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KEGG-expressed genes and pathways in intervertebral disc degeneration

Protocol for a systematic review and data mining

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Abstract

miRNAs and genes play significant roles in the etiology and pathogenesis of intervertebral disc degeneration (IDD). This study aimed to identify aberrantly expressed miRNAs, genes, and pathways in IDD through a comprehensive bioinformatics analysis.

Data of miRNAs expression microarrays (GSE63492) and genes microarrays (GSE23130) were obtained from GEO database. Similarly, aberrantly expressed miRNAs and genes were obtained using GEO2R. In addition, functional and enrichment analyses of selected miRNAs and genes were performed using the DAVID database. Meanwhile, protein–protein interaction (PPI) network was constructed using STRING, and then visualized in Cytoscape.

A total of 98 upregulated miRNAs were identified. They were enriched in biological processes of response to organelle, ion binding, cellular nitrogen compound metabolic process, biosynthetic process, small molecule metabolic process, cellular protein modification process, catabolic process, molecular function, neurotrophin TRK receptor signaling pathway, and protein complex. In addition, 1405 high expression protein genes were detected. It indicated enrichment in biological processes, such as translational initiation, nonsense-mediated decay, viral transcription, cell–cell adhesion, rRNA processing, translation, RP-dependent cotranslational protein targeting to membrane, nuclear-transcribed mRNA catabolic process, regulation of mRNA stability, and mRNA splicing via spliceosome and extracellular matrix organization. In addition, pathway analysis exhibited the common enrichment in focal adhesion, Hippo signaling pathway, ECM-receptor interaction, Wnt signaling pathway, PI3K-Akt signaling pathway, endocytosis, proteoglycans in cancer, and so on. The top 10 central genes of PPI network were POTEE, PPP2CA, RPL17, HSP90AA1, POTEF, RPL13A, ACTB, RPL18, RPS24, and HSPA1A.

In conclusion, our research proposed abnormally expressed miRNAs, genes, and pathways in IDD through bioinformatics methods, which may provide new insights into the pathogenesis of IDD. Thus, the Hub gene involving POTEE, PPP2CA, RPL17, HSP90AA1, POTEF, RPL13A, ACTB, RPL18, RPS24, and HSPA1A may be biomarkers for accurate diagnosis and treatment of IDD in the future.

Abbreviations: ACTB = Human Endogenous Reference Genes, CHTN = National Cancer Institute Cooperative Tissue Network, DEG = differentially expressed gene, ECM = extracellular matrix, GEO = Gene Expression Omnibus, GO = Gene ontology, HSP90AA1 = heat shock protein90AA1, HSPA1A = heat shock proteinA1A, IDD = intervertebral disc degeneration, NCBI = National Center for Biotechnology Information, POTEE = POTE Ankyrin Domain Family, Member E, POTEF = protein family member-F, PPI = protein–protein interaction, PPP2CA = recombinant bovine serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform, RPL13A = ribosomal protein L13A, RPL17 = ribosomal protein L17, RPL18 = ribosomal protein L18, RPS24 = ribosomal protein S24.

Keywords: bioinformatics, expression, genes, intervertebral disc degeneration, miRNA

1. Introduction

Intervertebral disc degeneration (IDD) affects the quality of life of middle-aged and elderly people. It not only causes physical suffering to patients,[1] but also increases the economic burden to family and society.[2] Many factors contribute to intervertebral disc degeneration. However, in this paper, the genetic factors are considered as the most important contributors,[3] such as abnormalities in miRNA[4] and genes. miRNA is a small molecule noncoding single-stranded RNA molecule encoded by an endogenous gene. It is involved in posttranscriptional gene expression control. As it can direct the expression of genes, it can inhibit translation or degradation. Thus, miRNA plays a key role in many biological processes, including proliferation, apoptosis, and differentiation. Hanaei[5] revealed that genetic variation in the disc assembly can cause changes in the normal homeostasis of the disc, thus leading to intervertebral disc degeneration. In addition, many other factors, including vitaminD receptor,[6]

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proteoglycans,[7] cytokines, enzymes,[8] and collagens, also play significant roles in the pathophysiology of intervertebral disc degeneration. In particular, there are many research genes that can also take part in this process in some way.

