

Original Article

Molecular Mechanisms of Intervertebral Disc Degeneration: Significance of SPARC Gene Expression

Jiancong Zeng^{1,2,3}, Song Yan^{1,2,3}, Wei Dong², Jinsong Wei³¹The First Clinical Medical College of Guangdong Medical University, Zhanjiang, China;²Department of Orthopedics, Shiyan People's Hospital, Bao'an District, Shenzhen, China;³Department of Spinal Degeneration and Deformity Surgery, Affiliated Hospital of Guangdong Medical University, Zhanjiang, China**Abstract**

Objectives: To explore the role of the *SPARC* gene in intervertebral disc degeneration (IDD) using bioinformatics analysis combined with *in vitro* experiments. **Methods:** Single-cell sequencing data from the GEO and CNGBdb databases were analyzed to identify differential *SPARC* expression across degeneration grades. *In vitro*, nucleus pulposus cells were treated with LPS and *SPARC*, followed by RT-qPCR, Western blot, immunofluorescence, and immunohistochemistry to assess gene and protein expression changes. **Results:** *SPARC* expression was significantly upregulated in degenerated nucleus pulposus cells and correlated with altered *COL2A1* and *ACAN* expression. **Conclusion:** *SPARC* may play a key role in IDD pathogenesis, offering a potential therapeutic target for future interventions. These findings contribute to our understanding of the molecular mechanisms underlying IDD and pave the way for targeted therapies.

Keywords: Extracellular Matrix, Intervertebral Disc Degeneration, Single-Cell Sequencing, SPARC

Introduction

Lower back pain (LBP) is a major contributor to the demand for rehabilitation services and poses a significant economic burden worldwide¹. LBP encompasses various types of pain, with intervertebral disc degeneration (IDD) being the most common cause^{2,3}. Over the years, numerous studies have investigated the etiological factors of degenerative disc disease and its pain-generating pathways to identify strategies for preventing further degeneration and discovering better therapeutic targets to alleviate pain^{4,5}. The intervertebral disc primarily consists of the nucleus pulposus, annulus fibrosus, and cartilaginous endplates⁶, with nucleus pulposus degeneration being the key driver of intervertebral disc degeneration⁷. Historically, research on the pathogenesis of degenerative intervertebral discs has

focused on histopathological changes⁸, gene expression, and signaling pathways in the nucleus pulposus^{9,10}. However, the differentially expressed gene markers involved in disc tissue degeneration have not been fully elucidated. A deeper investigation into these expression differences could provide novel insights into the treatment of lumbar disc degeneration. Therefore, understanding these differential expression changes and their biological mechanisms is crucial.

Previous studies have investigated marker genes in nucleus pulposus cells, including *COL2A1*¹, *ACAN*, and *SOX9*¹¹ in normal human nucleus pulposus cells, as well as *COL1A1*, *FN1*¹², *MMP13*, and *SPARC*^{13,14} in degenerated cells. Among these, *SPARC* (Secreted Protein Acidic and Cysteine-Rich), an extracellular matrix (ECM) component, is highly expressed in normal bone, wound repair sites, and tissue remodeling, where it plays a role in regulating cellular activity and ECM composition^{15,16}. *SPARC* is also involved in bone formation^{17,18} and promotes the accumulation of type I collagen^{19,20}. Despite evidence suggesting differential *SPARC* expression during nucleus pulposus tissue degeneration, its precise role in intervertebral disc degeneration remains unclear. While previous studies have indicated a trend in *SPARC* expression changes during degeneration, its specific involvement in disease progression and underlying biological mechanisms require further investigation.

Intervertebral disc degeneration is characterized by

The authors have no conflict of interest.

Corresponding author: Jinsong Wei, Department of Spinal Degeneration and Deformity Surgery, Affiliated Hospital of Guangdong Medical University, No. 57, South Renmin Avenue, Xiashan District, Zhanjiang 524001, Guangdong Province, China
E-mail: jinsong.wei@gdmu.edu.cn

Edited by: G. Lyritis

Accepted 14 March 2025



the progressive degeneration of nucleus pulposus tissue. Research into the marker genes of degenerating nucleus pulposus cells is essential for understanding this disease. In this study, we aim to explore *SPARC* expression changes during nucleus pulposus cell degeneration and its potential impact on cellular processes.

Materials and Methods

Data collection

The dataset GSE165722 was obtained from the Gene Expression Omnibus (GEO) database, while the CNPOO02664 dataset was sourced from the China National GeneBank (CNGBdb). These datasets contain single-cell sequencing data from normal and degenerated human nucleus pulposus tissues.

Cell Culture and Drug Intervention

Normal human nucleus pulposus cells (*iCell-0028a*, iCell Bioscience Inc., China) were used, and the culture medium was obtained from the iCell Primary Chondrocyte Culture System (PriMed-iCELL-O20, iCell Bioscience Inc., China). Cells were maintained in an incubator at 37°C with 5% CO₂ until they reached the second passage. Passaged nucleus pulposus cells were seeded into culture flasks and incubated until they reached 50–70% confluence. Cells were then treated with PBS (control), LPS, SPARC or LPS + SPARC in different experimental groups. After 48 hours of drug exposure, cells were collected for further analysis. The effective concentrations used for treatment were 10 µg/mL LPS²¹ and 2 µg/mL SPARC²².

