Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

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

Shiyu Hu
Shenzhen Second People's Hospital
Yucheng Fu
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital
Bin Yan
Shenzhen Second People's Hospital
Zhe Shen
Shenzhen Second People's Hospital
Tao Lan (275305207@qq.com)
Shenzhen Second People's Hospital

Research article

Keywords:

Posted Date: June 5th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32769/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published on September 1st, 2020. See the published version at https://doi.org/10.1186/s13018-020-01902-4.

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. The aim of this study is 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. In addition, 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 TGFB1. 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. A total of 23 samples, including 15 non-degenerative disc samples and 8 degenerative disc 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)[16]. 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[17]. 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[18] and Cytoscape[19] 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 with respect to 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. 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 three-dimensional 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 IVD, they led to a final common result of excessive degradation of extracellular matrix[20]. 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)[21-23]. 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[24,25]. 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[26]. On the contrary, TIMP-1 and TIMP-2 mRNA and protein expression increases in degenerated IVD tissue, antagonizing the effect of MMPs[27]. 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 conformed that activation of PI3K-Akt pathway increased SOX9 expression and activity, and consequently led to increase of aggrecan expression in NP cells[28]. 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[29]. Many recent studies also showed that resveratrol suppressed IL-1β-mediated NP cell apoptosis through activating the PI3K-Akt pathway[30-32]. 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[33]. Hence, gene therapy targeting PTEN may play an important role in treating IDD.

We further constructed a PPI network for better understanding 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[34]. 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-βsignaling may contribute to IVD

degeneration[35]. 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[36]. 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[37]. Tajerian M et al. Showed that the underlying mechanism of silence of SPARC gene during aging may attributed to DNA methylation[38]. A recent in vivo experimental study showed that stable expression of CTGF and TIMP1 genes by co-transfection adeno-associated virus 2 resulted in an increase of synthesis of aggrecan and type II collagen in degenerated intervertebral disc, which served as potential target gene for disc regeneration[39]. 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[40,41]. 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[42].

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/S
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 conferring tensile strength	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