At present, the gene chip technology has showed many advantages of automated, diverse, highly parallel, and miniaturized. Hence, it is widely used in clinical medicine to find meaningful gene of interest and discover biomarkers for early diagnosis and prognosis of IDD.[9] A lot of miRNAs and genes expression profiling microarrays have been obtained to discover various differentially expressed miRNAs and genes (DEGs) in IDD. However, the data of individual research are not sufficient to analyze key miRNAs and genes in the biological process or IDD. However, with the advanced bioinformatics analysis of available microarray data, more reliable and accurate screening results can be obtained by overlapping related data sets.

Until now, only few studies have been implemented for a comprehensive analysis information of both miRNAs expression profiling microarray and genes profiling microarray in the development of intervertebral disc degeneration. In our current research, data of miRNAs expression profiling microarrays (GSE63492) and gene profiling microarrays (GSE23130) were integrated and analyzed using different bioinformatics tools. Meanwhile, special miRNAs, genes, and pathways were revealed in IDD. We construct a protein–protein interaction network and reveal the hub genes. At the same time, we expect to find new abnormal genes and pathways in IDD and expose the underlying molecular mechanisms that tend to coordinate disc degeneration.

In this study, Gene ontology (GO) analysis, KEGG pathway and DAVID functional annotation enrichment analyses were used to analyze the bioinformatics of miRNAs and genes to find related miRNAs and genes causing degeneration of intervertebral discs.

2. Materials and methods

2.1. Microarray data

This study was approved by the Institutional Review Board of Ethics Committee of the First Afliated Hospital, GuangXi Medical University. The miRNAs and genes expression profiling data sets (GSE63492, GSE23130) were obtained from Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information (NCBI). In addition, comparison of miRNAs expression profiles of human nucleus pulposus from IDD patients with normal controls from cadaveric intervertebral discs as well as genes expression profiling of disc tissue samples were obtained from the National Cancer Institute Cooperative Tissue Network (CHTN). Also, surgical disc procedures were performed on patients with herniated discs and degenerative disc disease.

2.2. Data processing

GEO2R online tool was used to analyze microarray data provided by the original submitter and identify miRNAs and genes. GEO2R is an interactive web tool that allows users to compare different sample sets in the GEO series to screen for differentially expressed miRNAs and genes under experimental conditions. $P < .05$ and $|\log FC| > 1$ were used as the deadline standards to find miRNAs and genes. Subsequently, we identified miRNAs and genes overlapping GO analysis data and KEEP pathway using the MATCH function.

2.3. Functional and pathway enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/)[10] was used to perform functional and pathway Enrichment analysis. DAVID can provide researchers with a systematic and comprehensive functional annotation tool necessary to reveal the biological implications behind a large number of genes. In addition, GO analysis[11] including biological process, molecular function, and the cellular component, biological process, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis[12,13] were used for the selected downregulated miRNAs and upregulated genes by DAVID. $P < .05$ is considered to be statistically significant.

2.4. PPI network construction

The Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org/) database[14] is a precomputed global resource. It was designed to find out and predict protein–protein interactions. In the current research, the STRING online tool was used to analyze genetically validated protein proteins. The overall score of >0.7 was considered meaningful.

Previously obtained biological networks are characterized by scale-free. Therefore, the statistical analysis of the network is used to assess the degree of connectivity, and to identify important nodes is considered, which are called central proteins.

