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Basic Science

Quantitative proteomic analysis of normal and degenerated human intervertebral disc

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Abstract

BACKGROUND CONTEXT: Degenerative disc disease (DDD) is the most common disease of aging in humans. DDD is characterized by the gradual damage of the intervertebral discs. The disease is characterized by progressive dehydration of nucleus pulposus and disruption of annulus fibrosus of intervertebral disc.

PURPOSE: Even though it is highly prevalent, there is no effective therapy to regenerate the degenerated disc, or decrease or halt the disease progression. Therefore, novel monitoring and diagnostic tests are essential to develop an alternative therapeutic strategies which can prevent further progression of disc degeneration.

STUDY DESIGN: The study was designed to understand the proteome map of annulus fibrosus and nucleus pulposus tissues of intervertebral disc and its differential expression in patients with DDD.

METHODS: The proteome map of the annulus fibrosus and nucleus pulposus tissues of intervertebral disc was cataloged involving one-dimensional gel electrophoresis—Fourier transform mass spectrometry/ion trap tandem mass spectrometry (FTMS/ITMSMS) analysis. The altered proteome patterns of annulus fibrosus and nucleus pulposus tissues for DDD were identified using Isobaric tag for relative and absolute quantification (iTRAQ)–based quantitative proteomics coupled with FTMS/ITMSMS and network pathway analysis.

RESULTS: The study identified a total of 759 and 692 proteins from the annulus fibrosus and the nucleus pulposus tissues of the disc based on FTMS/ITMSMS analysis, which includes 118 proteins commonly identified between the two tissues. Vibrant changes were observed between the normal and the degenerating annulus fibrosus and nucleus pulposus tissues. A total of 73 and 54 proteins were identified as differentially regulated in the annulus and the nucleus tissues, respectively, between the normal and the degenerated tissues independently. Network pathway analysis mapped the differentially expressed proteins to cell adhesion, cell migration, and interleukin13 signaling pathways.

CONCLUSIONS: Altogether, the current study provides a novel vision in the biomechanism of human disc degeneration and a certain number of proteins with the potential biomarker value for the preliminary diagnosis and scenario of DDD.

Keywords: Annulus fibrosus; Degenerative disc disease; Human spine; Intervertebral disc degeneration; Network pathway; Nucleus pulposus; Proteome mapping; Quantitative proteomics

Introduction

Low back pain (LBP) is a major public health problem in India, with about 75% of the population estimated to experience LBP during their lifetime [1]. One of the principal causes of LBP is degeneration of the intervertebral disc (IVD).

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Intervertebral disc disease/degenerative disc disease (DDD) results in subsequent loss of disc height, resulting in the compression of nerve root. Compression of a nerve root by a herniated disc is characterized by radiating pain along the course of a nerve root in the affected dermatome with or without neurological deficits in the corresponding muscles [2]. The pain associated with DDD is due to adverse changes in the matrix, resulting from dehydration of the nucleus pulposus (NP). Disc degeneration generally remains asymptomatic, associated with the loss of water content and proteoglycans. Usually, the loss of shock-absorbing function in degenerated discs makes the discs fibrotic. Fissures appear inside the disc with disorganization of annulus fibrosus (AF). At times, disc degeneration becomes symptomatic, resulting in significant disability to the patient [3]. Current treatments aim at relieving symptoms of LBP, rather than treating the underlying cause. There is an urgent need to provide more-effective treatments for IVD degeneration. During degeneration of IVD, the NP is most affected, and many studies stress the use of cell-based tissue engineering to regenerate the NP [4–6].

The association of disc disease with advancing age is well documented [7–9], but there are reports showing an increased incidence of disc disease in young adults, among various populations [10–12]. Details of molecular mechanism(s) underlying DDD are not known because of the inherent difficulties in obtaining intact IVD tissues from patients. Only few attempts have been made to understand the molecular basis of DDD in humans. Studies reinforce and associate the role of genetic factors with DDD [13]. Asporin [14], ADAMTS 7 (a disintegrin-like and metallopeptidase with thrombospondin type 7 motif), and ADAMTS 12 (a disintegrin-like and metallopeptidase with thrombospondin type 12 motif) are expressed at higher levels in degenerated human IVD [15]. Genetic polymorphisms in genes such as extracellular matrix (ECM)–degrading enzymes; collagen I, IX, and XI; aggrecan; Fas/FasL; inflammatory cytokines, such as IL (interleukin)-1, IL-6, and tumor necrosis factors α; and vitamin D receptors are associated with the development of DDD [16,17]. There is a huge requirement in the identification of biomarkers, which facilitates diagnostic and management strategies and thereby helps to differentiate conditions with similar presentation.

Due to the characteristic differences of IVD in various species of animal models, the previous findings may not be translatable to the human research. The load applied to the rat spine is substantially smaller than the load applied to the human spine [18]. In the case of humans, studies on IVD samples were carried out after autopsy [19] or sporadically [20]. Studies on proteome analysis of blood samples were performed to establish the association of serum proteins with lumbar IVD herniation [21]. Degenerated human AF cultures showed only 10 proteins to be differentially regulated against normal AF based on two-dimensional gel electrophoresis differential proteomic analysis [22]. A better understanding of the proteome components between the normal and degenerated human IVD is expected to provide more insights in the biology of IVD and associated process of degeneration.

Mass spectrometry-based quantitative proteomics is a powerful tool to analyze the cellular protein expression patterns, protein-protein interactions, and posttranslational modifications of proteins. In the present study, we aimed to establish the proteome map of both AF and NP of human IVD and the differential proteomic analysis of AF and NP tissues during DDD using high-throughput proteomic analysis involving tandem mass spectrometric analysis and isobaric tag for relative and absolute quantification (iTRAQ)–based quantitative proteomic analysis.

Methods

Collection of human annulus fibrosus and nucleus pulposus samples

The fresh IVD samples were collected from patients with chronic LBP and sciatica (Fig. 1, Left). These patients were diagnosed with DDD in the lumbar spine based on magnetic resonance imaging (MRI) (Fig. 1, Right). The disc degeneration was graded based on MRI classification involving Pfirrman grading system [23]. These patients underwent fusion surgery in the lumbar spine, in the form of transforaminal lumbar interbody fusion after failure of the conservative treatment. Annulus and NP tissues were obtained during the surgical procedure. These patients were under general anesthesia, with the patient in prone position, after thorough aseptic preparation of the surgical site and draping (Fig. 1). The diseased level in the lumbar spine is identified with the help of image intensifiers; paravertebral muscles are separated from the laminae and the spinous processes. Laminectomy was done at the diseased level. After retracting the dural sheath, the disc space is visualized after making a small nick in the outer layer of the disc, ie, annulus; nucleus material is removed with the help of pituitary rongeurs. Later, a small piece of the annulus is excised with the help of Kerrison punches. The normal discs used in our experiments were obtained from young patients, who sustained burst fractures of the lumbar spine, with no previous history of LBP. The IVDs appear normal on the MRI. These patients underwent fusion surgery for the burst fractures, and during the anterior reconstruction of the lumbar spine, the disc materials were collected.

The samples were obtained with informed consent of the patients and with the approval of the Sunshine Hospitals Ethics Committee. After collecting the disc tissues, they were thoroughly washed in normal saline to get rid of any blood and other debris. The tissues were collected in sterile containers. The tissues were frozen on dry ice, as soon as discectomy was done and transported to Centre for Cellular and Molecular Biology, Hyderabad, within 1 h after collection for further processing. The protocol for the collection of tissues from patients and from normal subjects and the experimental pro-