References

1. Luoma K, Riihim?ki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A. Low Back Pain in Relation to Lumbar Disc Degeneration. *Spine.*25(4):487-492.
2. Jensen CE, Riis A, Petersen KD, Jensen MB, Pedersen KM. Economic evaluation of an implementation strategy for the management of low back pain in general practice. *Pain.* 2017;158(5):891-899.
3. Lin CC, Li Q, Williams CM, et al. The economic burden of guideline-recommended first line care for acute low back pain. *Eur Spine J.* 2018;27(1):109-116.
4. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nature Reviews Rheumatology.*10(1):44-56.
5. Deyo RA, Weinstein JN. Low back pain. 1991;18(2):48.
6. Emilio, González, Martínez, et al. Biology and mechanobiology of the intervertebral disc.
7. Yang S, Zhang F, Ma J, Ding W. Intervertebral disc ageing and degeneration: The antiapoptotic effect of oestrogen. *Ageing Res Rev.* 2020;57:100978.
8. Li Z, Shao Z, Chen S, et al. TIGAR impedes compression-induced intervertebral disc degeneration by suppressing nucleus pulposus cell apoptosis and autophagy. *J Cell Physiol.* 2020;235(2):1780-1794.
9. Molinos M, Almeida CR, Caldeira J, Cunha C, Goncalves RM, Barbosa MA. Inflammation in intervertebral disc degeneration and regeneration. *Journal of the Royal Society Interface.*12(104):20141191-20141191.
10. Wang H, Tian Y, Wang J, et al. Inflammatory Cytokines Induce NOTCH Signaling in Nucleus Pulposus Cells: IMPLICATIONS IN INTERVERTEBRAL DISC DEGENERATION. *Journal of Biological Chemistry.*288(23):16761-16774.
11. Buckwalter, Joseph A. Aging and Degeneration of the Human Intervertebral Disc. *Spine.*20(11):1307-1314.
12. Gruber HE, Ingram JA, Norton HJ, Hanley EN. Senescence in Cells of the Aging and Degenerating Intervertebral Disc. *Spine.*32(3):321-327.
13. McGill SM. The biomechanics of low back injury: Implications on current practice in industry and the clinic. *Journal of Biomechanics.* 1997;30(5):465-475.
14. Dougill, Gary. Mechanical behaviours of intervertebral discs : clinical implications of loading, injury and treatment. 2016.
15. Ji SC, Han N, Liu Y, Li G, Sun Z, Li Z. Identification of genes associated with disc degeneration using bioinformatics. *Biotechnic & Histochemistry.*90(5):353-360.
16. Ashburner M, Ball CA, Blake JA, Botstein D, Cherry JM. Gene ontology: Tool for the unification of biology. *Nature Genetics.* 2000;25(1):25-29.
17. Minoru K, Susumu G. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research.* 2000(1):1.
18. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Lars JJ. STRING V9.1: Protein-Protein Interaction Networks, with Increased Coverage and Integration. *Nucleic Acids Research.* 2012;41(D1).
19. Smoot Me OKRJWPLIT. Cytoscape 2.8: new features for data integration and network visualization.
20. Colombini A, Lombardi G, Corsi MM, Banfi G. Pathophysiology of the human intervertebral disc.40(5):0-842.
21. Le Maitre CL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J Pathol.* 2004;204(1):47-54.
22. Li Y, Li K, Han X, et al. The imbalance between TIMP3 and matrix-degrading enzymes plays an important role in intervertebral disc degeneration. *Biochem Biophys Res Commun.* 2016;469(3):507-514.
23. Zhang JF, Wang GL, Zhou ZJ, Fang XQ, Chen S, Fan SW. Expression of Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases, and Interleukins in Vertebral Cartilage Endplate. *Orthop Surg.* 2018;10(4):306-311.
24. Sztrolovics R, Alini M, Mort JS, Roughley PJ. Age-related changes in fibromodulin and lumican in human intervertebral discs. *Spine (Phila Pa 1976).* 1999;24(17):1765-1771.
25. Singh K, Masuda K, Thonar EJ, An HS, Cs-Szabo G. Age-related changes in the extracellular matrix of nucleus pulposus and anulus fibrosus of human intervertebral disc. *Spine (Phila Pa 1976).* 2009;34(1):10-16.

