INTRODUCTION

That contractile cells have been found in other cartilage tissues in healing and disease states prompted the investigation of the presence of α-smooth muscle actin (α-SMA), an actin isoform often associated with contraction, in human intervertebral discs (IVDs). Recently, the presence of this isoform was reported in human IVD specimens from autopsy (1). In other connective tissues, expression of α-SMA is elevated in pathological states. The objective of this study was to evaluate the cell density and percentage of α-SMA-containing cells in nucleus pulposus (NP) tissue obtained during disc surgery. NP material from several surgeries was also cultured to determine how α-SMA expression changed with time in the culture environment. The results were compared to those obtained from prior study of surgical specimens.

METHODS

Immunohistochemistry of Pathological Specimens: To determine in situ expression of α-SMA in the diseased state, 9 L4-L5 and 9 L5-S1 disc specimens were collected from 17 patients during disc surgeries (discectomies, microdiscectomies, revision discectomies, and anterior fusions). The patient age was 38 ± 12 years (mean ± standard deviation). Ten patients were male and seven were female. The history of acute symptoms prior to surgery was 16 ± 16 months. Five of the patients had had previous lumbar disc surgery. The tissue was separated macroscopically into NP and annulus fibrosus.

Specimens were fixed in formalin for at least 48 hours, decalcified, and embedded in paraffin. Seven µm thick sections were stained with H&E and a monoclonal antibody to α-SMA (clone 1A4; Sigma Chemical Co., St. Louis, MO)(1). During histological analysis, the edges of the pathological tissue were avoided due to hypercellularity, which was believed to be partially an inflammatory reaction. Cells were counted and categorized as to α-SMA phenotype (positive or negative) and divided by the area of analysis to yield a cell density.

NP Specimens in Culture: NP tissue was removed from 4 L4-L5 and 2 L5-S1 discs during surgeries (microdiscectomies, discectomies, and anterior fusions) and placed in PBS with 2% pen/strep and 1% fungizone. The patient age was 38 ± 6 years; 5 patients were female and 1 was male. The history of acute symptoms prior to surgery was 13 ± 13 months.

Seventy-six 5 mm-diameter explants were obtained from the NP tissue using a dermal punch. Within 2 hours of surgery, the explants were placed in 1 mL of medium (DMEM/F12 with 10% FBS, 2% pen/strep, 1% fungizone, 2% ascorbic acid, and 1% glucose) in 6-well plates. The medium was changed and cell outgrowth was assessed every 2 days. Explants from each subject were sacrificed at 1, 2, 4, and 6 weeks. For 26 explants, the wet mass was obtained at sacrifice. Explants were processed for histology and α-SMA immunohistochemistry as above. Cells were counted and categorized as to α-SMA phenotype (positive or negative), morphology (round or elongated), and whether they were in a group of cells (group: ≥ 2 cells), and cell density was determined.

RESULTS

Pathological NP Specimens: Every NP section exhibited the presence of α-SMA-containing cells. Approximately 25% of the cells contained this contractile actin isoform, and in some areas as many as 50% (Table 1). Greater than 95% of the cells in these nucleus specimens were round in shape, and they were often surrounded by a round pericellular matrix. Many cells were members of clusters or chondrocyte pairs.

Cell density (Table 1) did not correlate with gender and disc level, but was significantly higher in older individuals (p = 0.02).

NP Specimens in Culture: Explants were generally adherent after 1 day in culture. The average outgrowth time was 8.6 ± 5.0 days.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Surgical Specimens</th>
<th>Autopsy Specimens</th>
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<tbody>
<tr>
<td>Cell density (cell/mm²)</td>
<td>34 ± 8 (7-140)</td>
<td>28 ± 5 (2-140)</td>
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<tr>
<td>% of α-SMA of total cells</td>
<td>24 ± 3 (3-46)</td>
<td>15 ± 3 (0-63)</td>
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The wet mass at sacrifice, 53 ± 19 mg, was found to be independent of sacrifice time. Histologically, the total area assessed and the number of cells counted were 1.5 ± 0.7 mm² and 140 ± 98 cells per section. Approximately 60-70% of the cells in the explants stained positive for α-SMA. The percentage of cells that were round, the percentage that stained positive for α-SMA, the percentage in groups, and the cell density were not significantly different among times in culture. The percentage of α-SMA-containing cells that were round showed a significant difference among sacrifice culture times (p = 0.008), with values decreasing through 4 weeks and then slightly rising at 6 weeks.

DISCUSSION

The age ranges for the the current study population of surgical (pathological) specimens and the prior analysis of autopsy cases (63 ± 13 years; Table 1; ref. #1) were significantly different (p < 0.001). The cell density estimates for the tissues in the two groups were not, however, significantly different. In contrast to the current findings in pathological cases, not every IVD from autopsy showed positive staining for α-SMA. A Student's t test revealed that the p value for the comparison of the percentage of α-SMA-positive cells in the two groups was 0.05, with 50% more cells in the surgical cases containing α-SMA (Table 1). This suggests that the level of expression of α-SMA is elevated in pathological situations in the NP of human lumbar IVDS as it is in some other connective tissues; however, it should be recognized that the groups were not age-matched. Our data also indicate that the expression of α-SMA is relatively stable in NP explants out 6 weeks in culture. Although, of the cells that contained α-SMA, the percentage that were round decreased with time in culture.

Previous work (1) in quantifying α-SMA in human autopsy specimens revealed that the protein was expressed more in the nucleus than the annulus and positively staining cells were preferentially round. The role of this contractile actin isoform remains uncertain. Expression of this cytoskeletal protein may play some role in maintaining the round morphology of the cells in situ. That the percentage of α-SMA-positive cells that were round decreased with time in culture may reflect a different function of expression of this actin, such as contraction or migration.

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REFERENCES


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