RT-qPCR Analysis of Gene Expression in Nucleus Pulposus Cells

To evaluate gene expression changes induced by LPS and SPARC, human nucleus pulposus cells were treated for 48 hours, followed by RT-qPCR analysis. The expression levels of *COL2A1*, *COL1A1*, *ACAN*, and *SPARC* were quantified in each experimental group. By analyzing the expression changes of these target genes, differentially expressed genes in normal and degenerated nucleus pulposus cells were identified.

mRNA levels were quantified using the $2^{-\Delta\Delta Ct}$ method, with *GAPDH* as an endogenous control. Primer sequences used for amplification were as follows:

COL2A1

- Forward: 5'-CAAGAACAGCATTGCCTATCTG-3'
- Reverse: 5'-GATAACAGTCTTGCCCCACTTA-3'

COL1A1

- Forward: 5'-AAAGATGGACTCAACGGTCTC-3'
- Reverse: 5'-CATCGTGAGCCTTCTCTTGAG-3'

ACAN

- Forward: 5'-TCCTGGTGTGGCTGCTGTCC-3'
- Reverse: 5'-TCTGGCTCGGTGGTGAACCTAG-3'

SPARC

- Forward: 5'-CAAGAAGCCCTGCCTGATGAGAC-3'
- Reverse: 5'-TTCCGCCACCACCTCCTCTTC-3'

GAPDH

- Forward: 5'-GTCTCCTCTGACTTCAGCG-3'
- Reverse: 5'-ACCACCCTGTTGGTAGCCAA-3'

Western Blot Analysis of Protein Expression

Protein expression changes in nucleus pulposus cells were analyzed using western blotting. Cells from each experimental group were rinsed twice with PBS and lysed in RIPA buffer (R0010, Solarbio) containing 1% phenylmethanesulfonyl fluoride (PMSF) for 10 minutes. Lysates were then centrifuged at 15,000×g for 10 minutes at 4°C, and protein concentrations were measured using a BCA protein assay kit (P0010, Beyotime).

Proteins were separated by 12.5% SDS-PAGE and transferred onto PVDF membranes (TM-PVDF-R-45, LABSELECT). Membranes were blocked with Western Blocking Solution (P0023B, Beyotime) for 90 minutes at room temperature, followed by overnight incubation at 4°C with primary antibodies against ACAN (DF7561, Affinity), SPARC (DF6503, Affinity), COL2A1 (AF0135, Affinity), COL1A1 (AF7001, Affinity), and GAPDH (AF7021, Affinity).

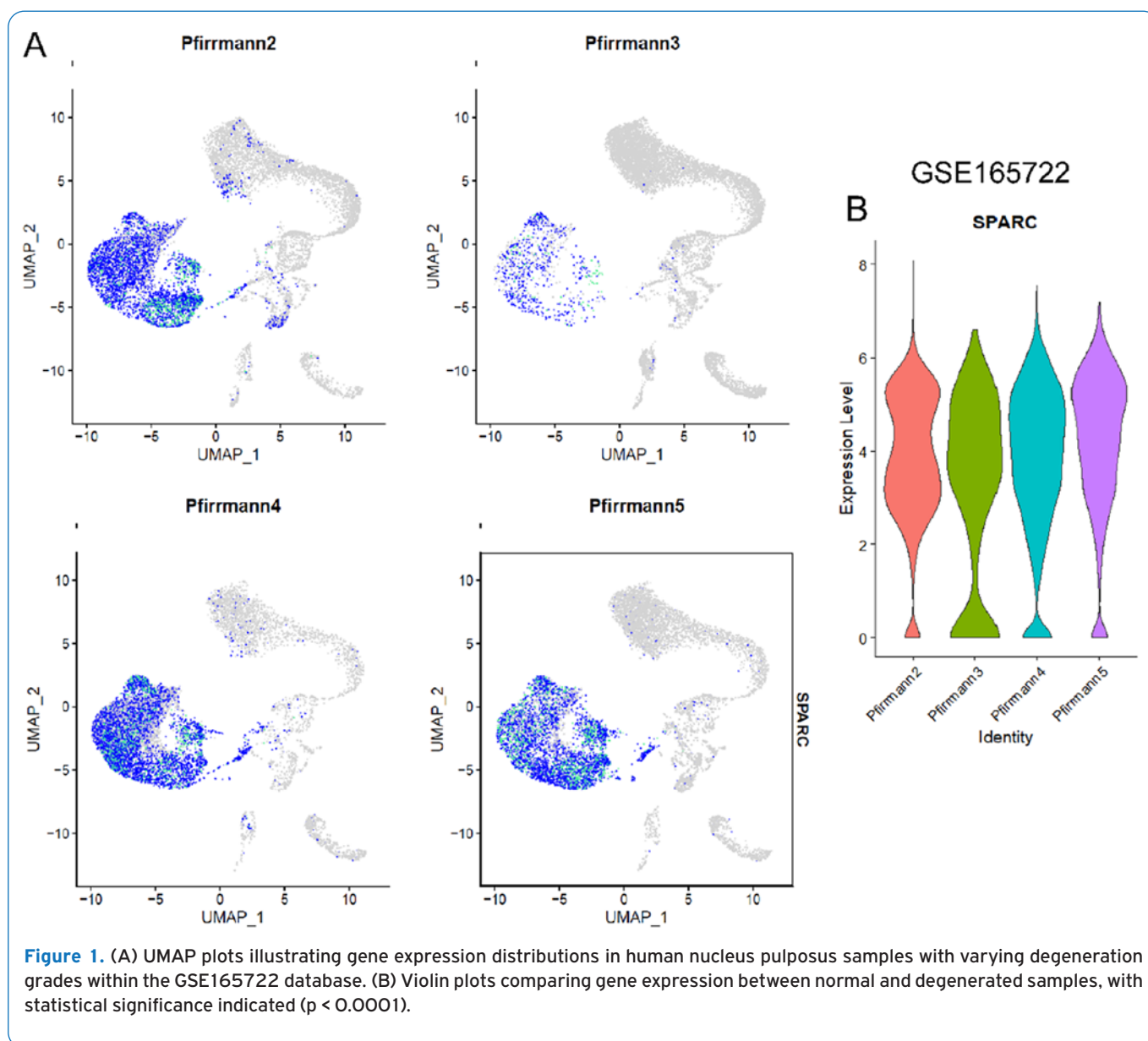
After incubation with secondary antibodies for 1 hour at room temperature, protein detection was performed using BeyoECL Plus (P0018FM, Beyotime). Band intensity was quantified using ImageJ software (version 1.8.0).

