Identification of biological processes and signaling pathways in the stretched nucleus pulposus cells

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Abstract

Low back pain is mostly caused by disc degeneration, which is due to the alterations in the osmotic pressure of nucleus pulposus cells. However, the knowledge about the mechanism and therapies for disc degeneration is not fully understood. Here, our objective is to identify significantly changed genes and biological processes in the stretched nucleus pulposus cells. The GSE175710 dataset was originally produced by using the Illumina HiSeq 4000 (*Rattus norvegicus*). The KEGG and GO analyses indicated that "MAPK signaling", "TNF signaling", "IL17 signaling", and the "NF-κB signaling pathway" are mostly affected in the stretched nucleus pulposus cells. Moreover, we identified several genes according to the PPI network such as *Mmp9*, *Cxcl12*, *Col1a1*, and *Col3a1* in the stretched nucleus pulposus cells. Thus, our study provides further insights into the study of disc deterioration.

Introduction

Low back pain affects nearly 80% of the population worldwide, and most cases can be defined as intervertebral disc degeneration¹. About two-thirds of adults suffer from back pain, and most pain is due to disc diseases². Unfortunately, the current medical strategies have no significant improvement in patients such as health status and disability³. Furthermore, the back pain surgery for degenerative discs was found to have negatively affected the mechanics of surrounding discs⁴. The intervertebral disc separates the vertebral bodies in the spine that are strained by various factors including compression, bending, and shear⁵. Certain spinal stress is associated with disc injury, degeneration, and damage⁶. Spinal loading on the disc is also affected by various conditions including oxygen tension, pH, water content, and permeability⁷.

The lack of nucleus pulposus cells in the disc is the early sign of disc degeneration⁴. The nucleus pulposus cells maintain the extracellular matrix through secreting proteoglycans, aggrecan, and collagens⁸. A mechanical loading system that applies cyclic stretch stress can qualify the cells' deformation and strain⁹. Since the capacity of nucleus pulposus cells is the reason for disc degeneration, it is a critical approach to restore or regenerate the nucleus pulposus cells¹⁰. A number of studies tested stem cell-based therapy for inhibiting disc degeneration by transplantation of mesenchymal stem cells

into the vertebral disc, which has been proven to decrease disc degeneration in animal models¹¹. Though disc degeneration is caused by mechanical stress on the nucleus pulposus, the mechanism and functions of nucleus pulposus cells in vitro have not been clarified¹².

In this study, we evaluated the effects of loading stress on the nucleus pulposus cells by analyzing the RNA sequence data. We identified a bunch of DEGs and several significant biological pathways. We also introduced the gene function enrichment and created the protein-protein interaction (PPI) network and Reactome map for finding the potential interacting proteins and pathways. These functional genes and biological processes will shed light on the treatment of low back pain.

Methods

Data resources

The data was created by using the Illumina HiSeq 4000 (*Rattus norvegicus*) (Beijing TongRen Hospital, Capital Medical University, No. 1, Dongjiaomin Lane, Dongcheng District, Beijing, China). The analyzed dataset includes three groups of control cells and three groups of stretched cells.

Data acquisition and preprocessing

We processed the raw data by the R package^{13, 14}. We performed a classical t-test to identify DEGs with $P < 0.001$ and fold change ≥ 1 as being statistically significant.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses

We performed the KEGG and GO analyses in this study by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (<http://david.ncifcrf.gov/>). We set the $P < .05$ and gene counts > 10 as the statistically significant cutoff.

Protein-protein interaction (PPI) networks

The PPI networks were constructed and verified by using the Molecular Complex Detection (MCODE)¹⁵. The significant modules were created from constructed PPI

networks. The biological pathways analysis was performed by Reactome (<https://reactome.org/>), and $P < 0.05$ was used as the cutoff criterion.

Results

Identification of DEGs between the control and elongated nucleus pulposus cells

To determine the molecular mechanisms of excessive mechanical strain on nucleus pulposus cells, we analyzed the DEGs from the control and elongated nucleus pulposus cells. A total of 484 genes were identified with the threshold of $P < 0.05$. The top ten of up-and-down-regulated genes for the control and elongated nucleus pulposus cells are shown by the heatmap and volcano plot (Figure 1). The top ten DEGs were listed in Table 1.

