Nucleus Pulposus Cells have Epithelial Cell-Like Cytoskeleton and Highly Express N-Cadherin

Robert Tower, PhD[1]
Zuozhen Tian, PhD[2]
Brian Cosgrove, PhD[1]
Robert Mauck, PhD[1,2,3]
Ling Qin, PhD[1]
Motomi Enomoto-Iwamoto, DDS PhD[4]
Yejia Zhang, MD PhD[1,2,3]

[1]Department of Orthopaedic Surgery University of Pennsylvania

[2]Department of Physical Medicine and Rehabilitation University of Pennsylvania

[3]Translational Musculoskeletal Research Center (TMRC) Corporal Michael J. Cresenz Veterans Affairs Medical Center Philadelphia, PA

[4]Department of Orthopaedics University of Maryland Baltimore, MD

Introduction

Back pain related to intervertebral disc (IVD) degeneration is a common condition and is believed to initiate in the nucleus pulposus (NP). Understanding the characteristics of the NP cells may help design strategies to prevent and/or revert IVD degeneration. In this study, we aim to examine actin cytoskeleton organization by staining filamentous actin (F-actin) with fluorescently-tagged phalloidin and analyzing gene expression profiles.

Methods

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the Corporal Michael J. Crescenz Veterans Affairs Medical Center in Philadelphia. For mouse tail injury model, twelve young adult (8 week old) female C57BL/6j mice (the Jackson Laboratory, Bar Harbor, ME, USA) were used in this study. Under anesthesia, the mouse tail IVDs were injured with a 26-G needle inserted under fluoroscopic guidance. Histological evaluation of Alcian Blue and Haematoxylin and Eosin (H&E) counter stained sections confirmed typical changes during injury. Tail IVDs with adjacent vertebral bodies were isolated, decalcified with 12.5% EDTA (Sigma), embedded in OCT compound (Tissue-Tek, Torrance, CA), and cryosectioned. Actin filaments were stained with Alexafluor 488 phalloidin (Thermo Fisher Scientific), covered with mounting medium containing DAPI (Vector Laboratories, Burlingame, CA, USA), and imaged with a confocal microscope (Nikon Eclipse Ti, Nikon, Japan). For gene expression analyses, the lumbar and coccygeal vertebrate of 4 mice were isolated with a scalpel under a dissecting microscope: the gelatinous NP was scraped off with a scalpel. Annulus fibrosis (AF) tissues, identified by their concentric rings, were shaved off the cartilaginous endplate with a scalpel. Total cellular RNAs were extracted and the Mouse Extracellular Matrix & Adhesion Molecules RT Profiler PCR Array (Qiagen, Gaithersburg, MD) was used to profile the expression of 84 genes important for cell-cell and cell-matrix interactions.

Results

In NP cells from intact IVDs, actin filaments are highly concentrated at the periphery of the cell, where they form a three-dimensional network beneath the plasma membrane (n=5; Figure 1A). This cell shape is reminiscent of that in the epithelium where cells exhibit cobblestone morphology. In injured IVDs, NP cells transition to a more oval shape and with a reduced cellular density (Figure 1B); actin filaments appear to have reorganized. Among the 84 genes examined, gene expression analyses showed cadherin 2 (cdh2; commonly known as neural (N)-cadherin) expressed higher in the NP than in AF, while secreted phosphoprotein 1 (spp1) was highly expressed in both the NP and AF. Thus, these genes were further examined by quantitative Real-Time PCR. NP, AF, and knee articular cartilage (AC) were isolated from a further 8 mice. The validated primer sets for cdh2, spp1 and b2m were purchased (QuantiTect Primer, Qiagen, Gaithersburg, MD). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to *b2m* as an endogenous loading control. Cdh2 gene expression was significantly higher in the NP compared to AF, which in turn was significantly higher than the AC. ($n = 8$ for NP and AF; $n = 4$ for AC, $p < 0.01$; Figure 2A). Spp1 was expressed at equally high levels in the NP, AF and AC ($n = 4, p > 0.05$; Figure 2B).

Discussion

We were surprised to find that the normal NP cells represented a cobblestone organization (epithelial cell-like), with cells becoming more oval shaped (considered chondrocyte-like) following injury. Cdh2 gene is expressed higher in the NP than AF or AC, which would be linked to the unique morphology of NP cells. Future studies will include examination of gene expression and protein distribution of Chd2 and other epithelial cell makers, to elucidate the role of this intriguing cell phenotype in IVD degeneration.