Immunofluorescence Staining

Nucleus pulposus cells were fixed with 4% paraformaldehyde (PFA) for 20 minutes, followed by permeabilization with 0.5% Triton X-100 for 20 minutes. After blocking with 5% bovine serum albumin (BSA) for 10 minutes at room temperature, cells were incubated overnight at 4°C with a primary antibody against COL2A1 (AF0135, Affinity). The following day, cells were incubated with a secondary antibody for 1 hour at room temperature. An anti-fade mounting medium (BY-1002) was used to preserve fluorescence and prevent DAPI (4',6-diamidino-2-phenylindole) signal degradation. Imaging was performed using a Zeiss LSM 880 confocal microscope.

Immunohistochemistry Analysis

Immunohistochemistry was performed to detect RUNX-2 protein expression in nucleus pulposus cells. Cells were seeded in a 6-well plate at a density of 10⁴ cells per well and divided into two groups: a control group and a SPARC-treated group. After 48 hours of co-culture, cells were resuspended in medium at a density of 5 × 10⁵ cells/mL. Cells were fixed with 4% PFA for 30 minutes, followed by



blocking with goat serum to prevent nonspecific antibody binding. Slides were then incubated overnight at 4°C with a primary antibody against SPARC (DF6503, Affinity), followed by a SPARC-conjugated secondary antibody (1:50 dilution) for 30 minutes at room temperature. Finally, cells were visualized, and images were captured using a microscope.

Statistical analysis

Bioinformatics analyses, including cluster analysis, differential expression analysis, and marker gene identification were conducted using R software (version 4.2.0). Experimental data were analyzed, and histograms

were generated using GraphPad Prism (version 8.0). Differences among groups were compared by one-way analysis of variance with Dunnett test. A P-value of < 0.05 was considered statistically significant.

Results

Differential Expression of SPARC in Normal and Degenerated Nucleus Pulposus Cells

By analyzing single-cell sequencing data from the GEO and CNGBdb databases, we explored the differential expression of SPARC in normal and degenerated human nucleus pulposus tissues (Figures 1 and 2). The results revealed distinct expression patterns of SPARC between normal