3. Results

3.1. Identification of abnormally differentially expressed miRNAs and genes in the nucleus

Data from each microarray were separately analyzed using the online software GEO2R to screen miRNAs and genes. For the expression profiles of miRNAs in IDD, 98 upregulated miRNAs and 106 downregulated miRNAs were identified in GSE63492, as summarized in Table 1. Meanwhile, for the expression profiles of genes in IDD, 1405 high expression protein genes and 124 low

Table 1

Top 20 downregulated miRNAs.

miRNAs	logFC	*P*
hsa-miR-125b-1-3p	−4.6927	.00044391
hsa-miR-1184	−4.849102	.00054438
hsa-miR-3648	−3.763601	.00061356
hsa-miR-4769-5p	−2.284078	.00091784
hsa-miR-1273g-3p	−2.592945	.00194021
hsa-miR-1827	−2.190244	.00237317
hsa-miR-4327	−3.01603	.00402083
hsa-miR-542-5p	−2.608877	.00298923
hsa-miR-4306	−1.81941	.00380366
hsa-miR-486-3p	−4.39189	.0080018
hsa-miR-5100	−1.987467	.0090823
hsa-miR-196b-5p	−3.434128	.01354683
hsa-miR-185-5p	−1.530935	.01396457
hsa-miR-4795-3p	−2.48218	.0152815
hsa-miR-3173-3p	−1.706725	.01561563
hsa-miR-486-5p	−1.641332	.01675904
hsa-miR-302a-3p	−1.925318	.01699907
hsa-miR-155-5p	−3.222864	.0175384
hsa-miR-4454	−2.969762	.01780317

Table 2

Top 20 upregulated protein genes.

Gene symbol	logFC	*P*
AAK1	1.25405	2.49E-06
FAM58A	1.043564	1.59E-05
RASEF	6.745318	1.72E-05
RASEF	6.513244	1.76E-05
FAM173B	1.21717	7.78E-05
FIP1L1	1.523102	1.11E-04
FKSG49	1.278856	1.20E-04
CMAS	1.121824	2.09E-04
ADAM17	1.005946	2.60E-04
MEG3	1.83586	3.32E-04
DPP8	1.075902	4.98E-04
TSTA3	1.310288	6.45E-04
GSTP1	3.917828	7.11E-04
CENPBD1P1	1.116456	8.90E-04
HBB	6.443948	1.19E-03
AMZ2	1.02862	1.25E-03
ACTR2	1.663938	1.39E-03
ZNF24	1.018518	1.55E-03
AMZ2	1.557192	1.56E-03
ND6	1.032714	1.57E-03
CISD2	1.123746	1.59E-03

Table 3

Gene ontology analysis of downregulated expression miRNAs in intervertebral disc degeneration.

GO Category	*P*	Genes	miRNAs
Organelle	6.40E-94	2023	34
Ion binding	2.71E-55	1265	34
Cellular nitrogen compound metabolic process	2.30E-53	1005	34
Biosynthetic process	2.08E-29	814	34
Small molecule metabolic process	1.23E-24	503	34
Cellular protein modification process	7.26E-18	472	33
Catabolic process	8.25E-15	399	31
Molecular_function	5.24E-14	3244	34
Neurotrophin TRK receptor signaling pathway	1.12E-13	67	24
Protein complex	2.86E-13	750	33
Nucleic acid binding transcription factor activity	6.97E-13	221	33
Symbiosis, encompassing mutualism through parasitism	1.41E-12	118	29
Enzyme binding	1.48E-12	277	32
Gene expression	1.65E-12	122	25
Cellular protein metabolic process	7.59E-12	104	31
Viral process	9.55E-12	104	28
Cellular component assembly	9.58E-11	269	32
Cellular_component	6.17E-10	3256	34
Cytoskeletal protein binding	7.93E-10	174	28
Response to stress	3.04E-09	440	33

expression protein genes were found in GSE23130, as exhibited in Table 2.

