Therapeutic Potential of Caspase 3 Silencing for Biomechanical Overload-induced Intervertebral Disc Degeneration

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Disclosures:

Introduction: Although human intervertebral disc (IVD) degeneration leads to most spinal diseases, the pathogenesis of IVD degeneration remains unclear. One characteristic event early in the degeneration is the apoptosis of nucleus pulposus (NP) cells. There is increasing evidence that biomechanical overload is also a major etiology of IVD degeneration. We used in vitro and in vivo models of compressive loading to elucidate the underlying mechanism of IVD degeneration. In addition, we investigated whether the inhibition of apoptosis targeting caspase 3 is a potential clinical therapeutic strategy for the treatment of IVD degeneration induced by biomechanical stress.

Methods: All procedures in this study were approved by an ethical committee in our institution. (In vitro study) Human NP cell-agarose 3D composites were subjected to mechanical loading in a custom-designed bioreactor (Fig. 1). A 25% uniaxial unconfined static compressive strain, calculated based on sample thickness after preload, was applied. NP cell-agarose composites were divided in 4 groups as following: unloaded control (unloaded; non-transfected), compression only (loaded; non-transfected), control short interfering RNA (siRNA) (loaded; scrambled siRNA-transfected) and caspase 3 siRNA (loaded; caspase 3 siRNA-transfected). Cell viability and apoptosis was analyzed and western blot analysis was performed. All experiments were performed on 3 independent agarose cultures from 3 different individuals. (In vivo study) Effects of caspase 3 siRNA on biomechanical overload-induced IVD degeneration were investigated using a rabbit external compression model (Fig. 2). An animal was attached to the custom-made external-loading device and a 150-N axial compressive force was loaded on the L4/5 IVD. One week after operation, caspase 3 siRNA or control siRNA was subcutaneously injected into the center of L4/5 NP under fluoroscopy. 7.0Tesla-MRI, histological analyses were performed 8 and 16 weeks after siRNA injection. All experiments were performed on 8 discs from each treatment group and time-point. Statistical analyses were performed with ANOVA or Student’s t-test. P values <0.05 were considered statistically significant.
Results: \textit{(In vitro study)} Cell viability was significantly reduced after mechanical compression compared to unloaded control as indicated by Calcein AM staining. In contrast, apoptosis significantly increased after loading, as indicated by the TUNEL-positive cells (Fig. 3). Western blot analysis revealed that mechanical compression increased expression levels of cleaved caspase 3, several extracellular matrix-degrading enzymes (MMP-3, -13, ADAMTS-4 and ADAMTS-5) and decreased TIMP-1 expression level. Inhibition of caspase 3 signaling improves NP cell viability and reduces the production of the matrix-degrading enzymes. Importantly, protein expression of integrin α5 and integrin β1 was increased by loading, but not by caspase 3 knockdown (Fig.
(In vivo study) MRI and histological evaluation showed that degenerative changes were significantly suppressed in the caspase 3 siRNA group 8 and 16 weeks after injection (Fig. 4 & 5). Quantification of TUNEL staining showed that caspase 3 siRNA group had significantly fewer apoptotic NP cells. Likewise, the number of MMP-3-positive cells was significantly lower in the caspase 3 siRNA group. The expression of integrin α5β1 was increased by loading, but not by caspase 3.
Figure 4

A: T2-weighted MR images of intervertebral disc (IVD)s. B: Pfirman grading score of IVD degeneration. C: MRI index of nucleus pulposus alterations. (*, P<0.05; **, P<0.01)

Figure 5

Histology, immunohistochemistry and TUNEL staining of intervertebral discs 8 and 16 weeks after injection of caspase 3 siRNA or control siRNA into the rabbit nucleus pulposus (*, P<0.05; **, P<0.01).
Discussion: A single local injection of caspase 3 siRNA protected rabbits against IVD degeneration during at least 16 weeks of sustained compressive overload. This approach is based on a breakthrough discovery on the molecular mechanism of mechanical stress-induced NP destruction, showing that the imbalance in matrix-degrading enzymes production is largely dependent on caspase 3 activation. Therefore, the manipulation local apoptotic events may constitute a simple and effective means to prevent and/or reduce IVD degeneration. This noninvasive treatment strategy is expected to significantly impact future clinical research on IVD degeneration diseases.

Significance: Mechanical overload initiates IVD degeneration through a caspase 3-dependent imbalance in the expression of extracellular matrix enzymes. Inhibition of apoptosis by targeting endogenous caspase 3 was effective in preventing IVD degeneration induced by biomechanical stress.

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