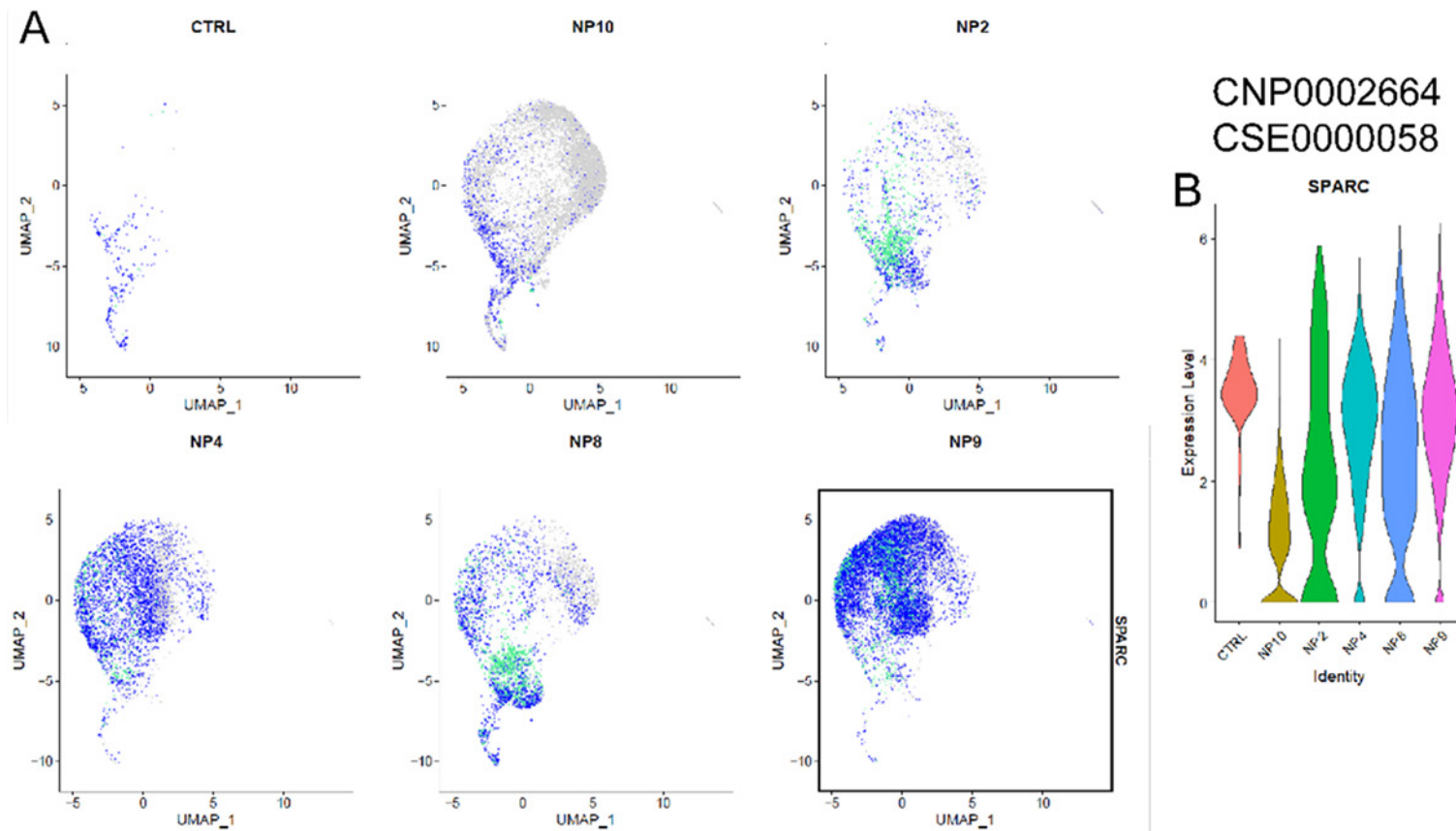


Figure 2. (A) UMAP plots illustrating gene expression distributions in human nucleus pulposus samples with varying degeneration grades within the CNP0002664 database. (B) Violin plots comparing gene expression between normal and degenerated samples, with statistical significance indicated ($p < 0.0001$).

and degenerated samples. In the GSE165722 dataset⁹, single-cell sequencing analysis was performed on nucleus pulposus tissues at four degeneration levels, corresponding to Pfirrmann grades 2, 3, 4, and 5. SPARC was detected in nucleus pulposus cells across all grades (Figure 1A), with a progressive increase in the number of highly expressing cells as degeneration worsened (Figure 1B). Similarly, analysis of the CNP0002664 dataset²³, which included one normal and five degenerated nucleus pulposus samples, demonstrated a comparable pattern

(Figure 2A). Both SPARC expression intensity and the number of expressing cells increased with degeneration (Figure 2B).

Gene Expression Changes in Nucleus Pulposus Cells Induced by LPS and SPARC Treatment

RT-qPCR was performed to analyze gene expression changes in nucleus pulposus cells treated with PBS ($n = 4$), LPS ($n = 4$), SPARC ($n = 4$), and LPS + SPARC

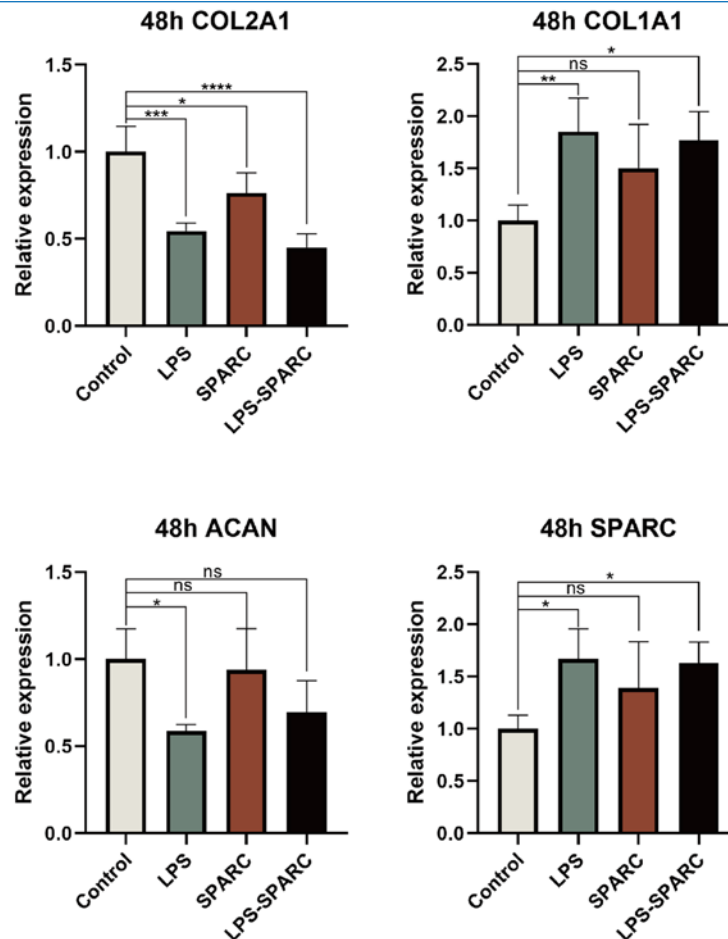


Figure 3. Gene expression changes in nucleus pulposus cells treated with each experimental group for 48 hours, detected by RT-qPCR. (**p < 0.05, *p < 0.01, ***p < 0.001, ****p < 0.0001, ns indicates no statistically significant difference).

(n = 4) for 48 hours (Figure 3). LPS-induced degeneration was confirmed by the downregulation of COL2A1 and ACAN, along with the upregulation of COL1A1 and SPARC, consistent with the findings from single-cell sequencing. In the group treated with SPARC alone, a similar expression pattern was observed: COL2A1 and ACAN were downregulated, while COL1A1 was upregulated. Notably, co-treatment with LPS and SPARC resulted in gene expression trends similar to those observed with individual treatments.