26. Vo NV, Hartman RA, Yurube T, Jacobs LJ, Sowa GA, Kang JD. Expression and regulation of metalloproteinases and their inhibitors in?intervertebral disc aging and degeneration. *Spine Journal Official Journal of the North American Spine Society.*13(3):331-341.
27. Bachmeier BE, Nerlich A, Mittermaier N, et al. Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration. *Eur Spine J.* 2009;18(11):1573-1586.
28. Cheng CC, Uchiyama Y, Hiyama A, Gajghate S, Shapiro IM, Risbud MV. PI3K/AKT regulates aggrecan gene expression by modulating Sox9 expression and activity in nucleus pulposus cells of the intervertebral disc. *J Cell Physiol.* 2009;221(3):668-676.
29. Gao XW, Su XT, Lu ZH, Ou J. 17beta-Estradiol Prevents Extracellular Matrix Degradation by Downregulating MMP3 Expression via PI3K/Akt/FOXO3 Pathway. *Spine (Phila Pa 1976).* 2020;45(5):292-299.
30. Yanhai, Jiang, Zhijie, et al. Resveratrol inhibits IL-1β-mediated nucleus pulposus cell apoptosis through regulating the PI3K/Akt pathway.
31. Gao J, Zhang Q, Song L. Resveratrol enhances matrix biosynthesis of nucleus pulposus cells through activating autophagy via the PI3K/Akt pathway under oxidative damage. *Biosci Rep.* 2018;38(4).
32. Wang W, Li P, Xu J, et al. Resveratrol attenuates high glucose-induced nucleus pulposus cell apoptosis and senescence through activating the ROS-mediated PI3K/Akt pathway. *Biosci Rep.* 2018;38(2).
33. Xi Y, Ma J, Chen Y. PTEN promotes intervertebral disc degeneration by regulating nucleus pulposus cell behaviors. *Cell Biol Int.* 2020;44(2):583-592.
34. Lehmann TP, Jakub G, Harasymczuk J, Jagodziński PP. Transforming growth factor β mediates communication of co-cultured human nucleus pulposus cells and mesenchymal stem cells. *Journal of Orthopaedic Research.* 2018;36(11):3023-3032.
35. Chen S, Liu S, Ma K, Zhao L, Lin H, Shao Z. TGF-beta signaling in intervertebral disc health and disease. *Osteoarthritis Cartilage.* 2019;27(8):1109-1117.
36. Gruber, E. H. Targeted Deletion of the SPARC Gene Accelerates Disc Degeneration in the Aging Mouse. *Journal of Histochemistry & Cytochemistry Official Journal of the Histochemistry Society.*53(9):1131-1138.
37. Millecamps M, Czerminski JT, Mathieu AP, Stone LS. Behavioral signs of axial low back pain and motor impairment correlate with the severity of intervertebral disc degeneration in a mouse model. *Spine J.* 2015;15(12):2524-2537.
38. Tajerian M, Alvarado S, Millecamps M, Dashwood T, Stone LS. DNA methylation of SPARC and chronic low back pain. *Molecular Pain.* 2011;7(1):65.
39. Liu Y, Kong J, Xi Y-M, Yu T, Hu Y-G. Effects of AAV2-mediated co-transfection of CTGF and TIMP1 genes on degenerative lumbar intervertebral discs in rhesus monkeys in vivo. *American Journal of Translational Research.* 2018;10(4):1085-1096.
40. Yuan B, Ji W, Fan B, Zhang B, Zhao Y, Li J. Association analysis between thrombospondin-2 gene polymorphisms and intervertebral disc degeneration in a Chinese Han population. *Medicine.*97(2):e9586.
41. Hirose Y, Chiba K, Karasugi T, et al. A Functional Polymorphism in THBS2 that Affects Alternative Splicing and MMP Binding Is Associated with Lumbar-Disc Herniation.82(5):0-1129.
42. Deguchi T, Hashizume H, Nakajima M, et al. A population-based study identifies an association of THBS2 with intervertebral disc degeneration. *Osteoarthritis Cartilage.* 2019;27(10):1501-1507.

