High-Mobility Group Box-1 Gene, a Potent Proinflammatory Mediator, Is Upregulated in More Degenerated Human Discs in Vivo and Its Receptor Is Upregulated by TNF-α Exposure in Vitro.

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Introduction: The mechanisms which control and enhance proinflammatory cytokine expression during disc degeneration are poorly understood. The high-mobility group box-1 gene (HMGB1) produces a protein which can itself act as a cytokine, or can function as a potent proinflammatory mediator. One previous animal study showed HMGB1 expression in rat caudal discs injected with lipopolysaccharide; HMGB1, IL-1β and TNF-α were significantly increased (1). Little is known about expression of HMGB1 in the human disc. Since proinflammatory cytokines increase significantly during human disc degeneration, we hypothesized that HMGB1 may show upregulation with advancing stages of degeneration, and upregulation in cells exposed to TNF-α.

Methods: Following IRB approval, annulus tissue was derived from 6 Thompson grade I-II discs, 9 grade III, 5 grade IV, and 3 grade V discs. Control (non-surgical) discs were obtained form the Cooperative Human Tissue Network. Total RNA was extracted using the TRIzol reagent, reverse transcribed to double-stranded cDNA, subjected to two rounds of transcription, and hybridized to the DNA microarray in the Affymetrix Fluidics Station 400. The GCOS Affymetrix GeneChip Operating System was used to determine expression levels. 3D cultured annulus cells from 4 grade I-II discs, and 6 grade IV-V discs were exposed to either control conditions or 10^3 pM TNF-α for 14 days (2), mRNA harvested and evaluated for gene array expression of the HMGB1 receptor (toll-like receptor 2). GeneSifterTM web-based software analyzed microarray data. Statistical significance was determined using the student t-test (2 tailed, unpaired, p<0.05 was taken as the significance level). Immunohistochemical studies were performed using paraffin-embedded disc specimens with anti-HMGB1 monoclonal antibody (MA5-17277, Pierce Biotechnology, Rockford, IL; 1:100 dilution for 120 min.). Three grade I, 4 grade II, 3 grade III, 4 grade IV, and 3 grade V discs were studied and digital images captured. Human brain tissue served as a positive control.

Results: Microarray analysis was used to search for HMGB1 gene expression in vivo in disc tissue. An 8-fold significantly greater expression level was seen in more degenerated Thompson grade V discs compared to healthier grade I/II discs (p=0.033, Figure 1A). We also tested for differences in control discs vs. herniated surgical disc specimens. Herniated specimens showed a 6.3-fold significantly greater expression level than that seen in control specimens (p=0.001, Figure 1B). Expression of the receptor to HMGB1, toll-like receptor 2, showed a 24-fold upregulation in vitro in cells exposed to TNF-α vs. controls (p=0.0003). Immunolocalization using an anti-HMGB1 monoclonal antibody showed that in human disc tissue there were abundant positive cells in the outer and inner annulus (Figure 2 A and B, single cells...
and cells in clusters, respectively; Figure 2C, negative control). Fewer cells showed positive localization in the nucleus (data not shown).

**Discussion:** HMGB1 can be passively released from necrotic or damaged cells, or actively secreted under conditions of stress. HMGB1 is known to be mediated by signaling pathways coupled to the receptor for advanced glycation end products (RAGE). The disc has two well recognized pathways which are activated by the toll-like receptors to HMGB1: p38 mitogen-activated protein kinase (MAPK) and nuclear factor-κB. Data presented here showed significantly greater expression of HMGB1 in more degenerated disc, and in herniated vs. non-herniated control discs. With both proinflammatory and immuno-stimulation properties, HMGB1 may be playing an important role as a proinflammatory mediator as reflected in our data showing a 24-fold significant increase in expression of its receptor, toll-like receptor 2, in TNF-α-exposed cells. HMGB1 may act on disc cells directly, amplifying proinflammatory signaling loops, or act as a late mediator in the development of inflammatory responses in annulus cells.

**Significance:** Novel data presented here demonstrated the presence of HMGB1 at the molecular and protein level in human intervertebral disc tissue and in cultured human annulus cells. With both proinflammatory and immuno-stimulation properties, the upregulation in HMGB1 identified here in more degenerated discs, coupled with our in vitro finding that its receptor was upregulated in cells exposed to TNF-α, strongly suggest that this gene plays a key role as a proinflammatory mediator during disc degeneration.
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