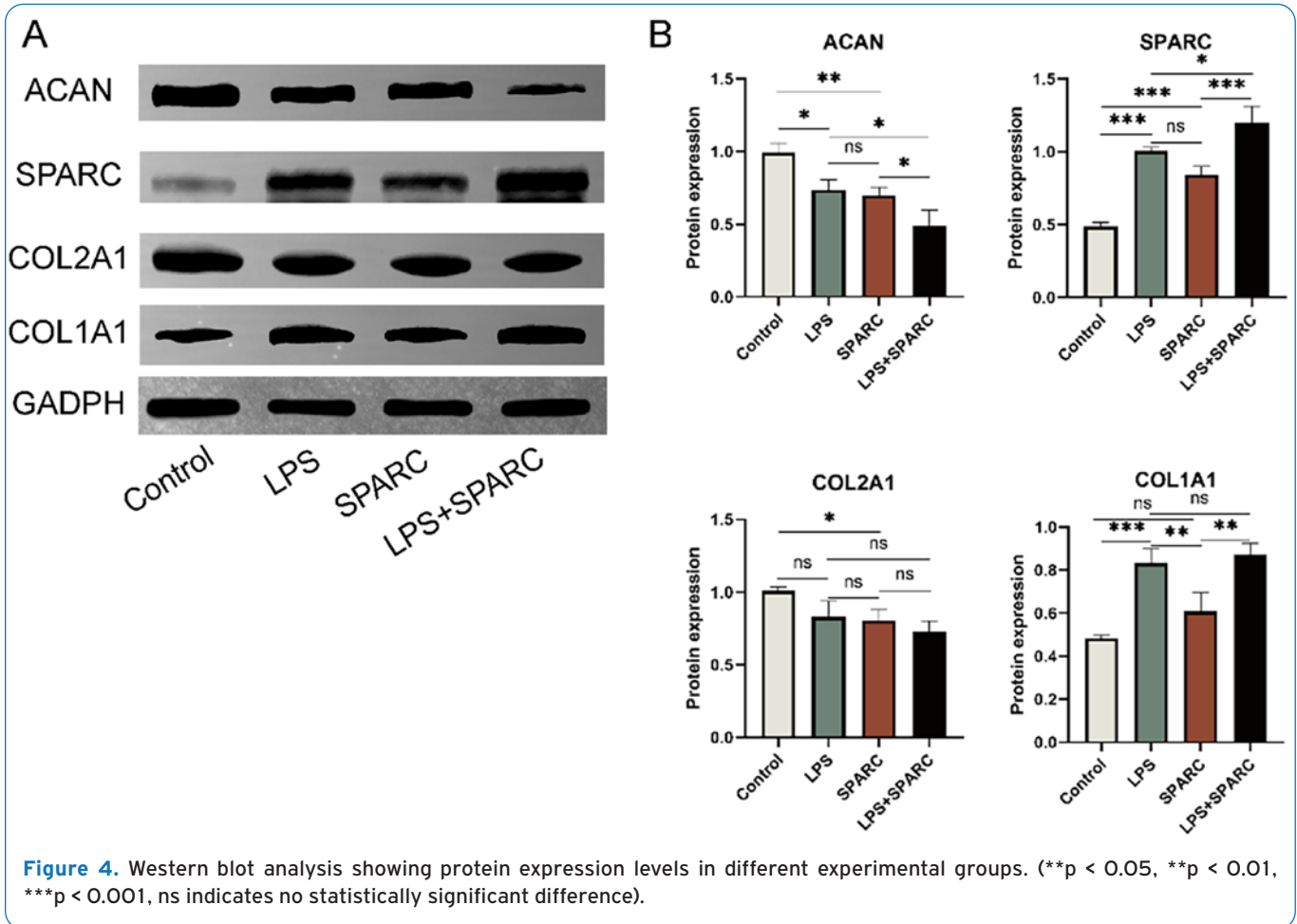
Quantitative Analysis of Protein Expression Changes Induced by LPS and SPARC

Western blot analysis was used to assess protein expression changes in nucleus pulposus cells treated with PBS (n = 3), LPS (n = 3), SPARC (n = 3), and LPS + SPARC (n = 3) for 48 hours (Figure 4A). Compared with the control group, the expression of COL2A1 and ACAN

decreased, while SPARC and COL1A1 were upregulated in both the LPS- and SPARC-treated groups (Figure 4B). Western blot analysis further confirmed that there was no significant difference in expression levels between the LPS and SPARC groups, indicating that SPARC may exert effects similar to LPS in nucleus pulposus cells.

Immunofluorescence Detection of Collagen Expression Changes in Nucleus Pulposus Cells After SPARC Intervention

Immunofluorescence was used to assess the protein expression of COL2A1 in nucleus pulposus cells treated with PBS (n = 3), LPS (n = 3), and SPARC (n = 3) for 48 hours (Figure 5A). The fluorescence intensity of COL2A1 was found to decrease in both the SPARC- and LPS-treated groups compared to the control group, with a more significant decrease observed in the LPS-treated group (Figure 5B).



Immunohistochemical Analysis of Aggrecan Expression in Response to SPARC Intervention

Immunohistochemistry was used to assess the distribution of Aggrecan (ACAN) in nucleus pulposus cells, identified by brown chromogen deposition. ACAN was detected in both the cytoplasmic and extracellular compartments of the cells. Following SPARC intervention, there was a notable reduction in the intensity of ACAN staining in the nucleus pulposus cells, in contrast to the control group, which exhibited stronger staining (Figure 6).

Discussion

While many scholars have proposed new marker genes for nucleus pulposus cells, such as FOXF1, OVOS2, and CA12^{24,25}, the most classical and widely recognized marker genes remain COL2A1, ACAN, and SOX9²⁶⁻²⁸. Recently, several studies have utilized advanced techniques like Single-

Cell Transcriptomics and Spatial Transcriptomics^{9,29,30} to characterize marker genes in nucleus pulposus cells. These studies have identified several differentially expressed genes during nucleus pulposus cell degeneration, including COL1A1, FN1, MMP13, and SPARC, which are upregulated in degenerating tissue¹¹⁻¹⁴. These findings have ushered in a new era of IDD research. However, the underlying biological mechanisms remain unclear. The precise role of these novel markers in nucleus pulposus degeneration, and their subsequent impact on the degenerative process, have yet to be fully understood, emphasizing the need for further investigation in this critical area of study.

