Analysis of key genes and pathways associated with the pathogenesis of intervertebral disc degeneration

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Research article

Keywords: intervertebral disc degeneration, gene, bioinformatics

DOI: https://doi.org/10.21203/rs.3.rs-32769/v2

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Abstract

Background: Intervertebral disc degeneration (IDD) is widely known as a main contributor to low back pain which has a negative socioeconomic impact worldwide. However, the underlying mechanism remains unclear. This study aims to analyze the dataset GSE23130 using bioinformatics methods to identify the pivotal genes and pathways associated with IDD.

Material/Methods: The gene expression data of GSE23130 was downloaded and differentially expressed genes (DEGs) were extracted from 8 samples and 15 controls. GO and KEGG pathway enrichment analyses were performed. Also, Protein–protein interaction (PPI) network was constructed and visualized, followed by identification of hub genes and key module.

Results: A total of 30 downregulated and 79 upregulated genes were identified. The DEGs mainly enriched in regulation of protein catabolic process, extracellular matrix organization, collagen fibril organization, and extracellular structure organization. Meanwhile, we found that most of DEGs were primarily enriched in PI3K-Akt signaling pathway. The top 10 hub genes were FN1, COL1A2, SPARC, COL3A1, CTGF, LUM, TIMP1, THBS2, COL5A2, and TGFBI.

Conclusions: In summary, key candidate genes and pathway were identified by using integrated bioinformatics analysis, which may provide insights into underlying mechanisms and offer potential target genes for the treatment of IDD.

Introduction

Low back pain (LBP) is increasingly recognized as a global public health problem associated with decreased quality of life and increased health-care expenditure[1-3]. It is estimated that 70-80% adult population suffer from at least one episode of LBP during their lifetime and 10% become chronically disabled[4]. With the changing of work and lifestyle, the incidence and prevalence of low back pain increase dramatically in the past few decades.

Intervertebral disc degeneration (IDD) is one of the most common source of low back pain[5]. Being the largest avascular structure of human body, intervertebral disc is a complex structure consisting of annulus fibrosus, nucleus pulposus and cartilage endplate. Extracellular matrix (ECM) is the main component of intervertebral disc which is responsible for maintaining both structure and function of intervertebral disc. Intervertebral disc degeneration is characterized by the excessive degradation of ECM, leading to reduced hydration, loss of disc height, and decreased ability to absorb mechanical force[6].

Management of IDD includes both conservative treatment and surgery. Although both methods have achieved satisfactory clinical outcomes, neither conservative nor surgical treatment can completely resolve lumbar disc degeneration. Hence, it is necessary to elucidate the underlying mechanisms of IDD in order to find out a curative method. Previous studies showed that IDD is most likely to be multifactorial, including apoptosis[7,8], inflammation[9,10], aging[11,12], and biomechanical loading[13,14]. However, the genetic factors are regarded as the most significant contributor[15].

Nowadays, the gene chip technology and bioinformatics methods have been widely used to obtain gene expression profile of disease. This study uses bioinformatics methods to analyze microarray of nucleus pulposus with the aim to identify differentially expressed genes (DEGs) and pathway related to progression of IDD. We also investigated some hub genes involved in progression of IDD based on protein-protein interaction (PPI) network. This study may provide new insights into pathogenesis of IDD and potential target candidates for new therapy.

Methods

Microarray data collection. The gene expression data of GSE23130 was downloaded from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) database, which was based on GPL1352 platform of Affymetrix Human X3P Array. Disc Tissue samples were obtained either by the National Cancer Institute Cooperative Tissue Network (CHTN) or surgical disc procedures performed on patients with herniated discs and degenerative disc disease. Thompson grade IV and V are considered to be IDD samples while Thompson grade I, II and III are control samples according to Thompson grading criteria[20]. A total of 23 samples, including 15 control samples and 8 IDD samples were contained in this dataset.

Differential Expression Analysis. Limma package version 3.28.21 (20) of Bioconductor 3.5 (http://www.bioconductor.org/packages/3.5/bioc/html/limma.html) was used to identify DEGs between the IDD samples and normal controls. Genes with fold change (logFC) >1 (upregulated) or < -1 (downregulated) and P value<0.05 were considered differentially expressed. Both heatmap and volcano plots were constructed to present expression profiles of differentially expressed genes using hierarchical clustering, which was performed using R software.

