Effects of Fluid Shear Stress on Human Intervertebral Disc Nucleus Pulposus Cells Based on Label-Free Quantitative Proteomics

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Objective. To explore the possible mechanism of fluid shear stress on human nucleus pulposus cells based on label-free proteomics technology. Methods. The human nucleus pulposus cell line was purchased and subcultured in vitro. The Flexcell STR-4000 multilow field cell fluid shear stress loading culture system was used to apply continuous laminar fluid shear stress (12 dyne/cm², 45 mins) to the monolayer adherent cells. Those without mechanical loading were used as the control group, and those subjected to fluid shear loading were used as the experimental group. Differential protein expression was identified using mass spectrometry identification technology, and bioinformatics analysis was performed using Gene Ontology GO (Gene Ontology) and Kyoto Encyclopedia of Genes and Genomes KEGG (Kyoto Encyclopedia of Genes and Genomes). Results. The proteomics results of the experimental group and the control group showed that the total number of mass spectra was 638653, the number of matched mass spectra was 170110, the total number of identified peptides was 32050, the specific peptide was 30564, and the total number of identified proteins was 4745. Comparing the two groups, 47 proteins were significantly different, namely, 25 upregulated proteins and 22 downregulated proteins. Bioinformatics analysis showed that significantly different proteins were mainly manifested in cellular process, biological regulation, metabolic process, binding, catalytic activity, cellular components (cell part), organelle part (organelle part), and other molecular biological functions. Conclusion. Using proteomics technology to screen human nucleus pulposus cells after fluid shear stress loading, the differential protein expression provides a basis for further exploration of the mechanism of mechanical factors on nucleus pulposus.

1. Introduction

Intervertebral disc degeneration (IVDD) is a common clinical symptom and one of the important causes of low back pain [1]. According to epidemiology, the global prevalence of low back pain is 7.3%, and there are about 540 million people every day who are suffering from lower back pain [2]. It seriously affects the normal work and life of patients and brings a heavy economic burden to individuals and society. Aging, trauma, genetics, and biomechanical factors can influence the progression of IVDD [3]. The intervertebral disc itself is affected by various mechanical stimuli, such as pressure, tension, and shear stress [4]. Different stress magnitudes, frequencies, and stress time have various effects on the intervertebral disc [5]. Mechanical stress itself has twin effects, and ordinary stress stimulation will have an effect on the normal physiological structure of the intervertebral disc and the internal microenvironment of the intervertebral disc, thereby accelerating the process of intervertebral disc degeneration, while appropriate mechanical stimulation can regulate the synthesis of extracellular matrix that is conducive to retaining the intervertebral disc itself. McMillan, Garbutt, and Adams [6] studied the loading of the human lumbar intervertebral disc and found that continuous...
loading reduces the fluid content of the intervertebral disc, and proper exercise can promote the water circulation in the nucleus pulposus [7].

The NP consists of a massive quantity of proteoglycan and type II collagen. It can shape a completely unique porous machine, and the flow of fluid inside the nucleus pulposus will have an effect on the change of vitamins and affect the anabolism and catabolism of materials. Within the early stage, our study group established a fluid-structure interaction numerical strain model through finite element analysis and used the pressure acquisition system to collect the load parameters of the subjects on the L4/L5 lumbar vertebrae during the 3-dimensional balance spine manipulation manner and amassed the lumbar backbone geometry data of regular humans.

Mimics 21.0 and 3-Matic were built 3D computational modeling and found that when the intervertebral disc tissue is subjected to tensile stress, the inflow and outflow of water in the NP will be triggered [8]. It has been reported that the pressure on the intervertebral disc can be converted into fluid shear stress inside the nucleus pulposus [9]. Excessive fluid shear stress can promote the increase of inflammatory factor secretion and affect the expression of anabolic- and catabolic-related proteins. As a not unusual mechanical form within the human body, the effect of fluid shear stress on human intervertebral disc nucleus pulposus cells involves the conversion of mechanical factors to biochemical factors, but the mechanical target and molecular mechanism of fluid shear stress acting on the intervertebral disc are still not clear, and further research is needed.

In this study, by creating a fluid stress environment for human NP cells and using proteomics technology to detect the expression of differential proteins after fluid shear stress loading, it is beneficial to explain the influence of mechanical effect on the intervertebral disc nucleus pulposus cells.