Significance

IVD degeneration is thought to initiate in the NP. NP cells reside in a high pressure and

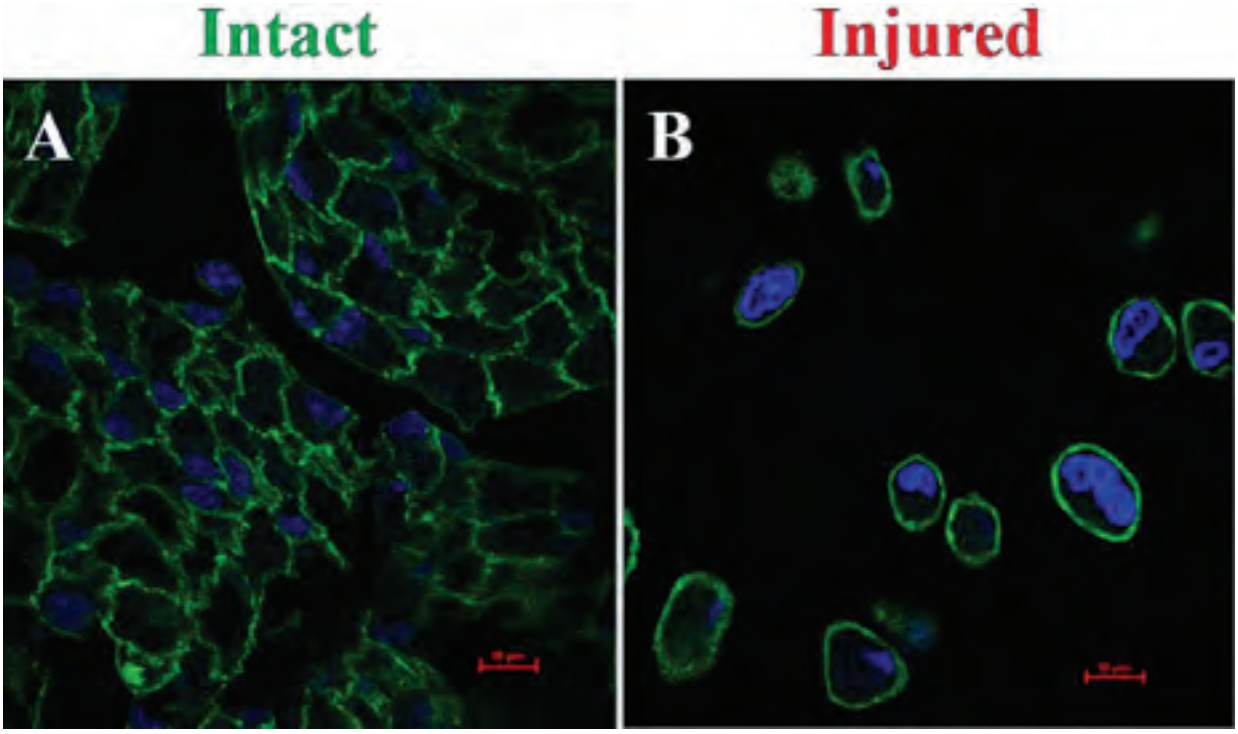

Figure 1. Injured tail nucleus pulposus cells proliferate. Green: F-actin cytoskeleton stained with phalloidin; Blue: cell nuclei stained with DAPI. Scale bar equals 10 um.

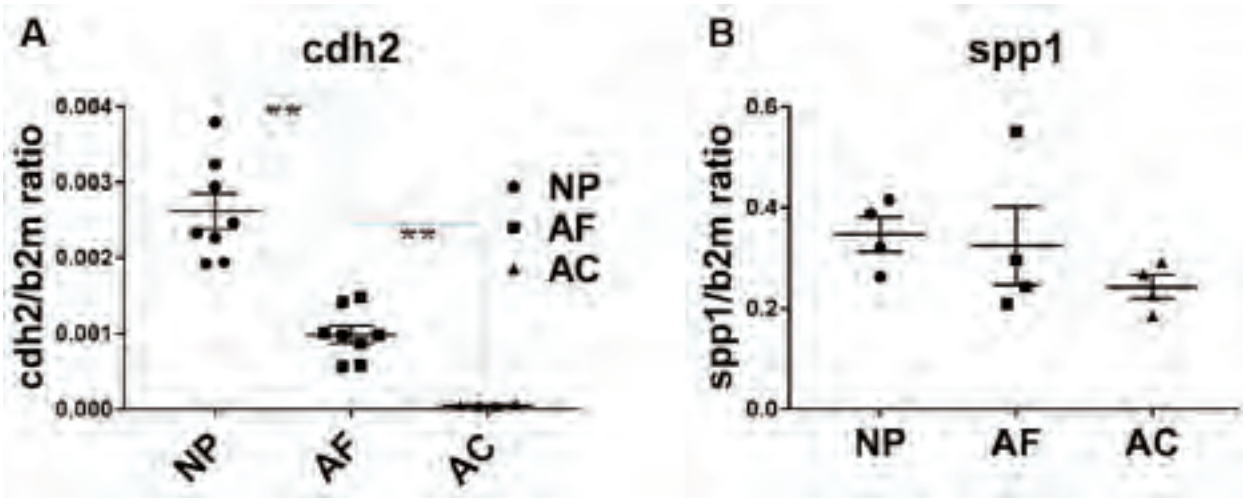

Figure 2. Gene expression by real time PCR in murine nucleus pulposus (NP), annulus fibrosis (AF) and articular cartilage (AC) tissue. Cdh2: cadherin 2; spp1: secreted phosphoprotein 1. Each point represents an individual animal; n = 4-8. **p < 0.01

low nutrient environment, and thus, require specialized physiological properties. A better understanding of the cytoskeleton and cell-cell interactions present in the normal NP, as well as the changes observed in response to injury may lead to better repair strategies for the degenerating disc.