Background Context

Fibronectin (FN) splice variants play important roles in regulating cell-matrix and matrix-matrix interactions in skeletogenesis and skeletal function in limbs and other sites. However, presence and possible roles of FN splice variants in human IVD have not been determined. FN is encoded by a single gene; alternative splicing produces multiple FN mRNAs, and as a result, up to 20 different isoforms and variants exist (Figure 1). "Full-length" isoforms of FN with molecular masses in the region of 250–280 kDa are produced by alternative splicing of the FN primary gene transcript at three major sites: extra domain A (EDA), extra domain B (EDB), and the variable region (V). In this study, we have characterized the FN alternative splice variants in normal and degenerative human IVD tissues.

Purpose

FN splice variants play particularly important roles in regulating cell-matrix and matrix-matrix interactions. The purpose of the present study is to determine the presence of FN and its isoforms in normal human IVD tissues and to examine whether changes in these patterns are associated with disc degeneration.

Study Design

This is a laboratory study where human IVD tissues were examined FN splice variants by molecular biology methods.

Methods

Fresh human IVDs with different degrees of degeneration were collected during spine surgery. The severity of degeneration was graded using MRIs according to Pfirrmann et al. Specifically, Pfirrmann Grade I disc is normal; Grade II-V discs are at progressively severe degrees of degeneration, with Grade V being the most severe. The grading was performed by three physicians independently, and the scores were averaged. Normal human disc tissues from a 34 day-old infant and a 25 year-old organ donor were acquired through the Cooperative Human Tissue Network (CHTN, Philadelphia, PA). Neither donor had any apparent musculoskeletal defect. The causes of death were neurodevelopmental dysfunction and renal failure, respectively.

The annulus fibrosus (AF) was separated from nucleus pulposus (NP). NP and AF tissues were stabilized in RNA later (Ambion, Austin, TX) following manufacturer’s instructions and stored at -70 degrees Celsius. Then, total cellular RNA was isolated using Trizol reagent (Life Technologies, Rockville, MD) and homogenizing the tissue using a rotor-stator device (Omni International, Marietta, GA). Total RNA was extracted using the RNA Easy kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. RNA was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers flanking the alternatively spliced regions: EDA, EDB and V. The V region PCR products were confirmed by sequencing.

Results

Both the EDB+ and EDB- splice variants were present in normal and degenerative IVD tissues. The EDB+ to EDB- ratio was highest in moderately degenerative tissue (Figure 2). The EDA+ domain was only expressed in infant but not adult tissue. All five variants of the variable-region (V) splice forms were present in all tissues studied. A splice form with the entire V-region, the 15th type III domain and 10th type I domain adjacent to the 3’ end of V region omitted (referred to as (V+-C) splice form) was present at higher levels in adult than in infant samples.

Conclusions

Our study is the first to describe the splice variants of FN using well characterized human IVD tissues. These variants may play a role in modulating cell-matrix or matrix-matrix interactions. Novel basic information gathered here will lead to a better understanding of pathological processes associated with disc malfunction and degeneration.