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Original Research

Comprehensive multi-factors reveal the pathogenesis of degenerative intervertebral disc

Kahaer Aikenmu[1] , Zhenbin Wang[1] , Qingcai Meng[2*]

[1] Department of Spine, The Fourth Affiliated Hospital of Xinjiang Medical University Urumqi, Xinjiang 830000, China
[2] Department of Orthopedic, The Fourth Affiliated Hospital of Xinjiang Medical University Urumqi, Xinjiang 830000, China

*Correspondence to: xjmengqingcai@163.com

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Abstract: Intervertebral Disc (IVD) is a moderately moving joint that provides load transfer and flexibility to the entire spine. Although healthy IVD can balance the turnover of slow-synthesis matrices, this balance is often disrupted that leading to the development of degenerative diseases. The pathogenesis and treatment mechanism of Intervertebral Disc Degeneration (IDD) has always been the focus of scientific research, but its pathogenesis is still unknown. Therefore, this study is based on a modular approach to in-depth analysis and explore the genes of IDD, intended to identify the molecular process of disc degeneration. Firstly, the data related to Intervertebral Disc Degeneration and normal intervertebral disc were downloaded from the GEO database. The differential analysis of two kinds of data was performed to obtain differential gene expression profiles. Secondly, mapping those differential genes to Cytoscape to construct protein-protein interaction networks (PPIs). Then, the module gene was subjected to enrichment analysis of GO function and KEGG pathway. Finally, non-coding RNAs (ncRNAs) and transcription factors that regulate the module are predicted based on hypergeometric testing. In summary, we identified 22 co-expression modules, and the enrichment analysis results revealed that the module genes were significantly involved in the regulation of definite biotic procedures. In conclusion, we recognized the ncRNA pivot (including miR-193b-3p, CRNDE, etc.) and TF pivot (including E2F1, E2F4, etc.) that significantly regulate dysfunction modules.

Key words: Intervertebral disc degeneration; Protein interaction network; Enrichment; Co-expression analysis; Regulatory factors.

Introduction

Intervertebral disc (IVD) has been known as the largest avascular structure in the human body and is known for its low cell content (1). Intervertebral disc degeneration (IDD) is a procedure that is supplemented by changes in cell, biochemistry, structure, and function and is largely responsible for low back pain (2). Current treatment strategies for degenerative disc disease, such as conservative treatment and surgery, do not treat the underlying pathogen, only relieve related symptoms such as low back pain. Surgical treatment will not reverse the degeneration of IDD, and may even accelerate degeneration (3). Degeneration of the intervertebral disc can lead to sciatica and back pain (4). Tissue weakening due to genetic factors, aging, nutritional damage and overload history is a fundamental factor leading to a degeneration of the intervertebral disc (5). Evidence revealed that mechanical stimulation is responded to by massive biological remodeling in the IVD, which may play a key role in degenerative intervertebral discs (6). In genetics, IL-37, IL-1β, IL-16, TGF-β1, and Smad3 are highly correlated with the degree of IVD degeneration, while IL-10 SNP is significantly associated with the IVD in the Iranian population (7, 8). Besides, the polymorphism of type I collagen (COLIA1) Sp1 gene may be a genetic risk factor for IDD in the elderly in Han (9).

At the present stage, researchers have explored the pathogenesis of IDD from the perspective of medicine and biology and made some progress. For example, autonomous circadian clocks established in mouse and human IVD cells can respond to age and cytokines and control critical pathways involved in IVD homeostasis (10). Cytokine and insufficient nutrient supply have been identified as one of the causes of IDD (11,12). In addition, disc degeneration is associated with changes in cytological behavior, including increased cell death and degradation of extracellular matrices (13). On the other hand, microRNA plays an important role in the degeneration of IVD, which can cause degeneration of IVD by regulating gene expression, thereby affecting cell apoptosis and proliferation, and increasing the production of inflammatory mediators and proteases (14). More importantly, microRNA stimulated by inflammation play a crucial role in promoting intervertebral disc degeneration by blocking chondroitin sulfate. This provides new insights into the pathogenesis and treatment of intervertebral disc degeneration (15). All of these findings have deepened our understanding of the pathogenesis of disc degeneration and provide guidance for our further research.