KEGG and GO enrichment analyses of DEGs. GO is a major bioinformatics tool to annotate genes, analyze gene products and sequences to underlying biological phenomena, including biological process (BP), molecular function (MF), and cellular component (CC)[21]. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a knowledge base for systematic analysis, annotation or visualization of gene functions and critical biological pathways closely related to intervertebral disc degeneration[22]. Both GO and KEGG enrichment analysis were conducted by Bioconductor. A P < 0.05 was considered to have statistical significance and to achieve significant enrichment.

PPI network construction and analysis of modules. Protein–protein interaction (PPI) enrichment analysis is useful to analyze the functional interactions between proteins which may provide insights into the mechanisms of generation or development of diseases. Online String[23] and Cytoscape[24] software were used to build the PPI network, and a confidence score >0.4 was set as the cut-off criterion. The most significant module was identified using Molecular Complex Detection (MCODE) using the following parameters: degree cut-off = 2, node score cut-off = 0.2, k-core = 2, and maximum depth = 100. GO and KEGG pathway enrichment analyses of genes in the most significant node were subsequently performed using String.
Hub gene selection and analysis. CytoHubba was used to select hub genes of PPI network with MCC method. The biological process of hub genes was then analyzed and visualized by the Biological Networks Gene Oncology tool (BiNGO; http://apps.cytoscape.org/apps/bingo) plugin.

Results

Differential Expression Analysis. A total of 19703 DEGs were identified from intervertebral disc samples in GSE23130. Among them, 30 were downregulated and 79 were upregulated within the P value <0.05 and |log2FC| >1 criterion. A heatmap plot and a volcano are shown in Figure 1 and figure 2 respectively, in which the red represents upregulated genes and the green represents downregulated genes. The top 10 upregulated and downregulated DEGs are shown in Table 1.

KEGG and GO enrichment analyses. We performed GO categories enrichment analysis to gain insights into the biological roles of the DEGs from degenerated versus non-degenerated disc samples. The DEGs mainly enriched in skeletal system development, regulation of protein catabolic process, extracellular matrix organization, collagen fibril organization, and extracellular structure organization in terms of biological process (BP). The DEGs mostly enriched in collagen-containing extracellular matrix, extracellular matrix, fibrillar collagen trimer, banded collagen fibril, and complex of collagen trimers regarding cellular component (CC). The DEGs primarily participate in extracellular matrix structural constituent, collagen binding, disordered domain specific binding, extracellular matrix structural constituent conferring tensile strength and structural constituent of post synapse concerning molecular function (MF). The top 5 BP, CC and MF enrichment analysis of DEGs are summarized in Table 2. The GO enrichment related bubble chart and circle plot are presented in Figure 3 and Figure 4. KEGG enrichment analysis showed that DEGs were enriched in ‘proteoglycans in cancer’, ‘platelet activation’, ‘PI3K-Akt signaling pathway’, ‘regulation of actin cytoskeleton’ and ‘thermogenesis’. The bubble chart and circle plot of KEGG enrichment were illustrated in Figure 5 and 6.

PPI network construction and analysis of modules. PPI network of DEGs was download from String and further analyzed by CytoScape. The PPI network included 110 nodes and 410 edges (Figure 7). The most significant module was identified from the PPI network using MCODE, and consisted of 17 nodes and 64 edges (Figure 8). GO and KEGG enrichment analysis of this module using String showed that these genes were mainly involve in extracellular matrix organization, skeletal system development, Protein digestion and absorption and ribosome.

Hub gene selection and analysis. The top 10 hub genes were identified by CytoHubba plugin using the Maximal Clique Centrality (MCC) method, including FN1, COL1A2, SPARC, COL3A1, CTGF, LUM, TIMP1, THBS2, COL5A2, and TGFb1 (Figure 9). All hub genes were upregulated in degenerated disc compared with control group. Analysis of hub genes were summarized in Table 3.

Discussion

Despite years of numerous clinical and experimental investigation, the underlying mechanisms of intervertebral disc degeneration remains unclear, which hinders the development of curative therapy. Genetic factors, mechanical factors, aging, inflammation and other potential factors may cause IDD whereas genetic factors play a critical role based on published literatures. Several studies indicate that genetic factors are critical contributors to the onset and progression of IDD[34,35]. For example, COL1A1 is a key gene encoding collagen I and polymorphisms of COL1A1 gene has been reported to increase the risk of IDD in different population studies[27,28]. In the present study, we identify a total of 109 DEGs between degenerative samples and controls, including 79 upregulated and 30 downregulated DEGs.