Figures

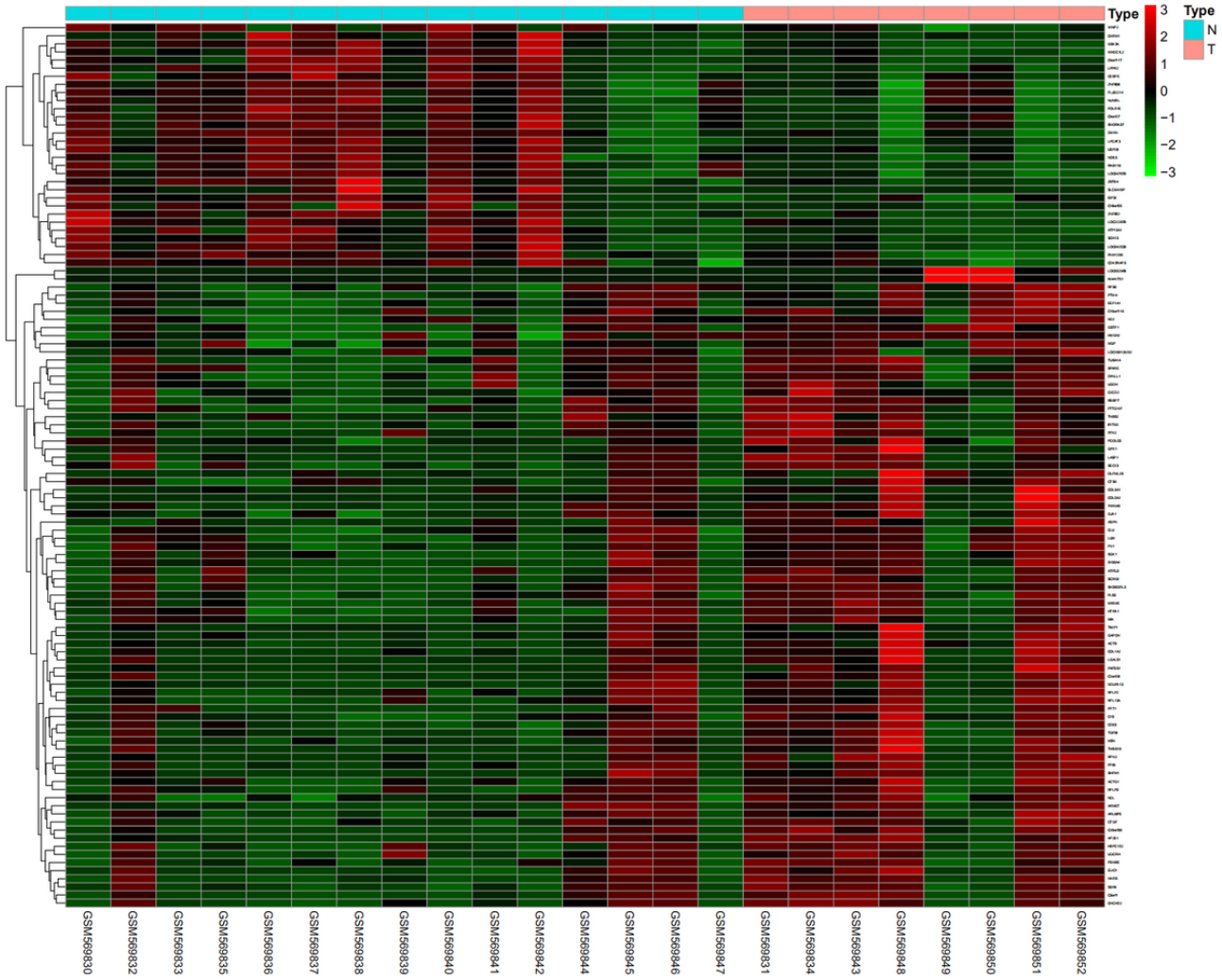

Figure 1

[No Legend]