Though earlier investigators have described a series of studies on disc degeneration, the general results remain elusive. In order to explore the mechanism of intervertebral disc degeneration comprehensively and deeply, we conducted a systematic modular analysis to determine the dysfunction modules and core molecules among the results. This provides a rich candidate resource for future experimental verification and drug

transfer and also provides theoretical guidance for future biological research of breast cancer.

Materials and Methods

Data resource

The NCBI (National Center for Biotechnology Information) Gene Expression Omnibus database (GEO Dataset) (16) consists of all types of high-throughput experimental data, including single-channel and dual-channel microarray-based assays for mRNA abundance, genomic DNA data and protein molecules data. Furthermore, it contains data from non-array-based high-throughput functional genomics and proteomics technologies. We first together with a set of gene expression profiles for intervertebral disc degeneration and non-degeneration of intervertebral discs from GEO, numbered GSE34095. The data set included three degenerative discs and three non-degenerative discs' samples. After that, we screened the ncRNA-mRNA interaction pairs with score >= 0.5 from the RAID v2.0 database (17), including 431937 interaction pairs involving 5431 ncRNA. All human transcription factor target data were downloaded and used in the general database TRRUST v2 database (18) for transcriptional studies, involving 2492 transcription factors and 9396 interaction pairs.

Difference analysis

The differential expression analysis of the gene expression profile data was accomplished by the R language limma package (19-21). First, use the background correct function to perform background correction and normalization of the data. Next, the control probe and the low expression probe were filtered by the Normalize Between Arrays function and quantile normalization way. Then, the differentially expressed genes of the data set were identified based on the lmFit and eBayes functions, adopting default parameters. Finally, the differential expression results were imported into Cytoscape (22) to construct a protein interaction network.

The protein-based network identification module

The modular approach is crucial to this research. Firstly, we mapped all differentially expressed genes into a human protein-protein interaction network, extracted interaction pairs only containing these differential genes, and constructed PPIs for IDD differential genes. Then, we apply the plug-in ClusterONE (23) with default parameters to classify the protein complex based on the cohesion algorithm and neighbor selection strategy, which is also the identification problem of dense substructure in the PPI network. In the ClusterONE algorithm, the higher the cohesion score, the protein is more likely a protein complex.

Function and pathway enrichment

Exploring the functions and signal pathways complicated in gene involvement often aids to investigate the molecular mechanisms of disease, and the enrichment analysis of the functions and pathways of genes in dysfunctional modules is an operative method to discover the underlying mechanisms of IDD. Consequently, we used the R language Cluster profiler package (24) for Go function and KEGG pathway enrichment analysis for the 22 modular genes of IDD. The cluster profiler is a Bioconductor software package that provides statistical analysis and visualization of the functional clustering of gene sets or gene clusters. Furthermore, we use Cytoscape's BinGO (25) application for pathway analysis of the integrated module network.

Regulator analysis

The transcription and post-transcriptional regulation of genes are often driven by non-coding RNA (ncRNA) and transcription factors (TF). Therefore, we have predicted and tested the role of the disc degeneration dysfunction module scientifically. Pivot regulators are defined as modulators that have significant regulatory effects on the module during disc degeneration, including ncRNA and TF. We require that there must be more than two control connections between each regulator and each module, and the significance of the enrichment target in each module calculated according to the hypergeometric test is p-value < 0.01.