3.2. GO functional enrichment analysis

To gain a deeper understanding of the function and enrichment pathways of miRNAs and genes, we used the online taxonomy tool, DAVID and obtained significant Enrichment results for these genes in KEGG and GO terms. In essence, downregulated miRNAs obtained from the nucleus pulposus specimens are mainly involved in GO terminology. They include organelle, ion binding, cellular nitrogen compound metabolic process, biosynthetic process, small molecule metabolic process, cellular protein modification process, catabolic process, molecular function, neurotrophin TRK receptor signaling pathway, and protein complex (Table 3). At the same time, enrichment analysis of nucleus pulposus genes showed that upregulated genes are mainly involved in translational initiation, nonsense-mediated decay, viral transcription, cell–cell adhesion, rRNA processing, translation, RP-dependent cotranslational protein targeting to membrane, nuclear transcribed mRNA catabolic process, regulation of mRNA stability, mRNA splicing via spliceosome, and extracellular matrix organization (Table 4).

3.3. KEGG pathway analysis

KEGG pathway Enrichment analysis suggested that downregulated miRNAs and high expression protein genes were significantly enriched in pathways. We also found 10 common adjustment pathways in KEGG pathway (Table 5).

3.4. PPI network construction and hub gene

To obtain a deeper understanding of the global interaction between DEGs and IDD, we constructed a PPI network for DEGs. There were 1,117 nodes (proteins) and 10,059 edges (interactions) in the PPI network. POTEE, PPP2CA, RPL17, HSP90AA1, POTEF, RPL13A, ACTB, RPL18, RPS24, and HSPA1A interacted with each other with the high degree of 110, 96, 83, 82, 80, 73, 73, 74, 70, and 64, respectively (Fig. 1).

4. Discussion

So far, the genetic mechanism of IDD has not been fully explained. This reveals that the underlying mechanisms of IDD development and progression can greatly facilitate diagnosis, treatment, and prognosis assessment. This study is the first to analyze the mechanism of IDD from the 2 levels of miRNAs and genes. In this study, therefore, we identified a total of 98 upregulated miRNAs and 106 downregulated miRNAs. We also obtained 1405 highly expressed genes and 124 low expressed genes by analyzing the available data for miRNA and gene expression (GSE63492, GSE23130) microarrays in IDD. Essentially, recent bioinformatic and experimental evidence have suggested that a remarkably large proportion of genes (>30%) are subject to miRNA-mediated regulation. Thus, it is clear that miRNAs function by suppressing protein production from targeted genes.[15] Therefore, the downregulated miRNAs and the upregulated genes were selected for further analysis. Furthermore, the enrichment of these miRNAs and genes exhibits certain pathways and central genes that are affected by aberrant expression, thus may provide new insights into the pathogenesis of IDD.

There is increasing evidence that miRNAs can regulate apoptosis in many cells. In the development of intervertebral disc degeneration, nucleus pulposus cells play a crucial role in apoptosis. Our results suggest that the downregulated miRNAs participate in organelle, ion binding, cellular nitrogen compound metabolic process, biosynthetic process, small molecule metabolic process, and other biological processes. In addition, miRNAs are involved in the modification or metabolic processes of many proteins. The results indicate that miRNA-143,[16] miRNA-27a, miRNA-155, and miRNA-127-5b[17] promote the progression of

Table 4

Gene ontology analysis of upregulated expression genes in intervertebral disc degeneration.