### 2. Materials and Methods

#### 2.1. Experimental Materials

Human normal nucleus pulposus cells (Wuhan Proser Biotech Biotechnology Corporation, China, batch number: CP-H097), fetal bovine serum (Gibco, USA, batch number: A3161001C), collagenase type II (Beijing Soleibo Technology Biotechnology corporation, China, batch number: C8150), 0.25% trypsin solution (Gibco, USA, batch number: 25200056), DMEM high-glucose liquid medium (HyClone, USA, SH30024.01), BCA protein quantification kit (Biyuntian Biotechnology Corporation, batch number: P0012), SDS-PAGE protein loading buffer (Biyuntian Biotechnology Corporation, batch number: P0015F), iodoacetamide (IAA, batch number: I1149-5G), formic acid (Thermo Fisher Scientific, batch number: A117), trifluoroacetic acid (TFA, Sigma, batch number: T6508), and acetonitrile (Merck, batch number: 1000304008) are the experimental materials.

#### 2.2. Experimental Apparatus

Fluid shear stress device STR-4000 (Flexcell, USA), NanoElute chromatography system (Bruker, Bremen, Germany), Agilent 1260 Infinity II HPLC system, low-temperature high-speed centrifuge (Eppendorf 5430R), electrophoresis (BIO-RAD), NanoDrop (Thermo Fisher Scientific, batch number: A117), trifluoroacetic acid (TFA, Sigma, batch number: T6508), and acetonitrile (Merck, batch number: 1000304008) are the experimental materials.

### Table 1: Upregulated protein expression in both groups (top 10).

| Number | Protein IDs | Gene name | FSS/CTR | P value | Regulation |
|--------|-------------|-----------|---------|---------|------------|
| 1      | O00622      | CCN1      | 12.63   | 0.006   | Up         |
| 2      | Q02413      | DSG1      | 7.73    | 0.014   | Up         |
| 3      | P31151; Q865G5 | S100A7; S100A7A | 5.97    | 0.004   | Up         |
| 4      | P07996      | THBS1     | 4.53    | 0.027   | Up         |
| 5      | P06454      | PTMA      | 4.35    | 0.019   | Up         |
| 6      | Q5T749      | KPRP      | 4.18    | 0.035   | Up         |
| 7      | P62633      | CNBP      | 3.87    | 0.040   | Up         |
| 8      | Q9Y5Q0      | FADS3     | 3.39    | 0.009   | Up         |
| 9      | Q14978      | NOLC1     | 3.39    | 0.0001  | Up         |
| 10     | Q9NS91      | RAD18     | 3.17    | 0.001   | Up         |

### Table 2: Downregulated protein expression in both groups (top 10).

| Number | Protein IDs | Gene name | FSS/CTR | P value | Regulation |
|--------|-------------|-----------|---------|---------|------------|
| 1      | O60907      | TBL1X     | 0.49    | 0.015   | Down       |
| 2      | O60507      | TPST1     | 0.49    | 0.020   | Down       |
| 3      | P09972      | ALDOC     | 0.48    | 0.022   | Down       |
| 4      | Q8NC96      | NECAP1    | 0.48    | 0.002   | Down       |
| 5      | O00213      | APBB1     | 0.48    | 0.023   | Down       |
| 6      | P06737      | PYGL      | 0.47    | 0.022   | Down       |
| 7      | P98082      | DAB2      | 0.47    | 0.003   | Down       |
| 8      | P49247      | RPIA      | 0.47    | 0.007   | Down       |
| 9      | Q9NWM8      | FKRP14    | 0.46    | 0.019   | Down       |
| 10     | Q7Z7L7      | ZER1      | 0.46    | 0.006   | Down       |
2.3. Cell Climbing. The nucleus pulposus cells were removed from liquid nitrogen and thawed. When the confluent density reached 85%–90%, the cells were washed with PBS. The NP cells were then dispersed using 0.25% trypsin (Gibco, USA) and resuspended in the appropriate culture plates. The cells were centrifuged at 1000 rpm for 5 min. Cell pellets were collected. 2 – 5 × 10^5 cells were seeded on 75 × 25 × 1 mm glass slides and then allowed to stand at room temperature for 3 hours. The NP cells were transferred to the culture, and the experiment was started when the cells grew to 85% confluence.

2.4. Creation of the In Vitro Fluid Environment. Multiflow field cells were examined in cultured cells loaded with fluid shear stress. The quick interface should be connected exactly. Fluid passages are not bent and sterilized with alcohol. With PBS perfusion, the fluidic tubing was kept moist. 2% complete medium was used as the perfusate for mechanical loading. Slides overgrown with cells were transferred into fluid chambers. The dampers were tilted to reduce air bubbles.