Results

Determine expression disorder molecules of IDD

Biologists have conducted many experiments on the pathogenesis of intervertebral disc degeneration and thus recognized the potential pathogenic genes. Nevertheless, the complex molecular connections and overall effects of these genes are uncertain. In order to detect the molecular changes in the process of IDD, we achieved differential expression analysis based on microarray data to identify differential gene expression (DEG) between disc degeneration and normal intervertebral disc, acquired genes that may lead to disc degeneration. The results revealed that 581 differential genes were gotten, and we believe that there are genes for disc degeneration in these differential genes. Subsequently, we increase the differential gene number to 2802.

Identify intervertebral disc-related functional modules

Modularity is a subsystem. It processes globally complex systems and decomposes and methodizes them in more detail. Each subsystem has its characteristics. For each basic gene, a module is a collection of genes with a series of co-expression relationships, and genes of the same module have consistent expression behavior. Besides, each module also has a certain interactive relationship. The overall effect of these interactions represents the global characteristics, and it is the bridge where each element gene plays a role in the global network. The expression behavior of genes related to intervertebral disc degeneration in patient samples is clustered into modules, which helps us to observe the complex synergy between these genes from the perspective of expression behavior. We introduced 2,802 genes into Cytoscape. Based on the cohesive guided clustering algorithm, we explored 22 functional modules (Figure 1). Anyone's role or the interaction between several of the 22 functional modules forms a functional pathway leading to disc degeneration. Therefore, the identification of gene function modules is an important method to explore the potential dysfunction of intervertebral disc degeneration.

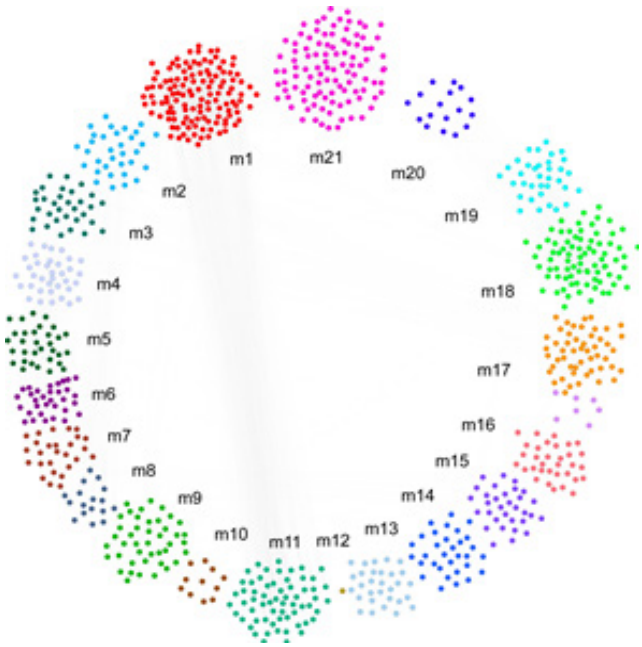

Figure 1. Identify the disc dysfunction module. There are 22 interaction modules, each color representing a module.

Functions and pathways involved in the gene of interested (GOI)

Functions and pathways are important mediators of disease physiological responses. Exploring the functions and pathways involved in dysfunctional module genes not only helps determine the upstream and downstream relationships of genes in the same pathway in the module but also helps build a molecular bridge between the module and disease based on systems biology Understanding of the underlying molecular mechanism of the disease. We performed GO function and KEGG pathway enrichment analysis on 22 modules and obtained 11853 biological processes, 1872 cells, 1920 molecular functions and 310 KEGG pathways (Figure 2). It was found that these functions are mainly concentrated on some biological processes, such as positive regulation of cell cycle, combined regulation, regulation of cell cycle phase transition, regulation of mitotic cell cycle phase transition and negative regulation of organelles. organization. At the same time, the enrichment results of the KEGG pathway indicate that the differential genes of IDD are mainly involved in signaling pathways such as viral carcinogenesis. In short, we integrated 22 module networks and used BinGO for path analysis (Figure 3).

