# ANNULUS FIBROSUS CELLULAR SENESCENCE IN INTERVERTEBRAL DISC DEGENERATION IS ASSOCIATED WITH BIOMECHANICS OF ANNULUS FIBROSUS

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**INTRODUCTION:** Degenerative disc degeneration (DDD) is the major cause of low back pain resulting from or associated with the degeneration of intervertebral discs, which seriously affects the life quality of patients [1]. Current surgical and conservative treatments only relieve the pain temporarily yet fail to restore the normal biomechanics and functions of healthy spine. Degenerative changes in biomechanical and structural properties of the intervertebral disc (IVD), including rarefaction in annulus fibrosus (AF) and volume loss of nucleus pulposus (NP), mainly contribute to DDD development [2]. AF plays a critical role in the biomechanical properties of IVD as its structural integrity is essential to confine NP and maintain physiological intradiscal pressure under loading. It has been known that ageing organisms accumulate senescent cells that are thought to contribute to body degeneration [3]. However, mechanism of cell senescence in AF within disc degeneration is unclear. Therefore, it is essential to understand the association of AF cellular senescence with biomechanical changes of AF during IVD, which can also provide valuable insights into the repairment and regeneration of AF to alleviate DDD.

**METHODS:** The clinical study was reviewed and approved by the Ethics committee of the Affiliated Hospital of Guizhou Medical University. Collection of human disc specimen from lumbar spine surgery was approved by IRB. We used degenerative disc specimen (n=20) from patients undergoing lumbar spine surgery. Control disc specimen (n=8) were collected from patients undergoing surgery for spine fractures or spine tumors (Grade I or Grade II Pfirrmann, non-degenerated discs on MRI). The degenerative discs were Pfirrmann Grade III to V. HE and Masson staining were performed to evaluate the AF structure, and Immunohistochemistry of the senescence marker p16INK4A was used to determine the cell senescence of AF. Atomic force microscopy (AFM) and the In Situ Three-Point Bending Test were used to test the elastic modulus of human AF tissue. Data represents mean ± SD. Statistical significance was calculated using student t-test analysis. Pearson Correlation and linear regression analyses were completed in GraphPad Prism V9.0.

**RESULTS:** Pfirrmann Grade I and II as control showed no degenerative changes compared with IVD patients with degenerative changes (**Fig.1a**). HE and Masson staining showed that degenerative AF with Pfirrmann Grade IV have a significant rarefaction relative to a non-degenerated AF with Pfirrmann Grade I disc (**Fig.b**). Immuno-positive AF cells of p16INK4A were significantly observed in human AF tissue particularly within those of high grades of disc degeneration (**Fig.2a**). AFM Imaging revealed that the elastic modulus of the collagen fiber significantly increases in degenerative AF compared with non-degenerated AF (**Fig.2b**). Moreover, AF senescent cells were also associated with the presence of Pfirrmann Grade (**Fig.2c**). Of note the number of immune positive cells for p16INK4A positively correlated with the elastic modulus of the collagen fiber of AF (**Fig.2d**).

**DISCUSSION:** Our findings indicated a role of AF cellular senescence that contributes to IVD and associated with biomechanical changes of AF. We provided evidence to support a central role of AF cellular senescence in this regulatory process in human IVD caused by the change of biomechanical and structural properties. Whether this AF biomechanical change is a directly result of replicative cell senescence is currently being investigated in vitro.

**SIGNIFICANCE**: IVD has been associated with approximately 40% of low back pain patients, however, to date no therapies target the pathogenesis of disc degeneration. Cellular senescence (aging) has been shown to be accelerated during disc degeneration but the mechanisms behind this are not known. This study implicates AF cellular senescence as a potential driver for AF biomechanical change within the IVD.

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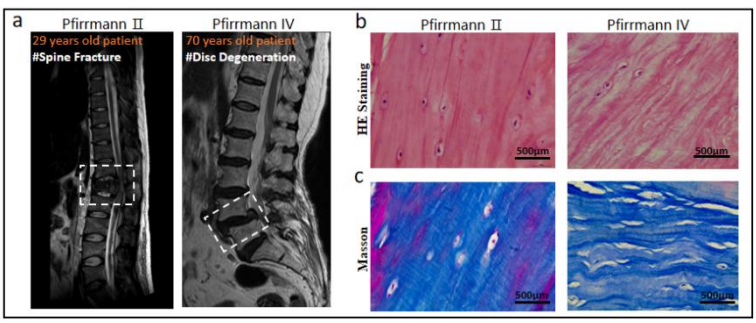
**ACKNOLEDGEMENTS**: This work was supported by NSFC (82360420); Doctor Start-up Fund of Affiliated Hospital of Guizhou Medical University (gyfybskj-2023-07); Guizhou Provincial Natural Science Foundation (Qiankehejichu[2023]387).



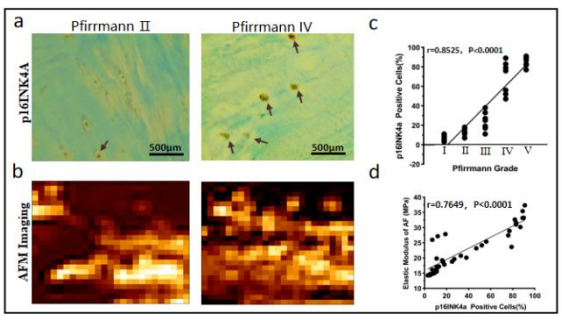




**Figure.1 Change of AF structure is associated with the degeneration of intervertebral discs. Fig.1a** MRI image from patients with different Pfirrmann Grade. **Fig.1b&c** HE and Masson staining of AF tissue from Pfirrmann Grade II and IV. Scar bar:500μm.

**Figure.2 AF senescent cells correlated with the structural properties of AF in IVD. Fig.2a** Immunopositively staining for p16INK4a. **Fig.2b** AFM Imaging of AF tissue from Pfirrmann Grade II and IV. **Fig.2c** Correlation of number of immune-positive cells for p16INK4A and Pfirrmann Grade in human AF of intervertebral discs. **Fig.1d** Correlation of number of immune-positive cells for p16INK4A and the elastic modulus of the collagen fiber of AF. Scar bar:500μm.