether notochordally-derived cells exist within the adult NP. The aim of this study was to investigate the gene expression of previously described notochordal cell (NC) markers in the adult NP.

METHODS:
27 lumbar (aged 25 to 75 years) and 27 cervical NP samples (aged 33-72 years) were obtained with informed consent during discectomy in accordance with university and ethical committee policy and HTA legislation. Tissue samples were partially processed for histological analysis or enzymatically digested with RNA extracted from cells and reverse transcribed to cDNA. Fixed specimens were then histologically graded1, whilst QPCR was performed on extracted samples for notochordal markers (Brachyury (T), Galectin-3 (LSGAL3), CD24, Chordin (CHRD), FOXA2 and NOGGIN). All data was normalised to expression of the housekeeping genes MRPL19 and EIF2B1, and analysed according to the ΔΔt method. Mann-Whitney tests were performed to assess statistical significance and p values equal to <0.05 were deemed significant.

RESULTS:
Expression of all NC markers was identified in both lumbar and cervical NP cells, although T and FOXA2 expression was not detected in every sample. In cervical NP samples, increased degeneration did not significantly alter expression of most NC markers, although a significant increase in NOGGIN expression was noted (Figure 1). A similar pattern of expression was noted in lumbar specimens. There were also no significant age-associated alterations in NC marker expression in lumbar samples (Figure 2). Interestingly, some differential expression between lumbar and cervical specimens was noted, with significantly increased expression of LSGAL3, CHRD and NOGGIN noted in cervical NP cells compared to lumbar NP cells, whilst expression of all other genes remained unchanged (Figure 3).

DISCUSSION:
This study corroborates other recent evidence regarding the developmental origins of the NP, as the finding of NC marker expression in adult NP is indicative of cells of notochordal derivation. The finding of continuous expression of most markers with ageing and degeneration may be indicative of an NC-like population of cells that persist in adult tissues. Interestingly, data shown here suggests that levels of NC marker expression vary with location within the spine, with cervical NP cells demonstrating significantly upregulated expression of multiple NC markers. The reasons for this are unclear, but might suggest that the persistence of NC-like cells is prolonged in cervical discs and NC cells of that region might be less susceptible to apoptosis as previously described. Further work may establish alterations in NC marker expression across a greater range of ages to include younger samples where NC cells are known to reside within the NP.

SIGNIFICANCE:
The findings presented here support other evidence suggesting that the NP derives from the notochord and not the surrounding AF. The detection of NC markers in adult human NP samples may also indicate the persistence of a sub-population of cells associated with development in adult tissues.

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