TF and ncRNA driving disc degeneration

From the perspective of systems biology and systems genetics, gene transcription and post-transcriptional gene regulation have long been considered as key regulators of disease occurrence and development, while transcription factors and ncRNA are co-expression and function regulators. Although many biologists value the regulation of IDD by single or several TFs and ncRNAs, few studies have focused on their overall global impact on dysfunction mechanisms and their role as bridges. Therefore, in this case, based on the targeted regulatory relationship between TF and ncRNA and module genes, we conducted a key analysis of auxiliary modules to explore key transcriptional regulators that regulate the progress of IDD. The results predicted

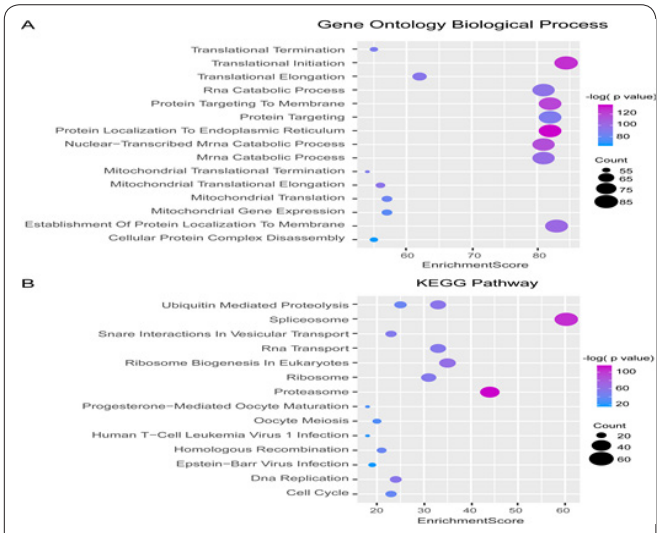

Figure 2. Extract of module gene function and pathway enrichment analysis. A. Excerpt analysis of module gene GO function enrichment analysis. The color increased from blue to purple, and the concentration increased significantly. The larger the circle, the greater the proportion of genes in the GO function module. B. Excerpt analysis of module gene KEGG pathway enrichment analysis. The color increased from blue to purple, and the concentration increased significantly. The larger the circle, the greater the proportion of the gene in the KEGG pathway entry.

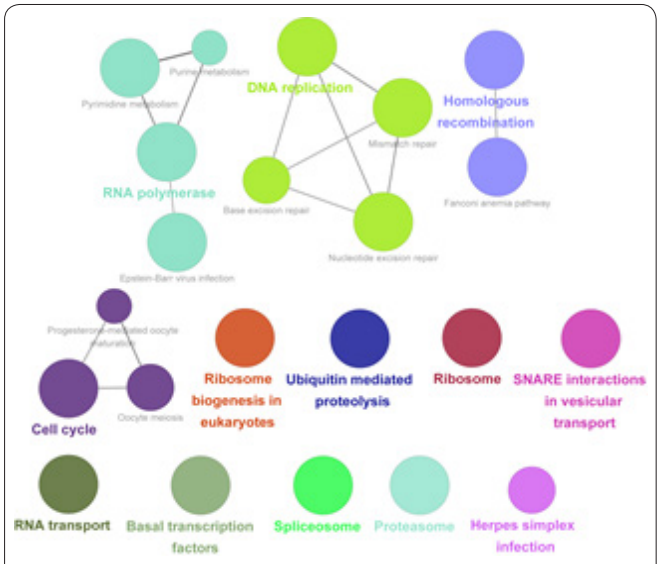

Figure 3. Path analysis of integrated module network.

that a total of 715 ncRNAs were associated with 1,213 ncRNA-module regulatory pairs, and 26 transcription factors were associated with 38 TF-module target pairs. Then import these data into Cytoscape to observed the adjustment of the regulator in the dysfunctional module (Figure 4A, B). In addition, the number of pivot control modules was statistically analyzed and the ncRNA modules (FENDRR, miR-19a-3p, miR-26b-5p, etc.) and TF (NFKB1, SP1, etc.) with the most abnormal functions were obtained. These transcription factors and ncRNA may regulate the process of IDD by mediating abnormal modules. Therefore, we identified these potential regulators as dysfunctional molecules during disc degeneration.