Category	Term	*P*
GOTERM_BP_DIRECT	GO:0006413~translational initiation	7.76E-20
GOTERM_BP_DIRECT	GO:0000184~nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	5.62E-18
GOTERM_BP_DIRECT	GO:0006614~SRP-dependent cotranslational protein targeting to membrane	3.43E-17
GOTERM_BP_DIRECT	GO:0019083~viral transcription	5.60E-13
GOTERM_BP_DIRECT	GO:0098609~cell-cell adhesion	7.82E-11
GOTERM_BP_DIRECT	GO:0006364~rRNA processing	7.35E-10
GOTERM_BP_DIRECT	GO:0006412~translation	9.65E-10
GOTERM_BP_DIRECT	GO:0043488~regulation of mRNA stability	2.04E-09
GOTERM_BP_DIRECT	GO:0000398~mRNA splicing, via spliceosome	2.20E-09
GOTERM_BP_DIRECT	GO:0030198~extracellular matrix organization	3.44E-07
GOTERM_CC_DIRECT	GO:0070062~extracellular exosome	2.80E-36
GOTERM_CC_DIRECT	GO:0005829~cytosol	5.63E-27
GOTERM_CC_DIRECT	GO:0016020~membrane	5.76E-24
GOTERM_CC_DIRECT	GO:0005925~focal adhesion	6.19E-24
GOTERM_CC_DIRECT	GO:0031012~extracellular matrix	1.18E-19
GOTERM_CC_DIRECT	GO:0005654~nucleoplasm	4.41E-18
GOTERM_CC_DIRECT	GO:0030529~intracellular ribonucleoprotein complex	1.13E-17
GOTERM_CC_DIRECT	GO:0022625~cytosolic large ribosomal subunit	4.09E-12
GOTERM_CC_DIRECT	GO:0005840~ribosome	1.29E-11
GOTERM_CC_DIRECT	GO:0005737~cytoplasm	3.73E-11
GOTERM_MF_DIRECT	GO:0044822~poly(A) RNA binding	1.27E-42
GOTERM_MF_DIRECT	GO:0005515~protein binding	2.76E-33
GOTERM_MF_DIRECT	GO:0003723~RNA binding	1.22E-14
GOTERM_MF_DIRECT	GO:0003735~structural constituent of ribosome	2.79E-11
GOTERM_MF_DIRECT	GO:0098641~cadherin binding involved in cell-cell adhesion	4.45E-11
GOTERM_MF_DIRECT	GO:0051082~unfolded protein binding	4.46E-11
GOTERM_MF_DIRECT	GO:0000166~nucleotide binding	2.43E-08
GOTERM_MF_DIRECT	GO:0003729~mRNA binding	9.27E-08
GOTERM_MF_DIRECT	GO:0008137~NADH dehydrogenase (ubiquinone) activity	5.32E-07
GOTERM_MF_DIRECT	GO:0031625~ubiquitin protein ligase binding	1.01E-05

nucleus pulposus cell apoptosis by binding to different genes of interest, providing potential therapeutic targets in the diagnosis and treatment of degenerative disc disease.

For high expression genes in IDD, GO analysis indicated that enrichment of biological processes were translational initiation, nuclear-transcribed mRNA catabolic process, nonsense-mediated decay, SRP-dependent cotranslational protein targeting to membrane, viral transcription, cell–cell adhesion, rRNA processing, translation, regulation of mRNA stability, mRNA splicing via spliceosome, and extracellular matrix organization.

We have found 10 common adjustment pathways in KEGG pathway. The results indicate that downregulated miRNAs and upregulated genes are involved in the PI3K/Akt pathway.

Table 5

The common pathways between downregulation miRNAs and upregulated protein genes in KEGG pathway.

Focal adhesion
Hippo signaling pathway
ECM-receptor interaction
Wnt signaling pathway
PI3K-Akt signaling pathway
Endocytosis
Proteoglycans in cancer
Prostate cancer
Regulation of actin cytoskeleton
Pathways in cancer