Previous research has implicated SPARC in facilitating type I collagen aggregation^{19,20}, as well as in regulating osseous development^{17,18}. While some studies have reported variations in SPARC expression during IDD^{13,14}, its exact role and underlying biological mechanisms in this condition remain unclear and require further investigation for clarification.

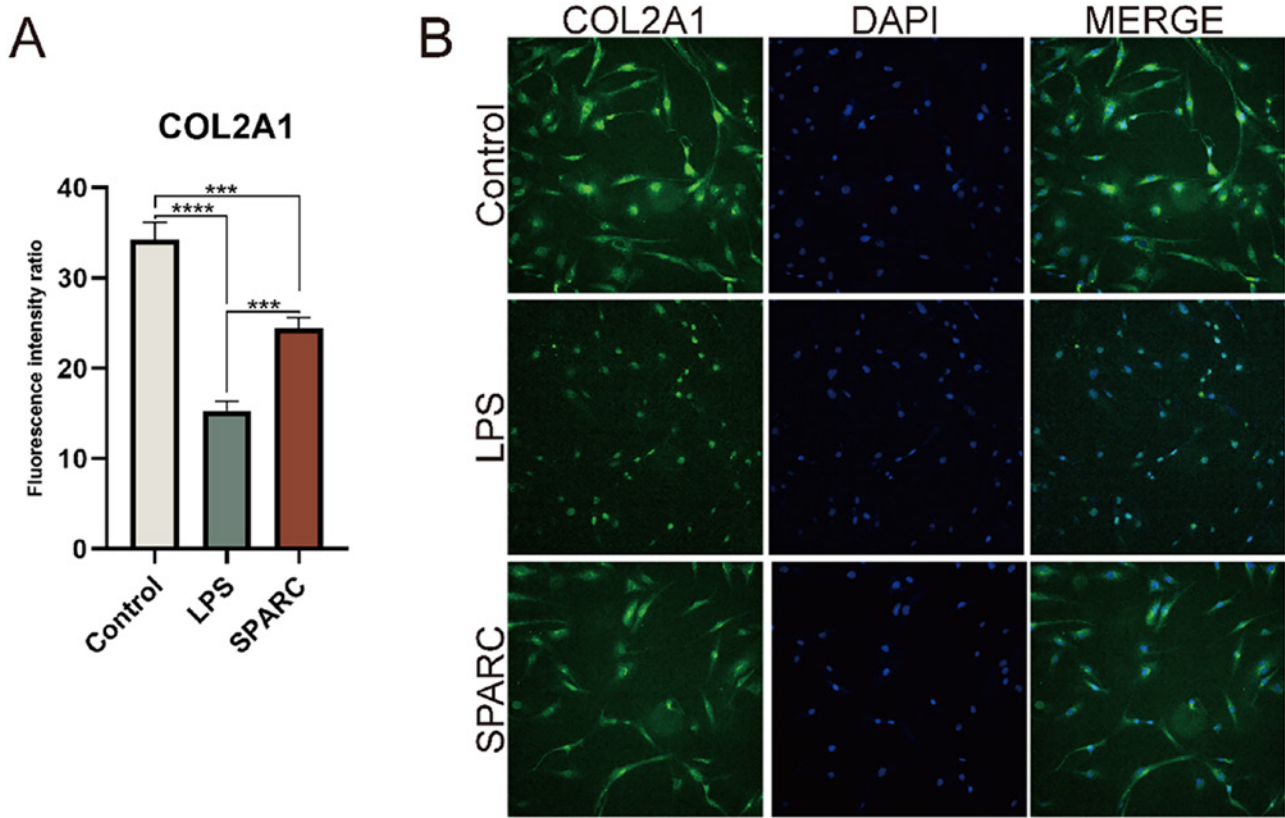


Figure 5. Immunofluorescence staining was used to determine the expression level of COL2A1 in nucleus pulposus cells for each experimental group. (** $p < 0.001$, **** $p < 0.0001$).