In terms of GO enrichment analysis, we found that most of DEGs were mainly involved in skeletal system development, regulation of protein catabolic process, extracellular matrix (ECM) organization, collagen fibril organization, and extracellular structure organization. Extracellular matrix (ECM) is a non-cellular threedimensional macromolecular network predominantly composed of collagens, proteoglycans and many other glycoproteins. ECM is crucial for maintaining structural and functional integrity of intervertebral disc. Previous studies showed that even though many potential mechanisms induced IDD, they led to a final common result of excessive degradation of the extracellular matrix[29]. The imbalance between anabolism and catabolism of ECM is regulated by ECM-modifying enzymes such as matrix metalloproteinases (MMPs) and their endogenous tissue inhibitors of metalloproteinases (TIMPs)[30-32]. Lumican (LUM) is the most significantly up-regulated gene in our analysis, which is one kind of keratan sulfate proteoglycan constituents of the ECM. Several studies showed that the abundance of lumican changed with the degeneration of intervertebral disc[33,34]. Study conducted by Vo NV et al. showed that ECM degradation increased by regulation of matrix metalloproteinases (MMPs), and ADAMTSs, leading to the development of IDD[35]. On the contrary, TIMP-1 and TIMP-2 mRNA and protein expression increases in degenerated IVD tissue, antagonizing the effect of MMPs[36]. Our bioinformatic analysis also showed that TIMP-1 increased in the IVD samples than controls.

Regarding KEGG pathway of our analysis, we found that most of DEGs were primarily enriched in PI3K-Akt signaling pathway. PI3K-Akt signal pathway showed protective effects on human nucleus pulposus under different pathological conditions. Activation of PI3K-Akt pathway protects against IDD by increase of ECM content, prevention of cell apoptosis and induction or prevention of cell autophagy. Studies confirmed that activation of PI3K-Akt pathway increased SOX9 expression and activity, and consequently led to increase of aggrecan expression in NP cells[37]. A study revealed that 17β-estradiol (E2) prevented the degradation of ECM by activation PI3K-Akt-FOXO3, which reduced the expression of MMP-3 and increased the expression of collagen II and aggrecan expression[38]. Many recent studies also showed that resveratrol suppressed IL-1β-mediated NP cell apoptosis through activating the PI3K-Akt pathway[39-41]. On the contrary, as the only known lipid phosphatase, tumor suppressor phosphatase and tensin homolog deleted from chromosome 10 (PTEN) can counteract the protective effect of PI3K-Akt pathway. Xi Y et al. showed that PTEN promoted intervertebral disc degeneration by negatively influence PI3K-Akt[42]. Hence, gene therapy targeting PTEN may play an important role in treating IDD.
We further constructed a PPI network for better understanding of the interaction between DEGs. The most significant module was extracted from the PPI network using MCODE plugin. Furthermore, top 10 hub genes—FN1, COL1A2, SPARC, COL3A1, CTGF, LUM, TIMP1, THBS2, COL5A2, and TGFBI, were identified from this network. To be mentioned, all hub genes also enriched in the most significant module. TGFBI is the seed DEG of the module. TGFBI is a protein secreted by many types of cells. It binds to collagen, forms part of the extracellular matrix (ECM), and interacts with integrins on cell surfaces. Study showed that TGF-β increased expression of COL1A1, ACAN, and SOX9 genes by mediating communication between nucleus pulposus cells and mesenchymal stem cells\[43\]. Activation of TGF-β signaling has a protective effect on intervertebral disc via inhibition of ECM degradation and increase of ECM synthesis, promotion of cell proliferation and inhibition of cell death, and alleviation of inflammatory response. However, excessive activation of TGF-β signaling may contribute to IVD degeneration\[44\]. SPARC is a matricellular glycoprotein involved in interactions between cells and matrix. Gruber HE et al. showed that deletion of the SPARC gene accelerated disc degeneration in the aging mouse\[45\]. Millecamps et al. demonstrated that inactivation of the SPARC gene led to early onset of both disc degeneration and behavioral indices of LBP in mice\[46\]. Tajerian M et al. showed that the underlying mechanism of silence of SPARC gene during aging may attributed to DNA methylation\[47\]. A recent in vivo experimental study showed that stable expression of CTGF and TIMP1 genes by co-transfection adeno-associated virus 2 increased synthesis of aggrecan and type II collagen in degenerated intervertebral disc, which served as potential target gene for disc regeneration\[48\]. Thrombospondin proteins (THBSs) are a class of glycoproteins that functions in maintaining homeostasis of ECM by regulating level of matrix metalloproteinase-2 (MMP-2) and MMP-9\[49,50\]. A Japanese population based genetic and functional data indicated that THBS2 played an important role in the pathogenesis of LDH by acting as modulator of MMP-2 and MMP-9 endocytosis\[51\].