2.5. Protein Extraction and Enzymatic Hydrolysis. Switching the glass slide to a square petri dish with a diameter of 100 mm, 0.25% trypsin was dropped to absolutely cover the floor of the glass slide. Allowing it to stand for 1 minute, the same amount of complete medium was set to terminate the digestion. Centrifuging at 1500 rpm for 5 minutes, the supernatant was collected. Uploading the right amount of SDT lysis buffer, we took a boiling water tub for 15 minutes and then centrifuged it at 14000 g for 15 minutes.

The BCA was used to quantify the protein in the control and experimental groups. Two groups of samples were taken 20 μg each; added to 6× loading buffer, respectively; bathed in boiling water for 5 min; and subjected to 12% SDS-PAGE electrophoresis (constant pressure 250 V, 40 min), and Coomassie brilliant blue staining was used for quality detection. 80 μg of protein solution was taken from each of the experimental group and the control group for FASP.

**Figure 1:** CTR1, CTR2, and CTR3 were the expression of differential proteins in the control group; FSS1, fss2, and fss3 are the differential protein expression of experiment group. Each column represents one sample, and each row represents the expression abundance of the same differential protein in different samples.
enzymatic hydrolysis, and DTT was added to the final concentration of 100 mM, and the solution was boiled for 5 min.

Adding 200 μl of UA buffer, mixed well, we transferred it to a 30 kD ultrafiltration centrifuge tube. Adding 100 μl of IAA buffer, the cells were set at room temperature in the dark for 30 min and centrifuged it at 12500 g for 15 min.

Adding 100 μl UA buffer and centrifuging it at 12500 g for 15 min, we then added 100 μl of 40 mM NH4HCO3 solution and centrifuge at 12500 g for 15 min. We changed to a new EP tube, added 40 μl trypsin buffer, shook it at 600 rpm for 1 min, and placed it at 37°C for 16–18 h. Adding 20 μl of 40 mM NH4HCO3 solution, we collected the filtrate. The

Figure 2: Column chart of differential protein GO notes.
protein was desalted, and the lyophilized protein was reconstituted with 40 µl of 0.1% formic acid solution, and the amount of protein at a wavelength of 280 nm was determined using a microplate reader.

2.6. Detection of Proteomics. The nucleus pulposus cells of the experimental group and the control group were detected by 4D label-free proteomics technology (Shanghai Jikai Gene Technology Biotechnology corporation), through protein extraction, protein quantification, SDS-PAGE, and protein enzymatic hydrolysis. The samples that passed the quality control were subjected to formal experiments using a high-resolution mass spectrometer to collect the original mass spectrometry data. According to the source of the sample, a specific protein database was built. Database matching was performed using Maxquant to obtain qualitative results.
of the protein, and FDR principle was used for data screening. Fisher’s exact test was used to determine whether the differentially expressed proteins had a significant enrichment trend in certain functional types.

3. Results

3.1. Protein Mass Spectrometry Identification. The experimental group and the control group were subjected to analysis after enzymatic hydrolysis. The peptides detected by mass spectrometry were mainly distributed in 7–20 amino acids, which conformed to the general rule based on trypsin enzymatic hydrolysis and HCD fragmentation. The results showed that the total number of peptides measured in the experimental group. The control group was 638653. The number of matching peptides was 170110, and the number of specific peptides in the two groups was 30564. The total number of identified proteins was 4745. The relative molecular mass of proteins is concentrated in 5–100 kDa, accounting for 80% of the total number of proteins. There are 2141 proteins whose coverage is less than 20%, accounting for about 70% of the total number of proteins; 1029 proteins whose coverage is between 20% and 40%, accounting for 28% of the total number of proteins; and 443 proteins whose coverage is more than 40% occupied 12% of the total protein.

3.2. Statistics of Differential Proteins. Using proteomics technology to detect the experimental and control groups, a total of 47 DEPs were found. Compared with the control group, 25 DEPs in the experimental group were upregulated and 22 were downregulated (Tables 1 and 2). The difference in expression abundance of DEPs in the loading group and the control group can be seen by cluster analysis of differential expression (Figure 1).