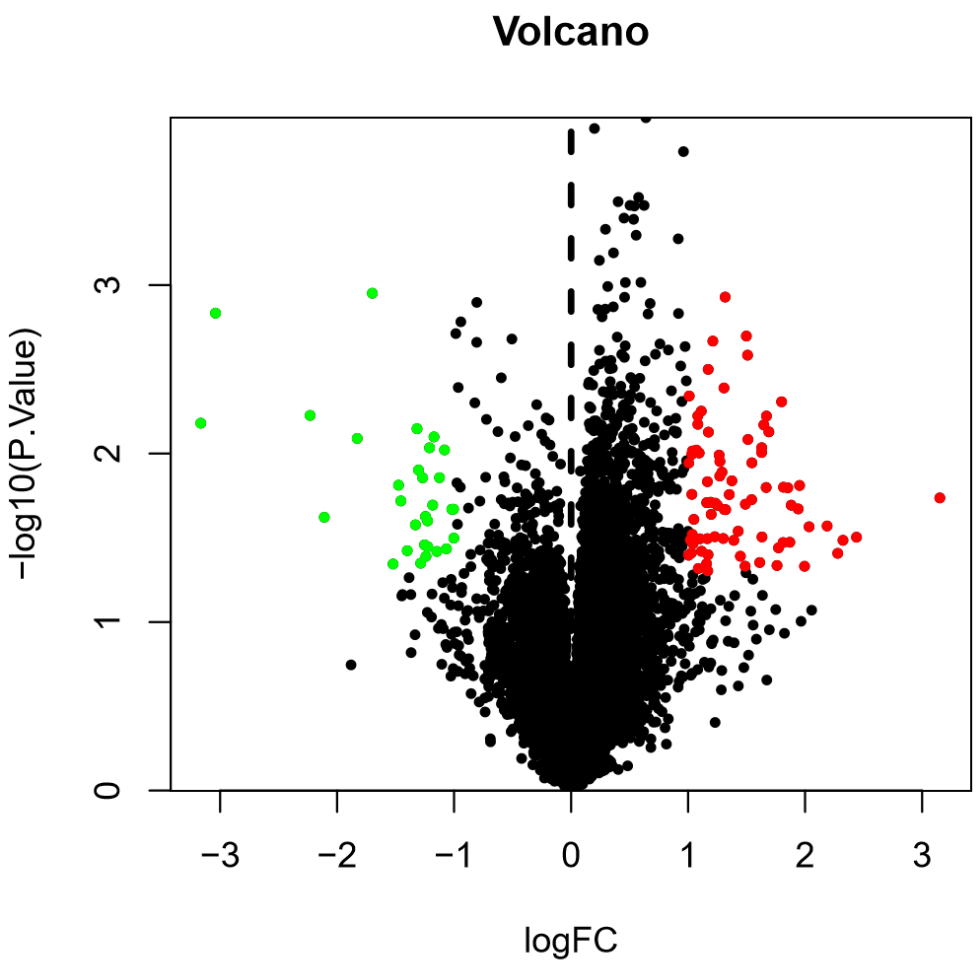

Figure 2

[No Legend]