**Conclusion**

In summary, the present study provides comprehensive analysis about the pathogenesis of IDD and offers potential target genes for the early diagnosis and treatment of intervertebral disc disease. PI3K-Akt signal pathway and related hub genes may play important roles in the progression of IDD that needs deeper investigation. Nevertheless, further experiments are required to validate their effects and mechanisms in IDD.

**Abbreviations**

LBP, low back pain; IVD, intervertebral disc; IDD, intervertebral disc degeneration; AF, annulus fibrosus; NP, nucleus pulposus; CEP, cartilage endplate; miRNA, microRNA; ECM, extracellular matrix; MMP, matrix metalloproteinase; DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

**Declarations**

**Ethics approval and consent to participate** This study is based on microarray analysis.

**Conflicts of interest statement** The authors have no conflicts of interest to disclose in relation to this article.

**Consent for publication** Not applicable.

**Data Availability Statement** The following information was supplied regarding data availability: The raw data can be found at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23130.

**Author Contribution** Conceived and designed the study: Tao Lan and Zhe Shen, analyzed the data: Yucheng Fu, wrote and revised the manuscript: Tao Lan and Shiyu Hu.

**Funding Statement** There are no funders to report for this submission.

**Acknowledgments** Special acknowledgment is due to May for assistance and support of life and work.

**Tables**

Table 1. The top 10 upregulated and downregulated DEGs.
| Group       | Gene symbol | logFC | P Value          |
|------------|-------------|-------|------------------|
| Downregulated | DMKN        | -3.168 | 0.006602318     |
|             | LOC647070   | -3.042 | 0.001467741     |
|             | GSK3A       | -2.233 | 0.005937805     |
|             | FLJ32214    | -2.113 | 0.023887151     |
|             | ATP13A1     | -1.831 | 0.008133858     |
|             | RAB11B      | -1.702 | 0.001118076     |
|             | ZNF808      | -1.525 | 0.045232746     |
|             | USP28       | -1.478 | 0.015404176     |
|             | LOC642533   | -1.458 | 0.019098623     |
|             | LOC222070   | -1.403 | 0.03768916      |
| Upregulated | LUM         | 3.147  | 0.018330652     |
|            | HTRA1       | 2.435  | 0.031358097     |
|            | SPARC       | 2.320  | 0.032710624     |
|            | RPLP0       | 2.275  | 0.03908414      |
|            | KIAA1751    | 2.184  | 0.026912864     |
|            | CLU         | 2.030  | 0.027190866     |
|            | ND2         | 1.991  | 0.046653239     |
|            | COL3A1      | 1.949  | 0.015460576     |
|            | S100A4      | 1.937  | 0.021301645     |
|            | MXRA5       | 1.877  | 0.020244629     |