3.3. GO Functional Annotation Analysis of Differentially Expressed Proteins. Map DEP to each GO entry in the GO...
database and count the number of upregulated and downregulated proteins under each GO secondary entry. 41 of the upregulated and downregulated proteins were mainly concentrated in cellular processes, 36 in biological regulation, and 35 in metabolic processes. 46 of the upregulated and downregulated proteins were concentrated in binding, and 22 differential proteins were concentrated in catalytic activity. 47 of the upregulated and downregulated proteins were concentrated in cellular composition, and 37 were concentrated in organelle composition (Figure 2).

3.4. GO Enrichment Analysis of Differentially Expressed Proteins. Blast2Go (https://www.blast2go.com/) software was used to perform GO functional enrichment analysis on the obtained differentially expressed proteins. The differentially expressed proteins can be obtained in BP, MF, and CC. Among them, there are top 10 in BP, such as keratinization, epidermal development, adhesion between endothelial cells, peptide cross-linking, regulation of tumor necrosis factor, lung development, negative regulation of vascular endothelial cells, cytoplasmic calcium ion transport, chondrocyte differentiation, and cell envelope organization. There are top 10 in MF, such as fibronectin binding, heparin binding, insulin-like growth factor binding, signaling receptor activator activity, protein glutamine gamma glutamyl transferase, extracellular matrix binding, calcium ion binding, structural composition of extracellular matrix, fibrinogen binding, and protein lysine 6 oxidase activity. There are top 10 in CC, such as basement membrane, keratinized envelope, fibrinogen complex, core binding factor complex, delta-DNA polymerase complex, BRCA1-BARD1 complex, extracellular matrix, integrin complex substances, sarcoplasmic reticulum, and insulin-like growth-binding protein (Figure 3).

3.5. Differential Protein KEGG Pathway Enrichment Analysis. Differential proteins are sorted in the pathway. The differential protein KEGG pathway annotation mainly includes metabolic pathways, biosynthesis of secondary metabolites, P13K-AKT signaling pathway, human papillomavirus infection, sphingolipids signaling pathway, AMPK signaling pathway, AGE-RAGE signaling pathway, and MAPK signaling pathway (Figure 4).

4. Discussion

The NP cells of the intervertebral disc faced complex mechanical load stimulation for a long time, and the mechanical stimulation can affect the structure and function of the intervertebral disc and the microenvironment inside the intervertebral disc. Our team found that the use of MechanoCulture FX2 like to load periodic stretch microstrain and found the stress of 100000 μ, 10% like tensile strain, frequency of 0.1 Hz, and 8640 cycles. It can significantly promote degenerated NP cell proliferation, inhibit the cell apoptosis, and promote the progression of the cell cycle [10]. Wang, Jie, and Jiwai [11] used low-, medium-, and high-frequency (3%, 9%, 19%) mechanical strains. They found that facing low-frequency mechanical strains and collagen type II and proteoglycan had no obvious effect. There were significant differences in increasing the expressions of TNF-α, IL-1β, and IL-6. The study showed that the effects of medium- and high-frequency mechanical strains were obvious. The results evidenced that low-frequency and high-frequency mechanical strains could promote intervertebral disc degeneration and the secretion of inflammatory factors through NF-kB p65. Chao, Ma, and Ma [12] found that low-intensity stretch stimulation had a positive significance in anti-inflammatory response. Wang, Yang, and Hsieh [13] used alginate gel to simulate the internal environment of the nucleus pulposus, while showing that there is continuous fluid flow inside the NP under cyclic stress conditions.

Cao et al. applied moderate fluid shear stress to the immortalized nucleus pulposus cells. The study found that the strength was 12 dyne/cm² and the loading time was less than 3 hours. The sGAG content and the aggrecan protein level were significantly increased, and the MMP13 protein level was significantly decreased. Fluid shear stress could slightly increase COL2a1 expressions and decrease ADAMTS5 expressions. Yichun et al. [14] found that when the strength of fluid shear stress is ≤12 dyne and the loading time is ≤45 minutes, the expression of BAD, Bax, and Caspase3 genes can be reduced in NP cells. As a special mechanical form, fluid shear stress can affect the proliferation, cycle, and apoptosis of various cells such as osteoblasts, osteoclasts, and chondrocyte-related phenotypes [15-17].