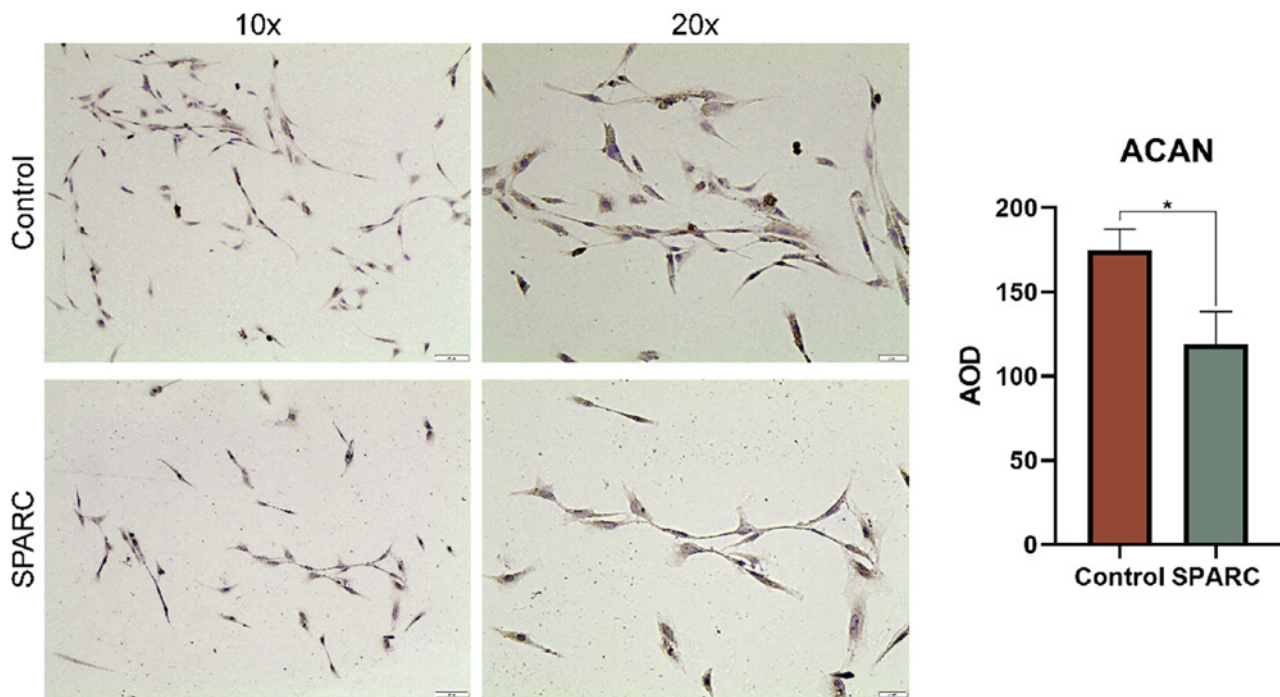


Figure 6. Immunostaining of nucleus pulposus cells after drug treatment. (* $p < 0.05$).

This study aimed to explore the changes in SPARC expression during IDD and its potential biological impacts. Using publicly available single-cell sequencing bioinformatics databases, we observed an upregulation of SPARC expression in nucleus pulposus cells at varying stages of degeneration. This finding was further corroborated in the LPS-induced degeneration model, suggesting that SPARC may play a crucial role in the pathogenesis of IDD. Our experimental results indicate that the upregulation of SPARC is closely associated with the remodeling of the extracellular matrix during IDD, particularly affecting the expression of key extracellular matrix components such as COL2A1 and ACAN1. These findings provide new insights into the role of SPARC in IDD and highlight potential molecular targets for future therapeutic strategies.

The study has also several limitations. First, this research primarily focused on *in vitro* models, and the applicability of these findings to *in vivo* environments requires further validation. More importantly, although we observed changes in SPARC expression, its precise mechanism of action and the signaling pathways involved in disc degeneration remain to be fully explored. Future studies should investigate how SPARC interacts with other extracellular matrix components and how it influences the function and degeneration of nucleus pulposus cells via specific signaling pathways.

The significance of this study lies in its potential to identify new therapeutic targets for IDD. By elucidating the role of SPARC in degeneration, it opens the door for the development of novel therapeutic strategies. However, the exact mechanism of SPARC in disc degeneration is still incompletely understood, and further *in vitro* and *in vivo* studies are necessary to clarify its role. Additionally, the interaction of SPARC with other extracellular matrix components, as well as the involvement of cell signaling pathways, requires deeper investigation. Future research should also explore the role of SPARC at different stages of degeneration and its differential expression across various populations and disease states to develop more effective treatments aimed at alleviating the lower back pain caused by IDD.

Conclusions

This study investigates gene expression changes in nucleus pulposus cells during intervertebral disc degeneration (IDD), with a specific focus on the expression pattern of the SPARC gene and its potential role in the degeneration process. The findings provide insights into the molecular mechanisms underlying IDD and highlight potential molecular targets for future therapeutic strategies. Overall, this research enhances our understanding of the molecular pathology of IDD and opens new directions for clinical treatment.

Authors' contributions

JZ designed the study and drafted the manuscript. SY and WD were responsible for collecting and analyzing the experimental data. JZ and

JW critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript.

Funding

This study was supported by Project initiated by high-level talent research in Affiliated Hospital of Guangdong Medical University (GCC2022007).

References

1. Cieza A, Causey K, Kamenov K, Hanson SW, Chatterji S, Vos T. Global estimates of the need for rehabilitation based on the Global Burden of Disease study 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2021;396(10267):2006-17.
2. Knezevic NN, Candido KD, Vlaeyen JWS, Van Zundert J, Cohen SP. Low back pain. *Lancet* 2021;398(10294):78-92.
3. Luoma K, Riihimäki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A. Low back pain in relation to lumbar disc degeneration. *Spine (Phila Pa 1976)* 2000;25(4):487-92.
4. Wong JJ, Côté P, Sutton DA, Randhawa K, Yu H, Varatharajan S, et al. Clinical practice guidelines for the noninvasive management of low back pain: A systematic review by the Ontario Protocol for Traffic Injury Management (OPTiMa) Collaboration. *Eur J Pain*. 2017;21(2):201-16.
5. Zawilla NH, Darweesh H, Mansour N, Helal S, Taha FM, Awadallah M, et al. Matrix metalloproteinase-3, vitamin D receptor gene polymorphisms, and occupational risk factors in lumbar disc degeneration. *J Occup Rehabil* 2014;24(2):370-81.
6. Mohd Isa IL, Teoh SL, Mohd Nor NH, Mokhtar SA. Discogenic Low Back Pain: Anatomy, Pathophysiology and Treatments of Intervertebral Disc Degeneration. *International journal of molecular sciences* 2022;24(1).
7. Yang RZ, Xu WN, Zheng HL, Zheng XF, Li B, Jiang LS, et al. Involvement of oxidative stress-induced annulus fibrosus cell and nucleus pulposus cell ferroptosis in intervertebral disc degeneration pathogenesis. *Journal of cellular physiology* 2021;236(4):2725-39.
8. Amelot A, Mazel C. The Intervertebral Disc: Physiology and Pathology of a Brittle Joint. *World Neurosurg* 2018;120:265-73.
9. Tu J, Li W, Yang S, Yang P, Yan Q, Wang S, et al. Single-Cell Transcriptome Profiling Reveals Multicellular Ecosystem of Nucleus Pulposus during Degeneration Progression. *Advanced science (Weinheim, Baden-Wurttemberg, Germany)* 2022;9(3):e2103631.
10. He R, Wang Z, Cui M, Liu S, Wu W, Chen M, et al. HIF1A Alleviates compression-induced apoptosis of nucleus pulposus derived stem cells via upregulating autophagy. *Autophagy* 2021;17(11):3338-60.
11. Tilotta V, Vadalà G, Ambrosio L, Cicione C, Di Giacomo G, Russo F, et al. Mesenchymal stem cell-derived secretome enhances nucleus pulposus cell metabolism

