Chondroadherin Fragmentation Mediated By HTRA1 Can Serve As A Biomarker For Early Stage Disc Degeneration

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Disclosures:

Introduction:
Previous data has associated CHAD fragmentation to disc degeneration present both in the adult with degenerative disc disease and premature degenerative disc disease at the apex of the spinal curves in adolescents with idiopathic scoliosis (1). At present, little is known about the molecular mechanisms involved in the degeneration of and how these may differ from normal turnover of the tissue. CHAD, a protein of the leucine rich repeat (LRR) family, is one of the proteins predominantly expressed in the extracellular matrix (ECM) of cartilaginous tissue, including that of the IVD. CHAD is primarily found close to the cells, where it can interact with collagen fibrils of the ECM and molecules at the cell surface, providing a mechanism for regulating cell metabolism and ECM structure (2, 3). The aims of this study were to determine whether CHAD fragmentation is unique to disc degeneration, to characterize if the cleavage observed within CHAD occurs at the same site in both the adult with degenerative disc disease (DDD) and the juvenile with adolescent idiopathic scoliosis (AIS), and to identify the protease capable of CHAD cleavage at this site.

Methods:
Healthy and degenerate lumbar IVDs were obtained through organ donations via Transplant Quebec. IVDs from patients with DDD and from patients with AIS were obtained at the time of surgery. 4 mm punches were taken and disc tissue then extracted using 15 volumes of extraction buffer (4 M GuCl, 10mM EDTA, COMPLETE™, 50mM NaAc, pH 5.8) on a wet weight per volume basis. Extracted proteins were ethanol precipitated, and CHAD fragmentation was studied using SDS-PAGE and western blotting in combination with specific antibodies. In order to characterize the CHAD cleavage site, a degenerate disc extract was subjected to CsCl density gradient centrifugation in order to remove proteoglycans. CHAD was purified from the protein fraction by chromatography through carboxymethyl cellulose. The CHAD-containing samples were then fractionated on an SDS-PAGE gel and stained with Coomassie blue. Gel portions containing the CHAD fragment were excised, then lyophilized, reduced and alkylated, and subjected to trypsin digestion. Peptides were then identified by liquid chromatography mass spectrometry. Anti-neoepitope antibodies specifically recognizing the fragmented CHAD were generated by immunizing rabbits with synthetic peptides conjugated to KLH. The peptides represented the terminal amino acid sequences at the site of CHAD fragmentation. Proteinase digests of IVD tissue were performed in order to replicate an identical site of cleavage in vitro.

Results:
Proteolytic fragmentation of CHAD was observed in IVDs from patients with DDD and in discs showing premature degeneration from individuals with AIS. Its presence appeared to be related to the degree of degeneration in both cases. This phenomenon was found to be specific to disc degeneration, as CHAD fragmentation was not observed in healthy adolescent and adult discs from organ donors. Within the degenerate disc, fragmentation was evident in tissue with signs of degeneration but not in tissue that had no signs of degeneration. Furthermore, CHAD fragmentation increased with increasing severity of degeneration. Upon analysis with an anti-neoepitope antibody specifically recognizing the fragmented CHAD, it was apparent that fragmentation occurred at the same site in degenerate surgical samples from adults with DDD and adolescents with AIS (Figure 1). Normal tissue samples showed no anti-neoepitope antibody binding, confirming that CHAD fragmentation at this site is not present in the healthy disc. Furthermore, proteinase digests of IVD tissue yielded a single proteinase, HTRA1, with the ability to cleavage native CHAD at an identical site to that seen in degenerate disc tissue. This was confirmed by immunoblotting analysis with the anti-neoepitope antibody (Figure 2).
Discussion:

CHAD fragmentation is associated with disc degeneration present both in the adult with DDD and the adolescent with AIS, and fragmentation is created by cleavage at the same site within both disorders. Fragmentation is not, however, present in normal disc tissue at any age. Thus, CHAD fragmentation may distinguish catabolic processes leading to disc degeneration from those associated with normal turnover and aging of the tissue, and as such serve as a marker of disc degeneration.

Significance:

It is necessary to recognize the biochemical processes that specifically contribute to disc degeneration, if degeneration is to be prevented or retarded, and if novel treatments aimed at initiating disc regeneration are to be developed. To this end, inhibition HTRA1 could be a possible therapeutic option. In addition, if the CHAD fragment diffuses from the disc tissue, it could be used to develop a potential immunoassay to detect early disc degeneration and assess the efficacy of therapy.

Acknowledgments:

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References:


![Figure 1. Comparison of the site of CHAD fragmentation in adult degenerate disc and idiopathic scoliotic degenerate disc using a general anti-CHAD antibody and an anti-neo-epitope antibody](image-url)
Figure 2. HTRA1 digestion of normal disc tissue comparing undigested to digested tissue using a general anti-CHAD antibody and an anti-neo-epitope antibody