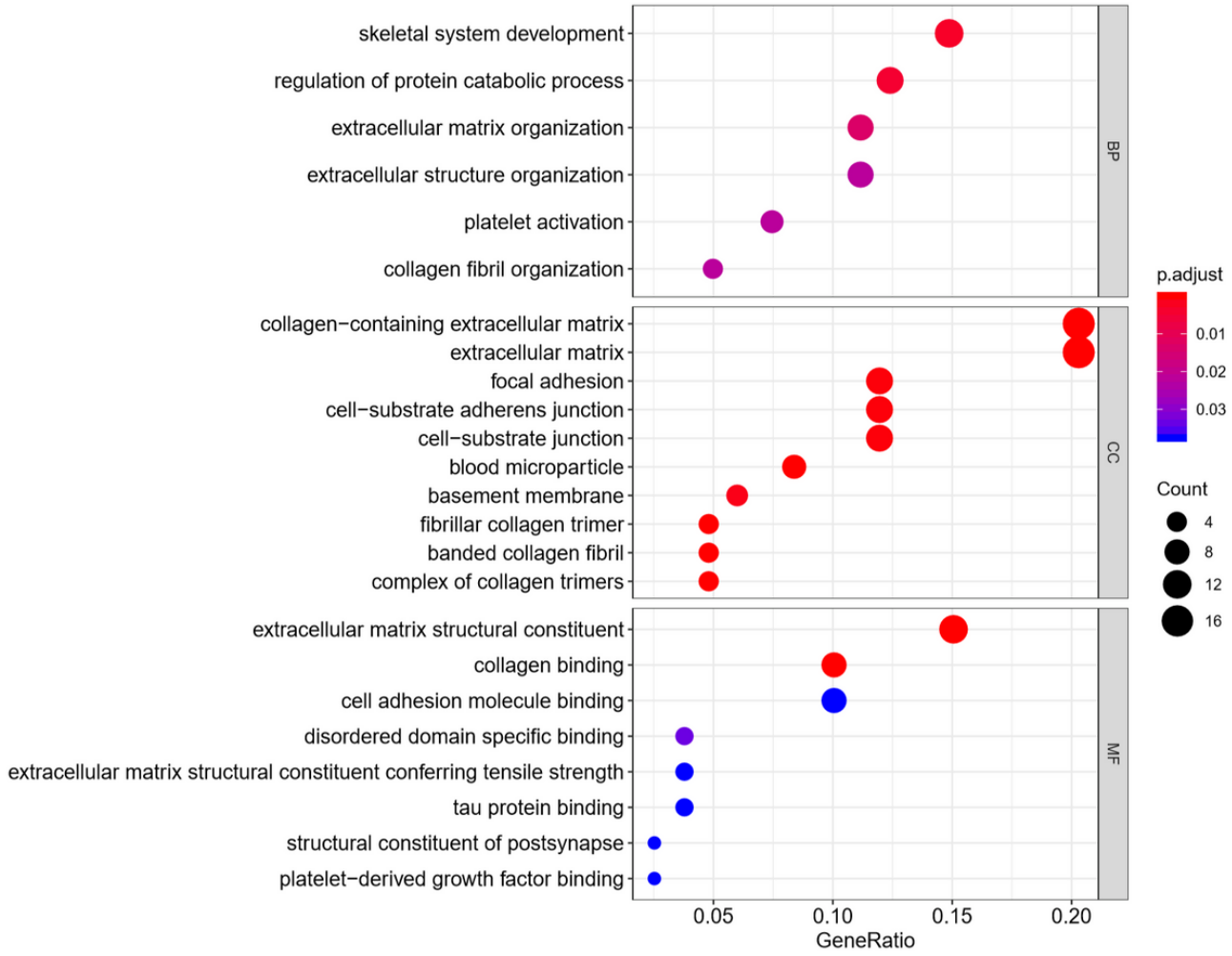

Figure 3

[No Legend]