- and modulates extracellular matrix gene expression *in vitro*. *Frontiers in bioengineering and biotechnology* 2023;11:1152207.
12. Li Z, Ye D, Dai L, Xu Y, Wu H, Luo W, et al. Single-Cell RNA Sequencing Reveals the Difference in Human Normal and Degenerative Nucleus Pulposus Tissue Profiles and Cellular Interactions. *Frontiers in cell and developmental biology* 2022;10:910626.
 13. Cherif H, Mannarino M, Pacis AS, Ragoussis J, Rabau O, Ouellet JA, et al. Single-Cell RNA-Seq Analysis of Cells from Degenerating and Non-Degenerating Intervertebral Discs from the Same Individual Reveals New Biomarkers for Intervertebral Disc Degeneration. *International journal of molecular sciences* 2022;23(7).
 14. Devraj VM, Vemuri SK, Banala RR, Gunda SK, Av GR, Gpv S. Evaluation of Anti-inflammatory and Regenerative Efficiency of Naringin and Naringenin in Degenerated Human Nucleus Pulposus Cells: Biological and Molecular Modeling Studies. *Asian spine journal* 2019;13(6):875-89.
 15. Rosset EM, Bradshaw AD. SPARC/osteonectin in mineralized tissue. *Matrix biology: journal of the International Society for Matrix Biology* 2016;52-54:78-87.
 16. Liu L, Hu F, Wang H, Wu X, Eltahan AS, Stanford S, et al. Secreted Protein Acidic and Rich in Cysteine Mediated Biomimetic Delivery of Methotrexate by Albumin-Based Nanomedicines for Rheumatoid Arthritis Therapy. *ACS nano* 2019;13(5):5036-48.
 17. Holland PW, Harper SJ, McVey JH, Hogan BL. *In vivo* expression of mRNA for the Ca⁺⁺-binding protein SPARC (osteonectin) revealed by *in situ* hybridization. *The Journal of cell biology* 1987;105(1):473-82.
 18. Termine JD, Kleinman HK, Whitson SW, Conn KM, McGarvey ML, Martin GR. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell* 1981;26(1 Pt 1):99-105.
 19. Murphy-Ullrich JE, Sage EH. Revisiting the matricellular concept. *Matrix biology: journal of the International Society for Matrix Biology* 2014;37:1-14.
 20. Bradshaw AD. The role of SPARC in extracellular matrix assembly. *Journal of cell communication and signaling* 2009;3(3-4):239-46.
 21. Li Y, Li K, Mao L, Han X, Zhang K, Zhao C, et al. Cordycepin inhibits LPS-induced inflammatory and matrix degradation in the intervertebral disc. *PeerJ* 2016;4:e1992.
 22. Ham SM, Song MJ, Yoon HS, Lee DH, Chung JH, Lee ST. SPARC Is Highly Expressed in Young Skin and Promotes Extracellular Matrix Integrity in Fibroblasts via the TGF- β Signaling Pathway. *International journal of molecular sciences* 2023;24(15).
 23. Han S, Zhang Y, Zhang X, Zhang H, Meng S, Kong M, et al. Single-Cell RNA Sequencing of the Nucleus Pulposus Reveals Chondrocyte Differentiation and Regulation in Intervertebral Disc Degeneration. *Frontiers in cell and developmental biology* 2022;10:824771.
 24. Minogue BM, Richardson SM, Zeef LA, Freemont AJ, Hoyland JA. Characterization of the human nucleus pulposus cell phenotype and evaluation of novel marker gene expression to define adult stem cell differentiation. *Arthritis and rheumatism* 2010;62(12):3695-705.
 25. Power KA, Grad S, Rutges JP, Creemers LB, van Rijen MH, O'Gaora P, et al. Identification of cell surface-specific markers to target human nucleus pulposus cells: expression of carbonic anhydrase XII varies with age and degeneration. *Arthritis and rheumatism* 2011;63(12):3876-86.
 26. Henriksson HB, Svanvik T, Jonsson M, Hagman M, Horn M, Lindahl A, et al. Transplantation of human mesenchymal stems cells into intervertebral discs in a xenogeneic porcine model. *Spine (Phila Pa 1976)* 2009;34(2):141-8.
 27. Richardson SM, Hughes N, Hunt JA, Freemont AJ, Hoyland JA. Human mesenchymal stem cell differentiation to NP-like cells in chitosan-glycerophosphate hydrogels. *Biomaterials* 2008;29(1):85-93.
 28. Le Maitre CL, Baird P, Freemont AJ, Hoyland JA. An *in vitro* study investigating the survival and phenotype of mesenchymal stem cells following injection into nucleus pulposus tissue. *Arthritis Res Ther* 2009;11(1):R20.
 29. Chen Y, Zhang L, Shi X, Han J, Chen J, Zhang X, et al. Characterization of the Nucleus Pulposus Progenitor Cells via Spatial Transcriptomics. *Advanced science (Weinheim, Baden-Wurttemberg, Germany)* 2024;11(18):e2303752.
 30. Rodrigues-Pinto R, Richardson SM, Hoyland JA. Identification of novel nucleus pulposus markers: Interspecies variations and implications for cell-based therapies for intervertebral disc degeneration. *Bone & joint research* 2013;2(8):169-78.