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Time course investigation of intervertebral disc degeneration in a rat-tail puncture model

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ABSTRACT

Aims: Intervertebral disc (IVD) degeneration was believed to contribute to lower back pain. The aim of the study was to investigate the pathogenesis and regulatory mechanism of puncture-induced IVD degeneration.
Main methods: We established a rat-tail puncture model using Kirschner wire and a homemade stopper. The progress of disc degeneration was evaluated by histological examination and the quantitative measurement of type I, type II collagen and other factors expression at 0.5, 1, 2, 6, and 12 weeks after puncture and was compared with control rats of the same age.
Key findings: Histological examination and Safranin-O staining revealed progressive degeneration of the punctured disc. Matrix metalloproteinase 13 (MMP13) was increased at 1 week after puncture but did not change in the control group. The interleukin-1 beta (IL-1β) mRNA expression level was elevated at the acute stage after puncture compared with the control group. The hypoxia inducible factor 2 (HIF-2) increased expression in punctured groups. Additionally, compare to adjacent non-punctured segments, *HIF-2α* expression level transiently increased and then decreased in the nucleus pulposus immediately following puncture, and it then increased 12 weeks after puncture.
Significance: The degenerative changes observed in this rat-tail puncture model are similar to human disc degeneration and that this model may be valuable for elucidating the molecular mechanisms and pathways underlying disc degeneration.

1. Introduction

Back pain is a widespread issue, with 70–85% of all people suffering from back pain at some point in their life [1]. Though multiple factors can contribute to back pain, it has been correlated with the degeneration of the intervertebral disc (IVD) [2]. The healthy and immature IVD of human is composed of dense connective tissue, which can be divided into the fibrocartilaginous annulus fibrosus (AF), cartilaginous endplate and the more gelatinous nucleus pulposus (NP), which is rich in notochordal cells (NCs) [3]. During growth and maturation, the IVD expands, and the NP becomes more fibrous as the cellular niche is altered to include a greater percentage of matrix, increased hypoxia, and reduced nutrient transport and these notochordal cells may eventually be lost and replaced by small nucleus pulposus cells (SNPCs) that migrate from the vertebral bodies through the endplate [4–6].

IVD degeneration is associated with an imbalance between catabolism and anabolism of extracellular matrix components and diversity of disc cells [7,8]. It is characteristic by elevated levels of inflammatory cytokines TNF, IL-1α, IL-1β, IL-6, and IL-17 and abnormal production of catabolic molecules [9,10]. Inflammatory cytokines such as IL-1β and TNF-α can increase the expression of matrix-degrading enzymes, such as matrix metalloproteinases (MMPs), and a disintegrin and metalloproteinase that contain thrombospondin motifs, and can promote

Abbreviations: ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; AF, annulus fibrosus; BMP, bone morphogenic protein; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; HIF, hypoxia inducible factor; HIF-2, hypoxia inducible factor 2; IL, interleukin; IL-1β, Interleukin-1 beta; IVD, intervertebral disc; MMP, matrix metalloproteinase; NP, nucleus pulposus; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor α.

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extracellular matrix degradation, chemokine production, and changes in IVD cell phenotype [11]. The loss of extracellular matrix may be linked to the expression and activity of major MMPs. Previous studies have suggested that MMPs (-1, -2, -3, -7, -9 and -13) are highly expressed during IVD degeneration [11–13]. It remains unclear which members of the MMP family are primarily responsible for the matrix disruption and for the decomposition and dynamic changes of MMPs during IVD degeneration. In contrast, factors such as TGF-β and bone morphogenic proteins can increase synthesis of type II collagen and proteoglycan [14–16]. The resulting imbalance in catabolic and anabolic responses leads to the degeneration of IVD tissues and to disc herniation and radicular pain.

The IVD is the largest avascular structure in the body which imposes a hypoxic stat on the NP cells [17,18]. Hypoxia inducible factor (HIF) is a transcription factor that initiates a coordinated cellular cascade in response to a low oxygen tension environment [18]. In nucleus pulposus, hypoxia-inducible factors can regulate survival of NP cells [19]. Furthermore, NP cells can expression HIF-1α under normoxic culture conditions and indicated that normoxic stabilization of HIF-1α is a metabolic adaptation of NP cells to a unique oxygen-limited microenvironment. The study confirmed that HIF-1α can be used as a phenotypic marker of NP cells [20]. In contrast, the role of HIF-2 is still unclear in the environmental regulation in NP cells.

Although the cellular components of the disc differ between humans and animals, numerous animal models have been established to study the mechanism of IVD degeneration. Physical injury of the IVD, which can cause physical disruption of the disc structure, is a relatively simple method to induce its degeneration [21–24]. Percutaneous needle puncture induces degenerative signs similar to those of human discs [25–27]. A recently developed needle puncture model has shown altered morphological and biochemical features similar to many of those found in human degenerative discs [28]. This model could enable the study of the pathological changes and underlying molecular mechanisms of IVD degeneration. In the present study, we established a tail puncture model in rats to induce IVD degeneration in 12 weeks. Histological and biochemical analyses were performed by time course investigation to examine the process of IVD degeneration in this model.