Meanwhile, activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway prevents IDD. This can be attributed to the increased ECM levels, prevention of apoptosis, promotion of cell proliferation, induction or prevention of autophagy, oxidative damage mitigation, and adaptation to anoxic microenvironment.[18] In addition, Zhang proves that the Hippo pathway is involved in natural IDD. Protein (YAP) activation and dephosphorylation were observed in young rat intervertebral discs, and their levels decreased with age. In addition, overexpression of YAP in acutely injured intervertebral discs can cause inhibition of the Hippo pathway. In addition, NPC was transfected with YAP and accelerated premature aging of cells by YAP interference with the Hippo pathway. Therefore, the results show that Hippo pathway can contribute to maintaining the homeostasis of the intervertebral disc and control NPC proliferation.[19] Meanwhile, the activation of WNT/$^{N}_{L}$-catenin signaling promotes cellular senescence and may modulate MMP and TGF/$^{N}_{L}$ signaling in NP cells. We hypothesize that the activation of WNT/$^{N}_{L}$-catenin signaling causes an increased breakdown of the matrix, thereby promoting IDD degeneration.[20] We also realized that the Proteoglycans in cancer pathway is involved in the IDD process.[21] At the same time, Zhu et al[22] found that upregulated genes are enriched in 9 pathways in mouse intervertebral disc tissue. They include cytokine–cytokine receptor interaction, apoptosis, chemokine signaling pathway, NOD-like receptor signaling pathway, Jak-STAT signaling pathway, Toll-like Receptor signaling pathway, pathways in cancer, purine metabolism, and small-cell lung cancer.