Discussion

Low back pain is a global health problem, more than

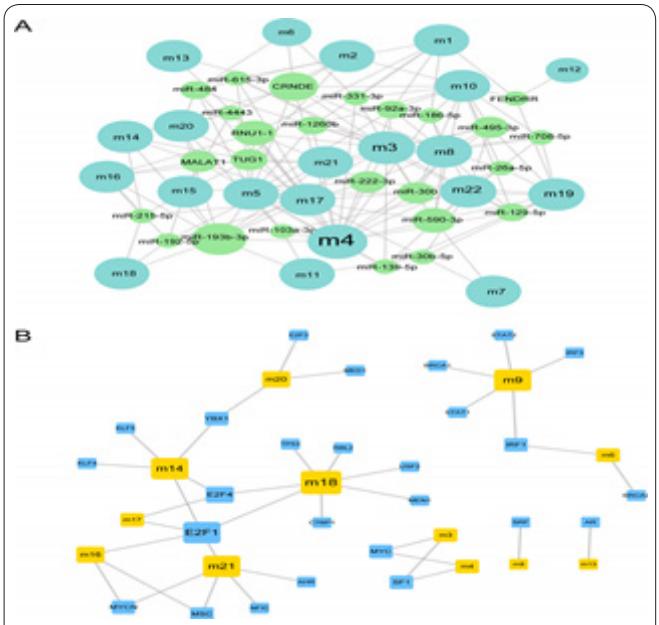

Figure 4. Modulators regulate the dysfunction module; **A.** The blue circle represents the module and the green circle represents the ncRNA, **B.** Orange squares represent modules and blue squares represent TF.

40% of which is caused by disc degeneration (26). Eliminating symptoms during the treatment of intervertebral disc degeneration is only a temporary solution and cannot be cured at all (27). Among them, loss of aggregating occurs in the early stage of intervertebral disc degeneration, and the degradation and loss of aggregating proteoglycan can lead to impaired function and degeneration of the intervertebral disc (28). Although researchers have studied IDD in various aspects and summarized it in the NCBI database, its pathogenesis remains unclear. In this research, we collected the genes for disc degeneration and intervertebral disc non-degeneration in the GEO Database (Gene Expression Omnibus Dataset) and analyzed the transcription factors and ncRNA regulators based on differential gene expression profiles in patients with disc degeneration. The driven differential expression module for intervertebral disc degeneration is intended to provide an in-depth understanding of the molecular mechanisms of intervertebral disc degeneration. At the functional module level, the module is significantly involved in some biological processes such as the positive regulation of the cell cycle, regulation of binding, regulation of cell cycle phase transition, regulation of mitotic cell cycle phase transition, and organelle fission and negative regulation of organelle tissue. It is also significantly involved in signal pathways such as viral carcinogenesis.

Disc degeneration is a common cause of back pain, which is associated with promoting cell senescence and reducing cell proliferation, whereas SUMO2 gene silencing promotes cell proliferation, and through down-regulation of NPC in rat IDD apoptosis and senescence-regulated p53 signal pathway (29). In addition, HNPSV-1 was successfully established using a recombinant SV40 adenoviral vector, indicating that human nucleus pulposus cells are immortalized and maintain primitive cell characteristics (30). More importantly, local adverse immune activity caused by the nucleus pulposus (NP) of the disc injury site is considered to be an important factor leading to disc degeneration (31). In addition, the module's genes are also involved in PI3K-Akt, Wnt, JAK-STAT, oxidative stress and other signaling pathways. Among them, phosphatidylinositol 3-kinase activates the PI3K/Akt pathway to prevent degeneration of the intervertebral disc, which is attributed to increased ECM content, prevention of apoptosis, cell proliferation, induction or prevention of autophagy, and facilitation of oxidative damage reduction, adapt to the hypoxic microenvironment (32). Silencing TUG1 not only protects human NPC from TNF-α-induced apoptosis and senescence but also promotes cell proliferation by blocking the Wnt/β-catenin pathway, providing a theoretical basis for clinical treatment of IDD (33). In addition, IL-21 interacts with TNF-α is positively correlated with intervertebral disc degeneration by the JAK-STAT signal pathway (34). IL-6 / JAK / STAT3 pathway is involved in the pathogenesis of IVD degeneration (35). On the other hand, oxidative stress-induced mitochondrial dysfunction is associated with the pathogenesis of intervertebral disc degeneration (IVDD) (36).