Enrichment analysis of DEGs between the control and elongated nucleus pulposus cells

To determine the biological roles of DEGs under the conditions of excessive mechanical strain for nucleus pulposus cells, we performed the KEGG and GO analyses as previously described^{16, 17} (Figure 2). The top KEGG pathways contain “MAPK signaling pathway”, “Focal adhesion”, “TNF signaling pathway”, “Fluid shear stress and atherosclerosis”, “IL-17 signaling pathway”, “AGE-RAGE signaling pathway in diabetic complications”, “NF-kappa B signaling pathway”, “ECM-receptor interaction”, “Rheumatoid arthritis”, and “Steroid biosynthesis”. We then identified the biological processes of GO, including “Positive regulation of cell adhesion”, “Ameboidal-type cell migration”, “Extracellular matrix organization”, “Extracellular structure organization”, “External encapsulating structure organization”, “Cell-substrate adhesion”, “Leukocyte migration”, “ERK1 and ERK2 cascade”, “Regulation of ERK1 and ERK2 cascade”, and “Peptidyl-tyrosine modification”. We also identified the top ten cellular components of GO, including “Collagen-containing extracellular matrix”, “Apical part of cell”, “Lytic vacuole”, “Lysosome”, “Neuron to neuron synapse”, “Cell leading edge”, “Receptor complex”, “Asymmetric synapse”, “Postsynaptic density”, and “Early endosome”. We further identified the molecular functions of GO, including “Nucleoside-triphosphatase regulator activity”, “DNA-binding transcription activator activity, RNA polymerase II-

specific", "GTPase regulator activity", "Phosphoric ester hydrolase activity", "Cell adhesion molecule binding", "Sulfur compound binding", "Glycosaminoglycan binding", "Heparin binding", "Extracellular matrix structural constituent", and "Integrin binding".

PPI network analysis

To further determine the relationship of the DEGs, we created the PPI network by using the Cytoscape software. The criterion of combined score > 0.2 was defined to create the PPI by using the 381 nodes and 1182 edges. Table 2 showed the top ten genes with the highest degree scores. The top two modules depicted the functional annotation (Figure 3). We further analyzed the DEGs and created the biological map by Reactome (Figure 4). We identified the top ten significant pathways including "Activation of gene expression by SREBF (SREBP)", "Regulation of cholesterol biosynthesis by SREBP (SREBF)", "Interleukin-10 signaling", "Collagen degradation", "Cholesterol biosynthesis", "Degradation of the extracellular matrix", "Assembly of collagen fibrils and other multimeric structures", "Metabolism of steroids", "Extracellular matrix organization", and "Collagen formation".

Discussion

Nucleus pulposus cells are located in the ecological niches without blood vessels and nerves where the osmotic pressure is higher than the plasma and is able to hold more proteoglycans¹⁸. The osmotic pressure alters with the age and degeneration of the intervertebral disc, which has a close relationship with the low back pain¹⁹.

The nucleus pulposus cells under the stress change the immune status in our studies. We found most of the immune pathways such as the MAPK signaling pathway, TNF signaling pathway, IL17 signaling pathway, and the NF-κB signaling pathway were related to the nucleus pulposus cells change. Similarly, the MAPK signaling pathway shows a critical role in controlling cell proliferation, differentiation, and apoptosis under the pathophysiological conditions of intervertebral disc²⁰. At the beginning of low back pain, there are significantly higher levels of TNF in the patient's group in comparison with the healthy group²¹. Shamji MF et al reported that the increased IL17 is closely related to the chronification of pain and the degenerated lumbar intervertebral discs²².

NF- κ B is a key inflammatory response mediator that regulates inflammation in chondrocytes and macrophages²³⁻²⁵, which can lead to osteoarthritis and rheumatoid arthritis^{26, 27}. Moreover, NF- κ B is able to regulate several inflammatory enzymes such as COX-2 to affect the pain sensitivity in arthritis^{28, 29}. Aisha S. Ahmed et al found that NF- κ B is also involved in the pain-Related neuropeptide expression in degenerative disc³⁰. Interestingly, we found the nucleus pulposus cells under the stress could affect rheumatoid arthritis and steroid biosynthesis by KEGG analysis, suggesting that nucleus pulposus cells may involve in the synthesis and secretion of proteins and liposomes under the loading condition.

By analyzing the GO, we found that cell migration and cell adhesion are the most important processes in the stretched nucleus pulposus cells. C.L. Gilchrist et al found that nucleus pulposus cells are originated from the embryonic notochord that exhibits strong cell-cell interaction and adherence³¹. Weiheng Wang et al showed the allogenic nucleus pulposus cells have the anti-apoptotic and migratory ability that lead to the disc regeneration³². GPCR signaling and RGS proteins regulate numerous physiological and pathological processes including inflammation, bone metabolism, and pain^{28, 33-36}. Strikingly, we found that nucleus pulposus cells can affect the GTPase regulator activity by analyzing the cellular components of GO, suggesting that RGS proteins may also regulate the functions of nucleus pulposus cells under the stress.