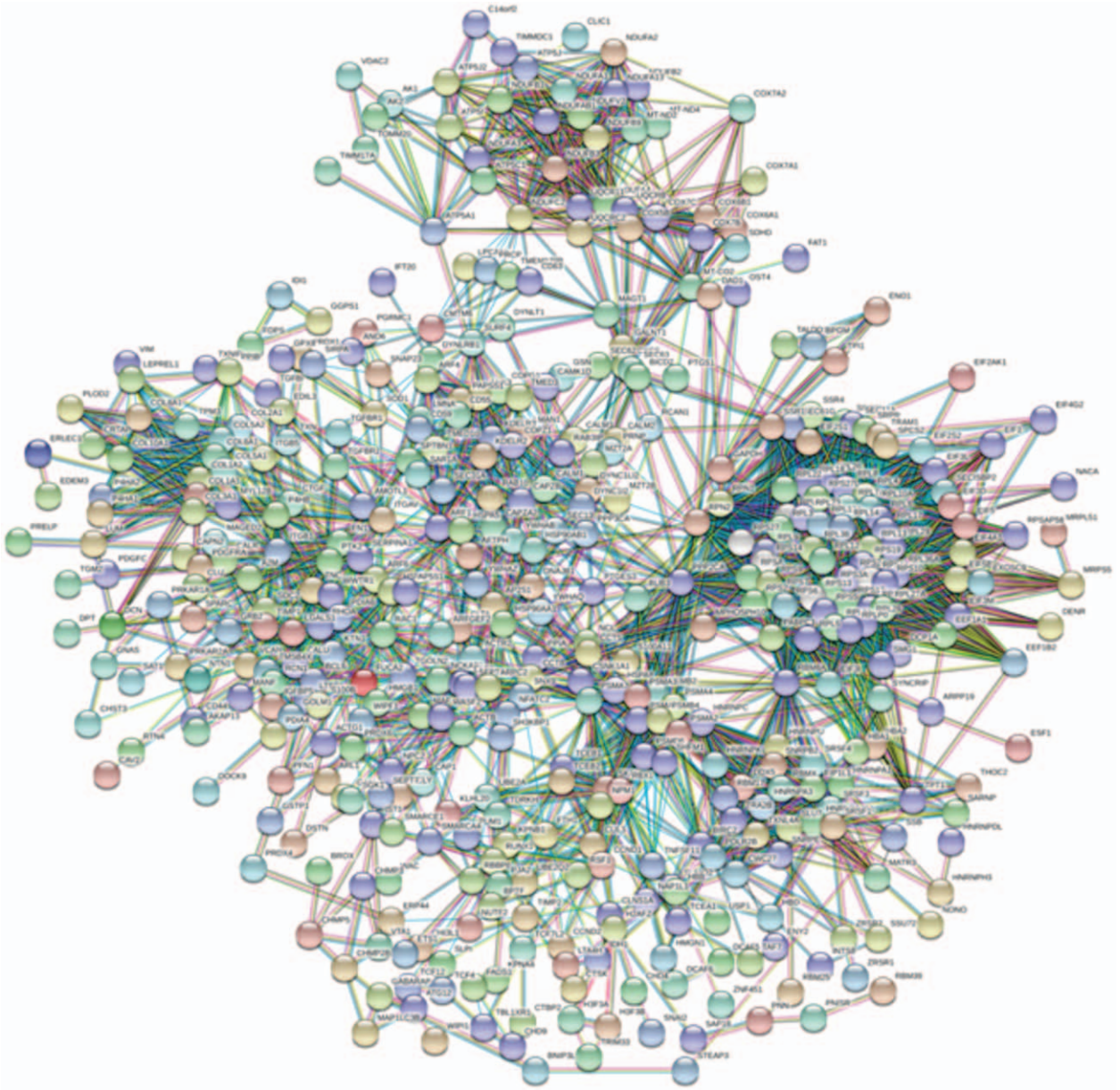

Figure 1. PPI network high expression genes. There were 1,117 nodes (genes) and 10,059 edges (interactions) in the PPI network. The top 10 central genes include POTEE, PPP2CA, RPL17, HSP90AA1, POTEF, RPL13A, ACTB, RPL18, RPS24, and HSPA1A.

Pathway in cancer is consistent with our findings among the pathways. As the rat's intervertebral disc is similar to the human human intervertebral disc, the pathways in cancer can be said to play a significant role in the degeneration of the mouse and human intervertebral disc.

The dysregulation of the top 10 central genes of the PPI network interacting genes may jointly lead to disorders in protein synthesis in IDD progression. PPP2CA is associated with Hippo signaling pathway and PI3K-Akt signaling pathway. Meanwhile, RPL17, RPL13A, RPL18, and RPS24 are associated with ribosome signaling pathway. Also, HSP90AA is associated with PI3K-Akt signaling pathway, pathways in cancer, and prostate cancer. In addition, ACTB is associated with focal adhesion, Hippo signaling pathway, proteoglycans in cancer, and regulation of actin cytoskeleton. Finally, HSPA1A is associated with the endocytosis signaling pathway.

Dongrim Seol et al[23] confirmed that ACTB is significantly expressed in normal adult intervertebral discs. Also, its expression increases in degenerative patients, which indicates that ACTB plays an important role in the degeneration of intervertebral discs. At the same time, Liu et al[24] found that transient expression of POTEE or POTEF induced apoptosis in Hela cells. These deductions indicate that the POTE gene family encodes a pro-apoptotic protein. In additon, Zhu et al[22] mentioned that the progress of IL6, VEGFA, THBS1, ITGA4, and collagen genes may be involved in IDD degeneration through bioinformatics methods. However, their results differed from the results of our analysis because the data samples they analyzed

were derived from cultured rat intervertebral discs under TNF-α, IL-1β, and serum-limited conditions, rather than using human intervertebral disc tissue specimens. The specimens used in this current research are all derived from human intervertebral disc tissue. Thus, our results are more credible and more conducive to IVD diagnosis and treatment.

In addition, with a deeper understanding of the interactions of the identified genes in IDD, it is possible to discover new treatment methods. A group of dysregulated ribosomal proteins (RPL13A, RPL17, RPL18, and RPS24) was mainly found to be related to protein synthesis processes. In particular, Cs-Szabo et al[25] demonstrated that in heavily degenerated tissues, the decline in the synthesis of aggrecan, modifying the metabolism of proteogly which might be an effective therapeutic strategy.

In summary, based on bioinformatics analysis of miRNA and gene expression profiles, we found that PPP2CA, as well as RPL17, RPL18, RPL13A and RPS24, P0TEE, HSP90AA, HSPA1A can take part in the process of protein synthesis during IDD development. This finding prompts the potential rise in the early diagnosis and prognosis of IDD. However, there is a need for further experimentation to include prospective clinical and mechanical experiments to confirm our results.

Author contributions

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