2. Materials and methods

2.1. Annulus fibrous needle puncture

All procedures for the rat experiments were approved by the Ethical Committee of Animal Experimentation of National Yang-Ming University, Taipei, Taiwan. Twelve-week-old male Sprague-Dawley rats were purchased from BioLASCO Taiwan Co., Ltd. The rats were anesthetized by an intramuscular injection of tiletamine/zolazepam (50 mg/kg). The tail skin was incised longitudinally, and the subcutaneous connective tissue and musculature were separated. Discs C6/7 and C7/8 were identified and punctured using a Kirschner wire (0.8 mm in diameter) with a stopper (Fig. 1A). The diameter of 12-week discs is around 10 mm, the depth of penetration (approximately 5 mm) was controlled by the stopper. After full penetration, the Kirschner wire was rotated 360° and held for 30 s (Fig. 1A). Rats of the same age that were incised longitudinally but not punctured served as a sham-operated control. After the operation, the wound was closed by suturing. The rats were allowed free activity in their cages.

2.2. Histological staining

Animals were sacrificed at 0.5, 1, 2, 6, and 12 weeks post-puncture by CO_2 inhalation. Six rats were examined in each group at each time point. Whole discs with the vertebrae adjacent to the punctured segments and non-punctured segments were removed and dissected. Tissue was fixed in 4% paraformaldehyde for 24 h, decalcified in 10% ethylenediamine tetra-acetic acid for 2 weeks, paraffin-embedded, and sectioned to 5-μm thickness with a microtome. The sections were stained with hematoxylin and eosin, and then scanned using Scanscope CS system (Aperio Technologies, Vista, CA, USA). The morphologic changes and histological appearance of the nucleus pulposus and annulus fibrosus were examined to evaluate the degree of degeneration. The cell clusters indicated the degeneration pattern.

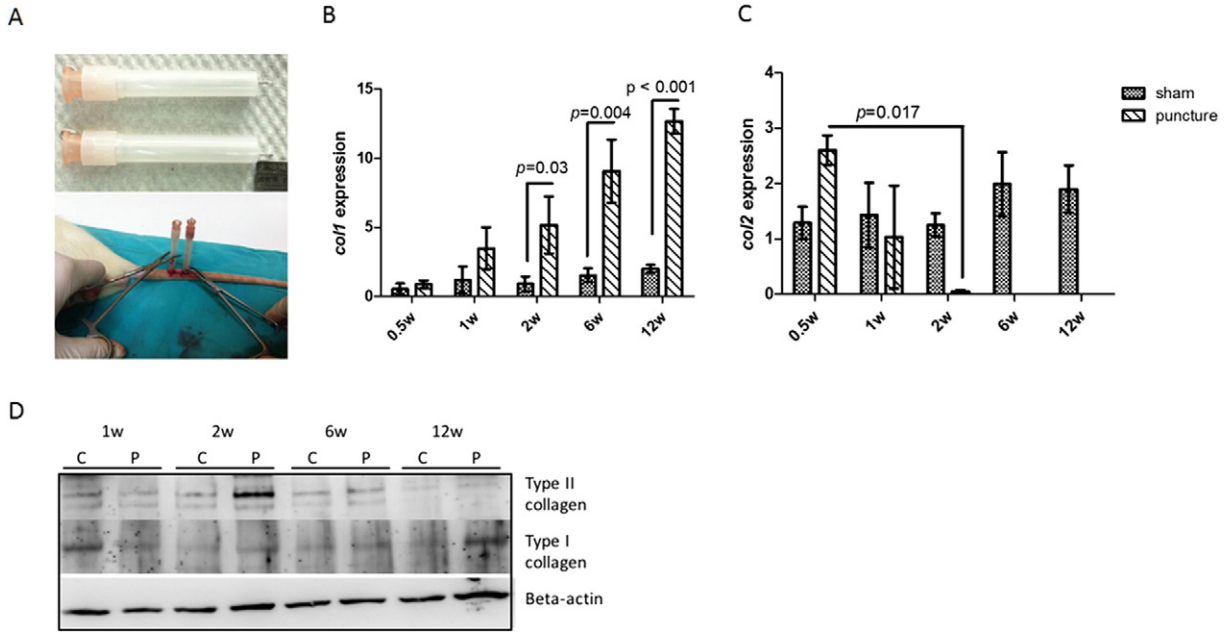

Fig. 1. Type I and II collagen expression in punctured nucleus pulposus and morphological changes to punctured discs. (A) Discs were punctured by Kirschner wire (0.8 mm in diameter) with a homemade stopper. *Col1a1*(B) and *Col2a1* (C) expression in the punctured and control NP.·(B) The continuous increase of *Col1a1* in the punctured NP compared to same-aged controls ($p < 0.05$; $n = 3$). (C)Expression of *Col2a1* was downregulated significantly from 0.5 weeks to 12 weeks after the puncture ($p < 0.05$; $n = 3$). Error bars in graphical data represent mean ± s.d. (D) Protein expression of Collagen I increased at 12 weeks after puncture, and Collagen II increased at 2 week after puncture and decreased gradually over time. (E) Expression of *Col2a1* was steady in control NP.