On the other hand, at the molecular level, we predicted that 715 ncRNAs participate in the mechanism of disc degeneration through a mediator module. According to statistical analysis, we determined that miR-193b-3p and CRNDE have significant effects on 11, 9 dysfunctional modules, while miR-590-3p and RNU1-1 significantly regulate 8 dysfunction modules. Among them, MiR-193b-3p is an important regulator of MMP-19 in human chondrocytes, which can alleviate the inflammatory response in osteoarthritis (37). CRNDE promotes proliferation, invasion and migration of osteosarcoma cells by regulating Notch1 signaling and epithelial-mesenchymal transition (38). MiR-590-3p promotes the proliferation and metastasis of colorectal cancer through the Hippo pathway (39). The mid-term fragility of the human RNU1 and RNU2 loci is induced by actinomycin D via the p53-dependent pathway (40). No effect on disc degeneration was found in the above studies of ncRNA. However, the results of our analysis show that it significantly regulates the dysfunction module of intervertebral disc degeneration, which is the focus of future research. Moreover, other ncRNAs that significantly regulate the disc degeneration dysfunction module may also be involved in the basic process of intervertebral disc degeneration, which can be used as a candidate for further molecular experiments. Finally, we identified 26 transcription factors that differentially expressed and significantly regulated the disc degeneration dysfunction module. According to the adjustment analysis, E2F1 and E2F4 significantly regulated 5 and 3 modules, respectively, and IRF1, MSC, MYC, MYCN, SF1, YBX1 significantly regulated 2 modules. These regulatory factors may play an important role in the degeneration of the disc. Among them, the transcription factor E2F1 is a core participant involved in cell cycle progression, DNA damage response and apoptosis (41). Transcription factors including TFAP2A, E2F4, SP3 and AR through mitogen-activated protein kinase, vascular endothelial growth factor and p53 pathway degeneration have the potential to regulate intervertebral discs (42). In the meanwhile, signal transduction and transcriptional activator 1 (STAT1), a key transcription factor in Janus kinase (JAK)-STAT signaling, regulates

the expression of a wide range of immune-related genes, including interferon (IFN) regulatory factor 1 (IRF1) (43). For exosomes, which are important carriers for information exchange between BM-MSCs and NPCs, individual exosomes or exosomes loaded with specific genes and drugs will be suitable candidates for cell-free treatment strategies for disc degeneration (44).On the other hand, MYC acts primarily as a transcription factor, promoting the expression of many target genes to coordinate death, proliferation and metabolism at the cellular, tissue and organism levels (45-50).

More importantly, the transcription factor MYCN is involved in the basic process control during embryonic development. The MYCN protein is located downstream of several signaling pathways, promoting cell growth, proliferation and metabolism of progenitor cells in different developmental organs and tissues (51-63). There have been relatively few studies on SF1 and YBX1, and no effect on disc degeneration has been found. Meanwhile, other transcription factors that significantly regulate the disc degeneration dysfunction module may also be involved in the basic process of intervertebral disc degeneration, which needs to be confirmed by experiments. This will promote biologists to further explore its regulation of intervertebral disc degeneration. Overall, our work deciphered a co-expression network involving differential gene regulation of disc degeneration. It helps reveal the core dysfunction modules and potential regulatory factors of this disease and enhances our understanding of its pathogenesis.

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