Table 2. The top 5 BP, CC, and MF enrichment analyses of the upregulated and downregulated DEGs.
| Ontology | ID         | Description                                      | Gene Ratio | P value   | P adjust | Gene ID                                      |
|----------|------------|--------------------------------------------------|------------|-----------|----------|----------------------------------------------|
| BP       | GO:0001501 | skeletal system development                      | 12/81      | 1.55E-06  | 0.003    | COL1A2/PAPSS1/GJA1/COL3A1/TIMP1/LUM/CTSK/MGP/PPIB/PLS3/|
| BP       | GO:0042176 | regulation of protein catabolic process          | 10/81      | 4.33E-06  | 0.004    | GSK3A/MSN/EEF1A1/GJA1/GPX1/TIMP1/CLU/NDUFA13/CDK5RAP3/P |
| BP       | GO:0030198 | extracellular matrix organization                | 9/81       | 1.96E-05  | 0.013    | COL1A2/COL3A1/TIMP1/LUM/COL5A2/CTSK/FN1/HTRA1/SPARC |
| BP       | GO:0030199 | collagen fibril organization                     | 4/81       | 5.02E-05  | 0.021    | COL1A2/COL3A1/LUM/COL5A2                       |
| BP       | GO:0043062 | extracellular structure organization              | 9/81       | 6.18E-05  | 0.021    | COL1A2/COL3A1/TIMP1/LUM/COL5A2/CTSK/FN1/HTRA1/SPARC |
| BP       | GO:0030168 | platelet activation                              | 6/81       | 6.50E-05  | 0.021    | ACTB/COL1A2/NOS3/COL3A1/ACTG1/CLIC1           |
| CC       | GO:0062023 | collagen-containing extracellular matrix         | 17/84      | 1.98E-12  | 5.40E-10 | COL1A2/PCOLCE/ASPN/LGALS1/COL3A1/TIMP1/THBS2/LUM/COL5A2 |
| CC       | GO:0031012 | extracellular matrix                             | 17/84      | 2.47E-11  | 3.37E-09 | COL1A2/PCOLCE/ASPN/LGALS1/COL3A1/TIMP1/THBS2/LUM/COL5A2 |
| CC       | GO:0005583 | fibrillar collagen trimer                        | 4/84       | 1.23E-07  | 8.37E-06 | COL1A2/COL3A1/LUM/COL5A2                       |
| CC       | GO:0098643 | banded collagen fibril                           | 4/84       | 1.23E-07  | 8.37E-06 | COL1A2/COL3A1/LUM/COL5A2                       |
| CC       | GO:0098644 | complex of collagen trimers                      | 4/84       | 1.11E-06  | 6.06E-05 | COL1A2/COL3A1/LUM/COL5A2                       |
| MF       | GO:0005201 | extracellular matrix structural constituent      | 12/80      | 9.73E-12  | 2.35E-09 | COL1A2/PCOLCE/ASPN/COL3A1/THBS2/LUM/COL5A2/MGP/MXRA5/F |
| MF       | GO:0005518 | collagen binding                                 | 8/80       | 4.10E-10  | 4.97E-08 | PCOLCE/ASPN/LUM/CTSK/PPIB/FN1/SPARC/TGFBI     |
| MF       | GO:0097718 | disordered domain specific binding               | 3/80       | 0.000412372 | 0.033   | GJA1/FN1/GAPDH                                    |
| MF       | GO:0030020 | extracellular matrix structural constituent      | 3/80       | 0.000945361 | 0.037    | COL1A2/COL3A1/COL5A2                           |
| MF       | GO:0099186 | structural constituent of postsynapse            | 2/80       | 0.000963809 | 0.037    | ACTB/ACTG1                                     |
| KEGG     | hsa05205   | Proteoglycans in cancer                          | 8/50       | 3.50E-05  | 0.004    | ACTB/COL1A2/MSN/CD63/LUM/FN1/ACTG1/RPS6       |
| KEGG     | hsa04611   | Platelet activation                              | 5/50       | 0.001031369 | 0.059   | ACTB/COL1A2/NOS3/COL3A1/ACTG1               |
| KEGG     | hsa04151   | PI3K-Akt signaling pathway                       | 8/50       | 0.001491165 | 0.059   | YWHAB/COL1A2/SGK1/NOS3/THBS2/FN1/RPS6/CHRM1  |
| KEGG     | hsa04810   | Regulation of actin cytoskeleton                 | 6/50       | 0.00205303 | 0.061    | ACTB/MSN/PFN2/FN1/ACTG1/CHRM1               |
| KEGG     | hsa04714   | Thermogenesis                                    | 6/50       | 0.003083363 | 0.069    | ACTB/ACTG1/NDUFA13/RPS6/ND2/UQCRH          |

Table 3. The top 10 hub genes.
| Rank | Name   | Score | degree |
|------|--------|-------|--------|
| 1    | FN1    | 18070 | 10     |
| 2    | COL1A2 | 17598 | 18     |
| 3    | SPARC  | 17455 | 17     |
| 4    | COL3A1 | 17338 | 17     |
| 5    | CTGF   | 16346 | 15     |
| 6    | LUM    | 15840 | 10     |
| 7    | TIMP1  | 12030 | 16     |
| 8    | THBS2  | 10106 | 10     |
| 9    | COL5A2 | 5796  | 11     |
| 10   | TGFBI  | 5040  | 7      |

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**Figures**
Figure 1

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