By creating a fluid shear stress environment for NP cells, this project used proteomics technology to screen the differential proteins between the experimental group and the control group. The results found that fluid shear stress can affect CCN1, DSG1, S100A7, THBS1, PTMA, KPRP, CNBP, and other proteins. Among them, CCN1 belongs to a member of the CCN family of stromal cell proteins, which is widely distributed in the extracellular matrix and is associated with various phenotypes such as cell proliferation, differentiation, senescence, and apoptosis. CCN1 has a significant role in promoting angiogenesis, and CCN1 exists in the embryonic mesenchymal layer during chondrogenesis and can promote chondrogenesis [18]. As a secreted matrix protein, CCN1 which can be affected by a variety of stimuli such as regulatory growth factors, cytokines, plasminogen, glycation end products, blood flow shear stress, and hypoxia. S100 protein, which exists in the cytoplasm or nucleus of various cells, can regulate cellular processes such as cell cycle and cell differentiation. Studies have found that compressive and shear mechanical stress can significantly upregulate the mRNA and protein levels of S100A7 and promoted resorption by inducing osteoclast differentiation and activity [19]. Relevant literature also showed that S100A7 can be affected by reactive oxygen species and played a role in the process of oxidative stress. Reactive oxygen species can induce the secretion of S100A7 [20]. THBS1 (thrombospondin-1) is an adhesion glycoprotein that mediates cell-to-cell and cell-to-matrix interactions, and it interacts with fibrinogen, fibronectin, laminin, type V collagen, and alpha-V/β-1 integrin binding. THBS1 had been extensively studied in renal, myocardial, and hepatic fibrotic diseases and had been found to play an important role in various ophthalmic diseases, namely,
The screening of differential proteins, as well as the enriched functional pathways, can further clarify the effect of fluid shear stress on NP cells. In the near future, we will continue to develop this part. Proteomics technology was used to clarify the differential expression of proteins under mechanical action and to further clarify the effect and mechanism of fluid shear stress on nucleus pulposus cells. It has positive significance for clarifying the mechanical target of biomechanical stimulation on NP cells.

5. Conclusion

This is the first time we have used proteomic techniques to find the effects of fluid shear stress on NP cells. Based on the discovered differential proteins, we will further explore the mechanistic target.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

All the authors declare no conflict of interests.

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References

[1] Q. Chensheng, N. Deng, X. Hongfei, Y. Zhao, and B. Chen, “Research Progress on riskfactors related to intervertebral disc degeneration,” Chinese Journal of Orthopaedics, vol. 41, no. 10, pp. 654–659, 2021.
[2] J. Hartvigsen, M. J. Hancock, A. Kongsted et al., “What low back pain is and why we need to pay attention,” The Lancet, vol. 391, no. 10137, pp. 2356–2367, 2018.
[3] Y. Wang, M. Che, J. Xin, Z. Zheng, J. Li, and S. Zhang, “The role of IL-1β and TNF-α in intervertebral disc degeneration,” Biomedicine & Pharmacotherapy, vol. 131, p. 110660, 2020.
[4] G. Yu, Z. Zhuang, P. Wang, F. M. Tian, W. Wang, and L. Zhang, “Research progress on the relationship between stress and intervertebral disc degeneration,” Chinese Journal of Orthopaedics, vol. 23, no. 9, pp. 823–826, 2015.
[5] E. González Martínez, J. García-Cosamalón, I. Cosamalon-Gan, M. Esteban Blanco, O. García-Suárez, and J. A. Vega, “Biological and mechanobiology of the intervertebral disc,” Neurocirugía (Asturias, Spain), vol. 28, no. 3, pp. 135–140, 2017.
[6] D. W. McMillan, G. Garbutt, and M. A. Adams, “Effect of sustained loading on the water content of intervertebral discs: implications for disc metabolism,” Annals of the Rheumatic Diseases, vol. 55, no. 12, pp. 880–887, 1996.
[7] H. Schmidt, S. Reitmaier, F. Graichen, and A. Shirazi-Adl, “Review of the fluid flow within intervertebral discs - how could in vitro measurements replicate in vivo?,” Journal of Biomechanics, vol. 49, no. 14, pp. 3133–3146, 2016.
[8] L. Huazhong, B. Wang, D. Wang, S. J. Liu, X. Chen, and B. Shi, “Fluid structure coupling effect in L4/L5 intervertebral disc under transient load,” Journal of Mechanics, vol. 53, no. 7, pp. 2058–2068, 2021.
[9] H. Y. Kim, H. N. Kim, S. J. Lee et al., “Effect of pore sizes of PLGA scaffolds on mechanical properties and cell behaviour for nucleus pulposus regeneration in vivo,” Journal of Tissue Engineering and Regenerative Medicine., vol. 11, no. 1, pp. 44–57, 2017.
[10] D. Wang, H. Weimin, C. Shengnan et al., “Study on the effect of stretch strain on the biological function and degeneration of nucleus pulposus cells,” *Chinese Journal of Orthopaedics*, vol. 41, no. 4, pp. 242–252, 2021.