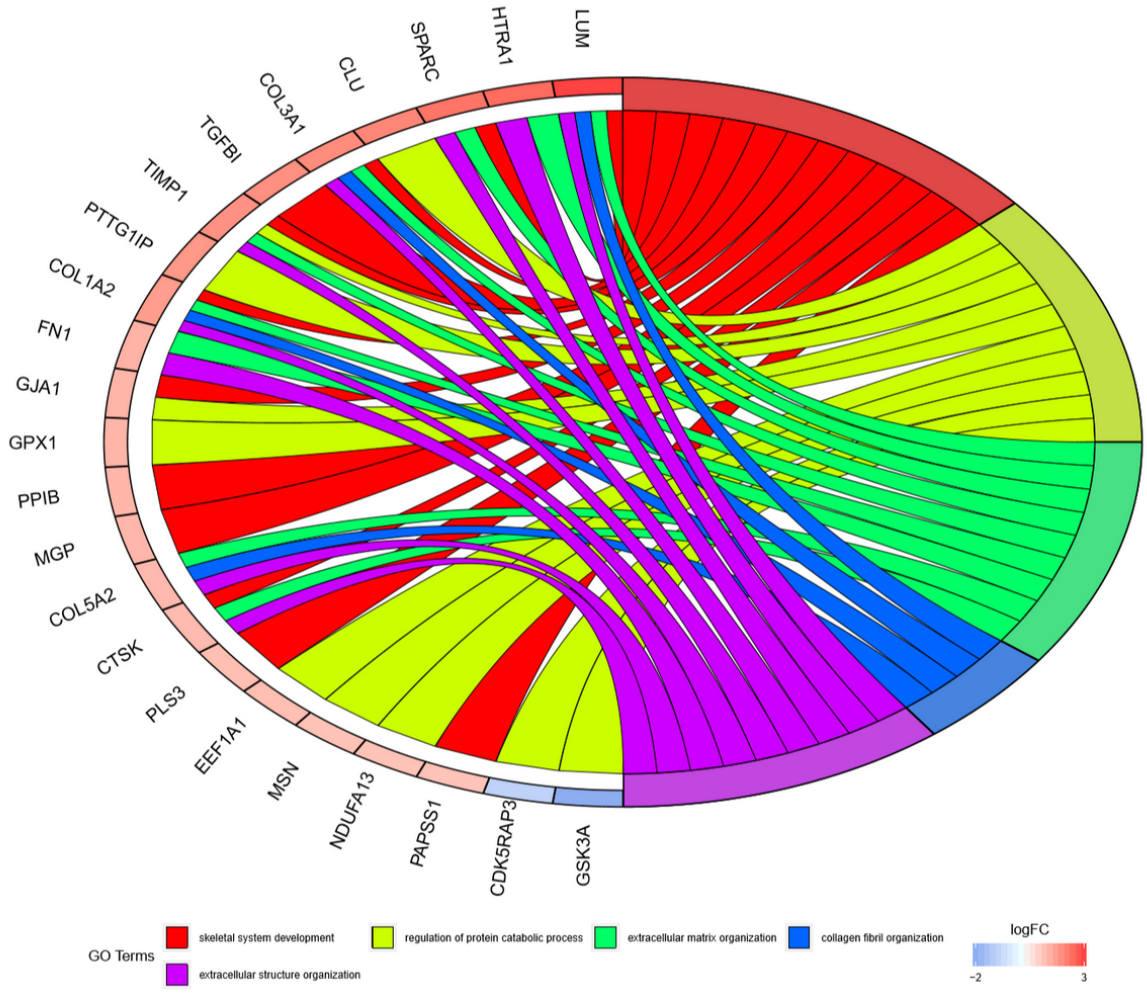

Figure 4

[No Legend]

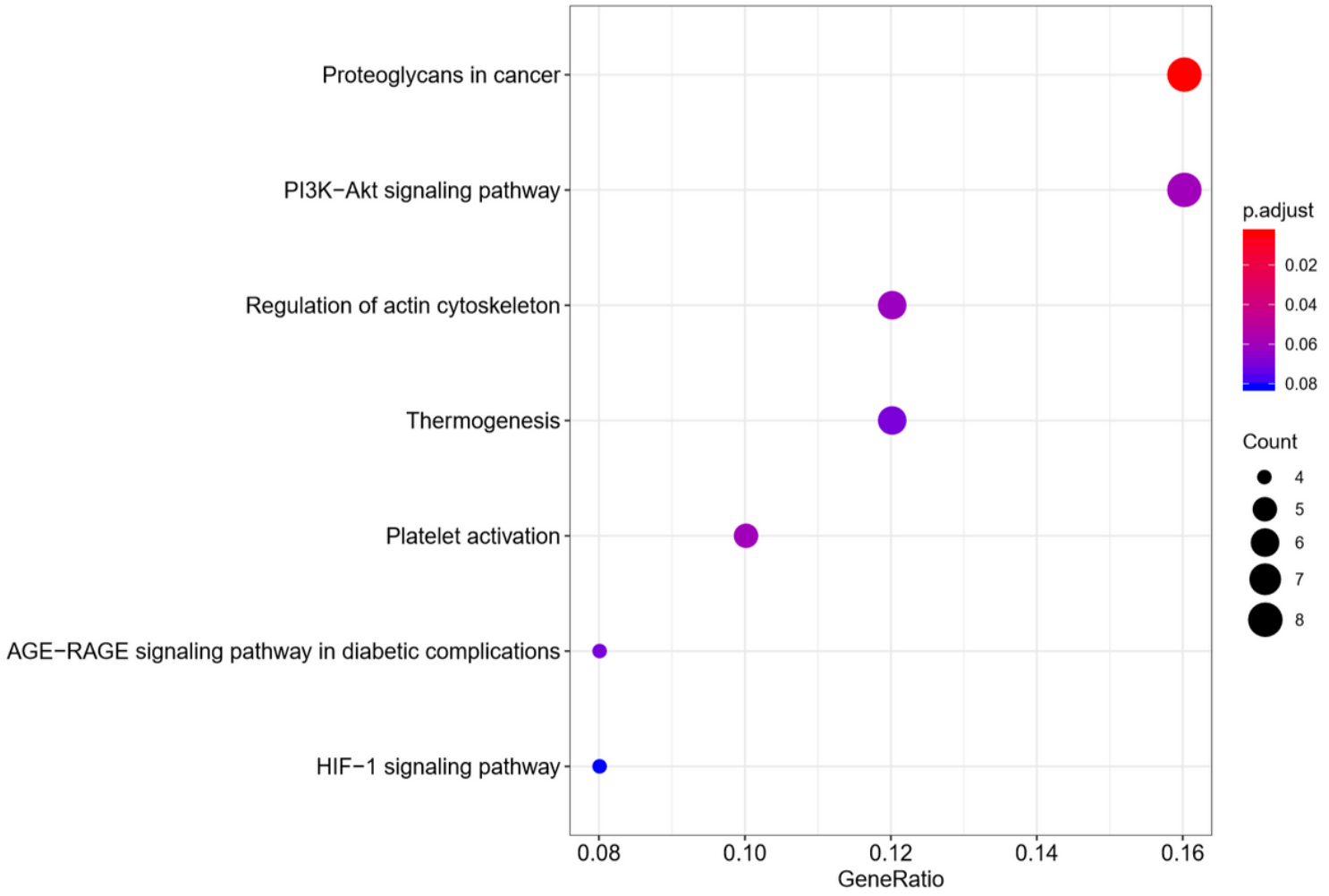

Figure 5

[No Legend]

