COLLAGEN AND PROTEOGLYCAN ABNORMALITIES IN GDF-5 DEFICIENT MICE MAY BE CORRECTED BY TREATING OF DISK CELLS WITH RECOMBINANT GROWTH FACTOR

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Introduction. Single gene abnormalities have been useful in defining the effects of specific proteins on tissue function. In this abstract, we report intervertebral disk abnormalities caused by the deficiency of Growth and Differentiation Factor-5 (GDF-5). GDF-5 is a member of the Growth and Differentiation Factor family of proteins, which are closely related to the Bone Morphogenetic Proteins (BMP's). GDF-5 is also called Cartilage-Derived Morphogenetic Protein-1 (CDMP-1) [1] and BMP-14. It has been shown to play a role in a variety of musculoskeletal processes including joint formation, endochondral ossification, and tendon and ligament maintenance and repair. Because of the known chondrogenic phenotype of the central regions of the intervertebral disk, we hypothesized that GDF-5 deficiency would lead to tissue-level abnormalities of the intervertebral disk.

Materials and Methods. Nineteen 16 week-old female GDF-5 deficient mice and 21 wild-type animals were obtained from the Jackson Laboratory (Bar Harbor, ME). With Institutional Animal Care and Use Committee approval, studies of the lumbar region of the spines were performed at age 20 weeks. After MRI, intervertebral disks were researched for histology, biochemistry, and gene expression analysis. Disk cells from GDF-5 deficient mice were isolated and treated with different concentrations of recombinant GDF-5 protein, collagen and aggrecan gene expression were analyzed with real-time PCR.

Results. MRI. MRIdemonstrated significantly lower T2-weighted signal intensity in the central region of the GDF-5 deficient disks compared with wild-type controls (p<0.05). This finding is consistent with decreased hydration of the nucleus pulposus region of the disk (Fig. 1).

Histology: The wild-type lumbar disk is composed of an outer fibrous annulus fibrosus (AF) with prominent concentric fibrous lamellae and an inner nucleus pulposus (NP) with an amorphous matrix and a heterogeneous cell population. Safranin-O staining of the disk normally creates a deep, red appearance to both regions due to the presence of proteoglycans. Only the outermost fibers of the annulus failed to stain red due to a lack of significant proteoglycan in this region. In contrast, GDF-5 deficiency demonstrated loss of the normal lamellar organization and replacement with chondroid tissue resembling fibrocartilage. (Fig. 2) The disks from GDF-5 deficient animals were noted to have smaller, less cellular NP regions with a disorganized matrix.

GAG and Hypro
Glycosaminoglycan (GAG) was measured to estimate the total proteoglycan within the disk while hydroxyprolene (hypro) was used to estimate the total collagen content.

Discussion. Our study demonstrates significant histological and biochemical differences between the disks from GDF-5 deficient mice compared with the wild type strain. In many respects the changes seen are similar to degenerative disk disease. To date, most of the research on the musculoskeletal effects of GDF-5 deficiency has focused on tendon [2], joints, and bone [3]. This is the first report detailing the effects of GDF-5 deficiency on the intervertebral disk. Genetic linkages and single gene defects leading to disk degeneration have also been described in some humans. Because this study focused on the changes in the disc in the GDF-5 deficient animals at a single time point, the course of disk changes over time in this animal model remains unknown. It is likely that the observed abnormalities of the GDF-5 deficient disk are remnants of an abnormality during embryonic development, rather than a purely degenerative process. However, because these tissue-level changes are dependent on the deficiency of a single gene, they will offer a unique opportunity to study the response of the disk to correction of an underlying metabolic defect. In turn the repair potential of a structurally abnormal disk is important for the development of strategies for the treatment of degenerative disk disease.

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