[11] S. Wang, J. Li, J. Tian et al., “High amplitude and low frequency cyclic mechanical strain promotes degeneration of human nucleus pulposus cells via the NF-κB p65 pathway,” *Journal of Cellular Physiology*, vol. 233, no. 9, pp. 7206–7216, 2018.

[12] H. Chao, X. Ma, J. Ma et al., “Effect of low-intensity traction on the expression of inflammatory factors in rat tail intervertebral disc,” *Chinese Journal of Orthopaedics*, vol. 31, no. 5, pp. 556–562, 2011.

[13] P. Wang, L. Yang, and A. H. Hsieh, “Nucleus pulposus cell response to confined and unconfined compression implicates mechanoregulation by fluid shear stress,” *Annals of Biomedical Engineering*, vol. 39, no. 3, pp. 1101–1111, 2011.

[14] Y. Yichun, *Experimental Study of ERK5 Mediated Fluid Shear Stress on Apoptosis of Nucleus Pulposus Cells*, Jinan University, 2014.

[15] W. Xingwen, G. Bin, W. Hong et al., “Fluid shear stress-induced down-regulation of microRNA-140-5p promotes osteoblast proliferation by targeting VEGFA via the ERK5 pathway,” *Connective Tissue Research*, vol. 63, no. 2, pp. 156–168, 2022.

[16] B. Zhang, L. An, B. Geng et al., “ERK5 negatively regulates Kruppel-like factor 4 and promotes osteogenic lineage cell proliferation in response to MEK5 overexpression or fluid shear stress,” *Connective Tissue Research*, vol. 62, no. 2, pp. 194–205, 2021.

[17] G. Yang, Y. Guo, P. Tu, C. Wu, Y. Pan, and Y. Ma, “Research progress of different mechanical stimulation regulating chondrocytes metabolism,” *Journal of Biomedical Engineering*, vol. 37, no. 6, pp. 1101–1108, 2020.

[18] F. Wang and Z. Wang, “The role of matrix cell protein ccn1/cyr61 in bone regeneration,” *Journal of Oral and Maxillofacial Surgery*, vol. 31, no. 5, pp. 324–326, 2021.

[19] H. Charoenpong, T. Osathanon, P. Pavasant et al., “Mechanical stress induced S100A7 expression in human dental pulp cells to augment osteoclast differentiation,” *Oral Diseases*, vol. 25, no. 3, pp. 812–821, 2019.

[20] L. Ye, S. Wenyan, L. Jiangan, and J. Liu, “Biological characteristics of S100A7 and its relationship with diseases,” *Journal of Medical Postgraduates*, vol. 27, no. 7, pp. 767–771, 2014.

[21] D. Jiang, B. Guo, F. Lin, Q. Hui, and K. Tao, “Effect of THBS1 on the biological function of hypertrophic scar fibroblasts,” *BioMed Research International*, vol. 2020, Article ID 8605407, 8 pages, 2020.

[22] R. Zhao, W. Liu, T. Xia, and L. Yang, “Disordered mechanical stress and tissue engineering therapies in intervertebral disc degeneration,” *Polymers (Basel)*, vol. 11, no. 7, p. 1151, 2019.

[23] K. Tzavlaki and A. Moustakas, “TGF-β signaling,” *Biomolecules*, vol. 10, no. 3, p. 487, 2020.

[24] S. Chen, S. Liu, K. Ma, L. Zhao, H. Lin, and Z. Shao, “TGF-β signaling in intervertebral disc health and disease,” *Osteoarthritis and Cartilage*, vol. 27, no. 8, pp. 1109–1117, 2019.

[25] Z. Wang, J. Shen, E. Feng, and Y. Jiao, “AMPK as a potential therapeutic target for intervertebral disc degeneration,” *Frontiers in Molecular Biosciences*, vol. 8, p. 789087, 2021.

[26] R. A. Alharbi, “Proteomics approach and techniques in identification of reliable biomarkers for diseases,” *Saudi Journal Of Biological Sciences*, vol. 27, no. 3, pp. 968–974, 2020.