**Introduction:** Autophagy is an important intracellular process by which cytoplasm and organelles are degraded; this adaptive response to sublethal stresses (such as nutrient deprivation) supplies needed metabolites. Autophagosomes (specialized double membrane vesicles) encapsulate material and fuse with lysosomes for degradation. The molecular and ultrastructural events during autophagy in human disc degeneration are unexplored. We hypothesize that expression of autophagy-related genes increases with increasing stages of disc degeneration, accompanied by ultrastructural changes typical of autophagy. Our objectives were: 1) To utilize microarray analysis and ontology searches to identify genes associated with autophagy, and 2) To identify fine structural evidence for autophagy in the degenerating human annulus.

**Methods:** Our Institutional Review Board prospectively approved these studies. Annulus tissue for molecular studies was derived from 6 Thompson grade I-II discs, 9 grade III, 4 grade IV, and 2 grade V discs. Total RNA was extracted using the TRIzol reagent, reverse transcribed to double-stranded cDNA, subjected to two rounds of transcription, and hybridized to the DNA microarray in the Affymetrix Fluidics Station 400. The GCOS Affymetrix GeneChip Operating System was used for determining gene expression levels of genes in the “autophagy” ontology group. Data were normalized, and GeneSifterTM web-based software used to analyze microarray data. Statistical significance was determined using the student t-test (2 tailed, unpaired, \( p<0.05 \) was taken as the significance level). Annulus tissues from 8 grade I, 3 grade II, 30 grade III, 16 grade IV and 2 grade V discs were fixed, thin-sectioned, grid-stained and viewed with a Phillips CM10 transmission electron microscope.

**Results:** Our molecular study design compared expression patterns in more degenerated Thompson grade IV and V discs vs. patterns in healthier grade I, II and III discs. Analyses showed significant upregulation of 10 genes related to autophagy in more degenerated annulus tissue, including beclin 1 (upregulated 1.6 fold, \( p=0.04 \)), microtubule-associated protein 1 light chain 3 beta (upregulated 2.0 fold, \( p=0.028 \)), the ATG12 autophagy related 12 homolog gene (upregulated 4.0 fold, \( p=0.005 \)), presenilin 1 (upregulated 1.6 fold, \( p=0.024 \)), and cathepsin B (upregulated 4.5 fold, \( p=0.036 \)). Ultrastructural evidence of macroautophagy (marked with *, X 35,470-56,380) in annulus cells in vivo included autophagic vacuolization (Fig. 1A, B), and autophagosomes with complex, redundant whorls of membrane-derived material (Fig. 1C-F). Fig. 1D shows an autophagosome containing a mitochondrial remnant. Microautophagosome formation (invagination of lysosomal membranes engulfing material within autophagosomes) was also present (data not shown).

**Discussion:** This work presents the first molecular and morphologic evidence for autophagy in the degenerating human annulus. Well-recognized genes related to autophagy were identified with significant upregulation in more degenerated tissues. Autophagy may be a survival mechanism which provides cells with alternative substrates during periods of nutrient deprivation, and it can also protect cells by eliminating damaged mitochondria. We have previously identified mitochondrial dysfunction in the annulus (1,2); production of reactive oxygen species, which can damage mitochondria, has previously been documented in the disc (3-6).

**Significance:** Findings point to the importance of future studies which further investigate autophagy in the human intervertebral disc.

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**References:**
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