Activation of Integrated Stress Response in the Nucleus Pulposus results in Disc Degeneration

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INTRODUCTION: Intervertebral disc degeneration (IVDD) is a leading cause of low back pain and is closely associated with abnormal mechanical loading of the spine. However, the molecular mechanisms linking mechanical stress to cell phenotypic alterations in degenerative nucleus pulposus (NP) remain poorly understood. The integrated stress response (ISR) is a conserved signaling pathway activated by diverse cellular stresses. We hypothesized that activation of the ISR mediates mechanical stress-induced cellular reprogramming and contributes to cell heterogeneity within the degenerative NP.

METHODS: We performed single-cell RNA sequencing (scRNA-seq) on nondegenerative and degenerative human intervertebral disc tissues to identify disease-associated molecular pathways and validated the key findings by immunostaining. To investigate these mechanisms *in vivo*, two complementary mouse models were used. The first was a genetically modified mouse line carrying a 13-base pair deletion in *Col10a1* (13del mouse), which develops progressive disc degeneration due to abnormal physiological mechanical loading, modeling endogenous stress-induced pathology. The second model involved applying asymmetric spinal loading to wild-type (WT) mice using the tail-looping method to induce artificial mechanical stress. The human study was approved by the Institutional Review Board, with informed consent obtained from all participants. All animal procedures were followed protocols approved by the Committee on the Use of Live Animals in Teaching and Research at the University of Hong Kong. Both sexes were included in all analyses (mouse samples: n = 4–6 per group; human samples: n = 3 per group).

RESULTS: ScRNA-seq revealed the ISR activation in NP cells of degenerative human intervertebral discs (Figure 1). ISR activation was associated with reduced notochordal- and chondrocyte-like cell populations and marked expansion of fibroblast-like cells, findings validated by immunofluorescence staining in degenerative human disc sections (Figure 1). Histological analysis demonstrated that 13del mice developed progressive disc pathology closely resembling human IVDD. By 16 months of age, these mice exhibited NP condensation, annulus fibrosus disruption, increased fibroblast-like cells, and loss of notochordal cells (Figure 2). Immunofluorescence confirmed sustained ISR activation (ATF4, CHOP, and XBP1s) and fibroblast marker expression (αSMA) in 13del mice as early as 6 months of age, indicating that molecular changes precede overt structural degeneration (Figure 2). Nuclear accumulation of YAP/TAZ was observed in NP cells from both degenerative human discs and 6-month-old 13del mice discs, implicating mechanotransduction signaling in ISR activation (Figure 3A–D). Moreover, tail-looping-induced asymmetric spinal loading in WT mice reproduced the 13del phenotype, confirming that mechanical stress alone is sufficient to activate the ISR and drive disc degeneration (Figure 3E).

DISCUSSION: Our study establishes the ISR as a key molecular mechanism linking mechanical stress to IVDD. Through human tissue analysis and mouse models, we demonstrate that abnormal mechanical loading activates the ISR, driving cellular reprogramming from notochordal/chondrocyte-like to fibroblast-like phenotypes. The persistent ISR activation observed in both human degenerative discs and mechanically stressed mouse models reveals a convergence of YAP/TAZ mechanotransduction and stress response pathways. These findings identify the ISR as a promising therapeutic target for preventing or relieving stress-induced disc degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: By uncovering the ISR as a central molecular pathway connecting biomechanical loading to NP cell reprogramming, this work advances understanding of IVDD pathogenesis and identifies a translationally relevant therapeutic target. ISR inhibitors such as ISRIB represent potential interventions for degenerative spine diseases that could significantly impact clinical management of chronic low back pain.

IMAGES AND TABLES:

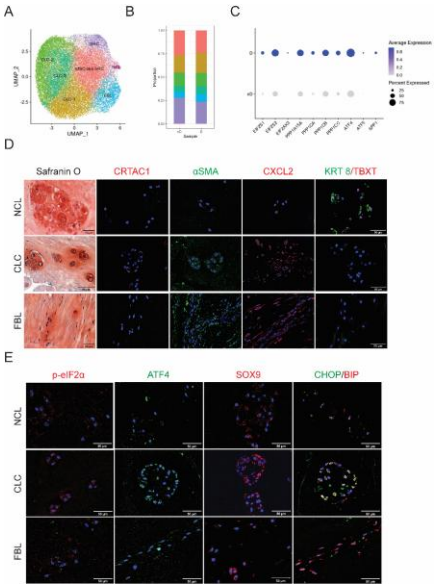

Figure 1 ISR activation in human degenerated nucleus pulposus cells.

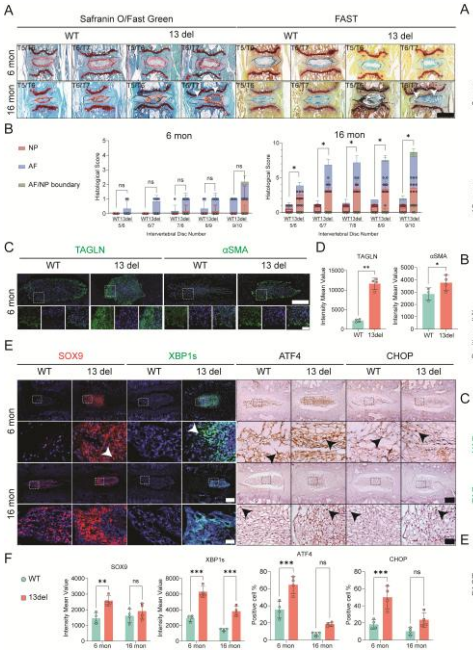

Figure 2 Mouse model of spontaneous disc degeneration exhibits ISR activation in the nucleus pulposus.

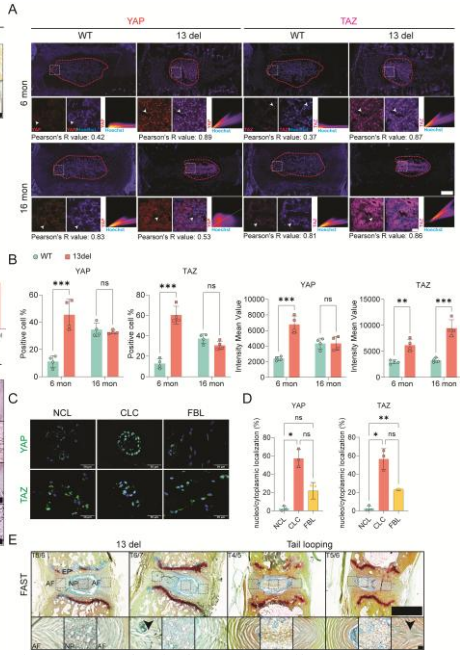

Figure 3 Mechanical stress contributes to intervertebral disc degeneration