Besides the KEGG and GO analyses, we also identified the significant PPI networks and genes in nucleus pulposus cells with the loading stress. P-B Li et al found that the up-regulation of MMP9 is strongly related to the degenerated lumbar disc tissues³⁷. Yang Yu et al found the CXCL12 was increased in the dorsal root ganglion after chronic compression³⁸. The study by C Tilkeridis et al showed the sequence changes of the COL1A1 gene indicate a strong relationship with lumbar disc diseases³⁹. Zongde et al found the decreased COL3A is associated with disc degeneration by bioinformatic methods⁴⁰. Louise S.C. Nicol found the inhibition of GM-CSF is analgesic in neuropathic pain⁴¹. The circadian clocks and their downstream molecules play central roles in various physiological functions⁴² such as metabolism, immune, apoptosis, and ER stress, protein synthesis, and its disruption has been related to many diseases⁴³⁻⁵¹. As a circadian gene-controlled gene, CCL5 regulates the most neuroprotective effects^{52, 53}.

The Study by Jiangtao Wan et al showed that MMP3 is a destabilizing factor in bone diseases⁵⁴. Rangel L Silva et al found that CXCL1 enhances nociceptor and central sensitization through CXCR2, which is a target for analgesic drugs⁵⁵. Zhenpeng Song et al found that STAT1 leads to cancer pain as a downstream regulator of ERK signaling⁵⁶. Zhonghui Guan et al showed the microglial expression genes that are related to pain through the CSF1 singling for survival⁵⁷.

In conclusion, this study provided information on nucleus pulposus cells under loading conditions. MAPK signaling, TNF signaling, IL17 signaling, and NF-κB signaling are the major biological pathways in the stretched nucleus pulposus cells. This study may shed light on the treatment of low back pain and disc deterioration.

Author Contributions

Min Zhang, Jing Wang: Methodology and Writing. Hanming Gu: Conceptualization, Methodology, Writing- Reviewing and Editing.

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Declarations of interest

There is no conflict of interest to declare.

Figure 2. KEGG and GO analyses of DEGs

(A) KEGG analysis, (B) Biological processes, (C) Cellular components, (D) Molecular functions.

Figure 3. The PPI network analysis of DEGs between the control and elongated nucleus pulposus cells

Cluster 1 (A) and cluster 2 (B) were the top two clusters and were constructed by String and MCODE in Cytoscape.

Figure 4. Reactome map representation of the significant biological processes between the control and elongated nucleus pulposus cells

Table 1

Entrez gene	Gene Symbol	Fold-change	Regulation
Top 10 down-regulated DEGs			
681355	LOC681355	-4.308594983	Down
1240	Cmklr1	-4.188048629	Down
2487	Frzb	-3.826703132	Down
103689971	LOC103689971	-3.823545803	Down
80168	Mogat2	-3.58056859	Down
2619	Gas1	-3.374662642	Down
7060	Thbs4	-3.239004297	Down
55220	Klhdc8a	-3.198784255	Down
2637	Gbx2	-3.191774195	Down
25992	Sned1	-3.184824365	Down
Top 10 up-regulated DEGs			
6348	Ccl3	7.38862333	up
6352	Ccl5	6.817080418	up
969	Cd69	6.331380669	up
4318	Mmp9	6.218007819	up
1437	Csf2	6.203278857	up
445	Ass1	6.153898019	up
3488	Igfbp5	6.150743666	up
1440	Csf3	5.895138107	up
5055	Serpinb2	5.815539174	up
4319	Mmp10	5.64306114	up

Table 2. Top ten genes demonstrated by connectivity degree in the PPI network

Gene Symbol	Gene title	Degree
Mmp9	Matrix metalloproteinase 9	58
Cxcl12	C-X-C motif chemokine ligand 12	38
Col1a1	Collagen type I alpha 1 chain	37
Col3a1	Collagen type III alpha 1 chain	32
Csf2	Colony stimulating factor 2	30
Ccl5	C-C motif chemokine ligand 5	29
Mmp3	Matrix metalloproteinase 3	28
Cxcl1	C-X-C motif chemokine ligand 1	28
Stat1	Signal transducer and activator of transcription 1	26
Csf1	Colony stimulating factor 1	26

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