Pathophysiological Role of ADAM8 (A Disintegrin And Metalloproteinase 8) in intervertebral disc degeneration

Yeja Zhang, Miersalijiang Yaseen, Robert Mauck, Maurizio Pacifici, Lachlan Smith, Howard S. An, MD, Motomi Enoto-Iwamoto

INTRODUCTION:
Chronic back pain related to intervertebral disc (IVD) degeneration is a significant problem, costing billions in the U.S. alone. Despite the staggering disease burden, there is no current effective treatment to retard IVD degeneration and reduce associated pain because the etiology of IVD degeneration remains unclear. IVD degeneration is characterized by increased extracellular matrix degradation and a variety of cellular responses. ADAM8 (A Disintegrin And Metalloproteinase 8) is a membrane-anchored proteinase and involved in cell-matrix and cell-cell interactions in physiological and pathological processes. Our results lead to the hypothesis that ADAM8 is a key enzyme in the degenerative cascade in IVD tissues and represents a major, novel and potentially far-reaching step ahead in understanding disease etiology.

METHODS:
To examine expression and distribution of ADAM8 in IVD, degenerative annulus fibrosus and nucleus pulposus tissues were collected from patients undergoing surgery for back pain with appropriate institutional review board (IRB) approval. Degree of IVD degeneration was determined by MRI (grade V being the most degenerative). Distribution of ADAM8 in the IVD tissues was analyzed by immunostaining. ADAM8 and its specific proteolytic product, fibronectin fragments (FN-f) (VRAA271), were quantified by Western blotting (n=3-4 each grade). To examine the role of ADAM8 in IVD degeneration, we performed gain-of-function experiments in mice. Mature human ADAM8 ectodomain was generated in HEK293T cell line (BioZyme, Apex, NC), labeled with the infrared dye (IRDye, LI-COR Biosciences) and injected into the wild type mouse tail IVD. The ADAM8 or vehicle-injected IVD tissue was harvested 1-4 weeks after injection and subjected to histological inspection and immunostaining with an antibody to VDIPEN (neocutepop of cleaved aggrecan, a generous gift from Dr. J. Mott). We have also performed loss-of-function experiments in mice. The ADAM8 gene-inactivation mutant mice that harbor a point mutation, replacing the Histidine acid at position 330 with a Glutamine (ADAM8H330Q mice, generously provided by Dr. Anne Marie Maluff) was used to examine requirement of ADAM8 for IVD. We dissected IVDs from wild type and the ADAM8H330Q mice at 9 months of age and examined the neocutepop of the fibronectin fragment (FN-f) (VRAA271) by Western blotting. Aggrecan cleavage was examined by immunostaining.

RESULTS:
Immunostaining demonstrated that ADAM8 was expressed in human IVD tissues. Both ADAM8 and its proteolytic products (fibronectin fragments) were increased with IVD degeneration. These findings establish the clinical significance of ADAM8 in IVD degeneration. We observed that disorganized chondrocyte proliferation in IVDs was much more evident in the ADAM8-injected IVDs than in the PBS-injected IVDs in wild type mice. ADAM8-injected IVDs showed higher immunoreactivity to the antibody against the neocutepop of cleaved aggrecan, compared with the PBS-injected IVDs. These findings suggest that the ADAM8 proteolytic domain stimulates IVD degeneration.

There was no apparent developmental defect in the ADAM8H330Q mouse IVD. Fibronectin neocutepop VRAA271 resulting from ADAM8 cleavage was not observed in the IVDs of ADAM8H330Q mice but was present in wild type control mice, suggesting that ADAM8 is required for production of FN-f (VRAA271). Immunostaining study showed that wild type IVDs contained cleaved aggrecan (the neocutepop VDIPEN) in the nucleus pulposus. Such a phenotype was not detected in the ADAM8 mutant IVDs, indicating that inactivation of ADAM8 function suppresses aggrecan degradation in IVD.

SIGNIFICANCE:
The findings outlined above implicate ADAM8 as a critical mediator in IVD degeneration and support our central hypothesis that an excessive increase in ADAM8 proteolytic activity induces fibronectin cleavage and subsequent IVD degeneration, and that inhibition of ADAM8 could represent a therapeutic tool to delay or even halt the degenerative cascade in the IVD.