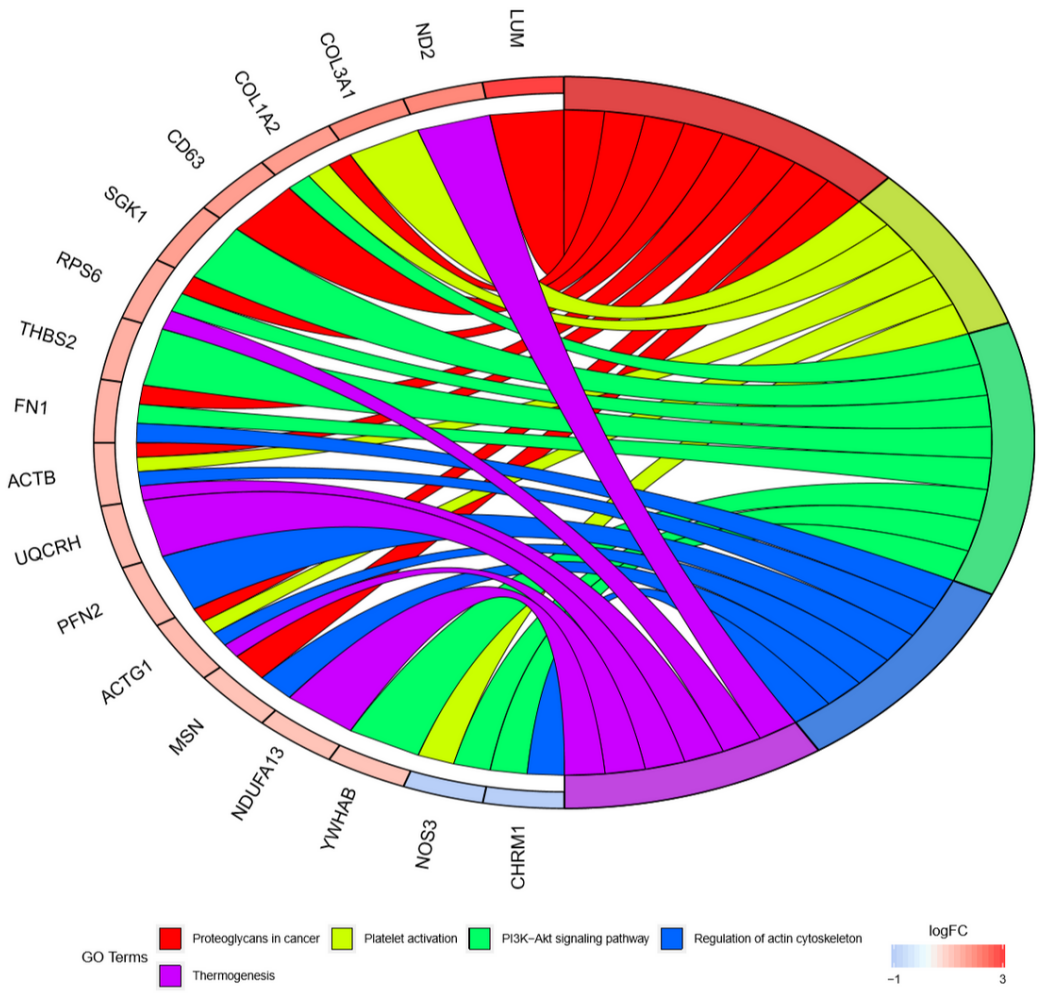

Figure 6

[No Legend]

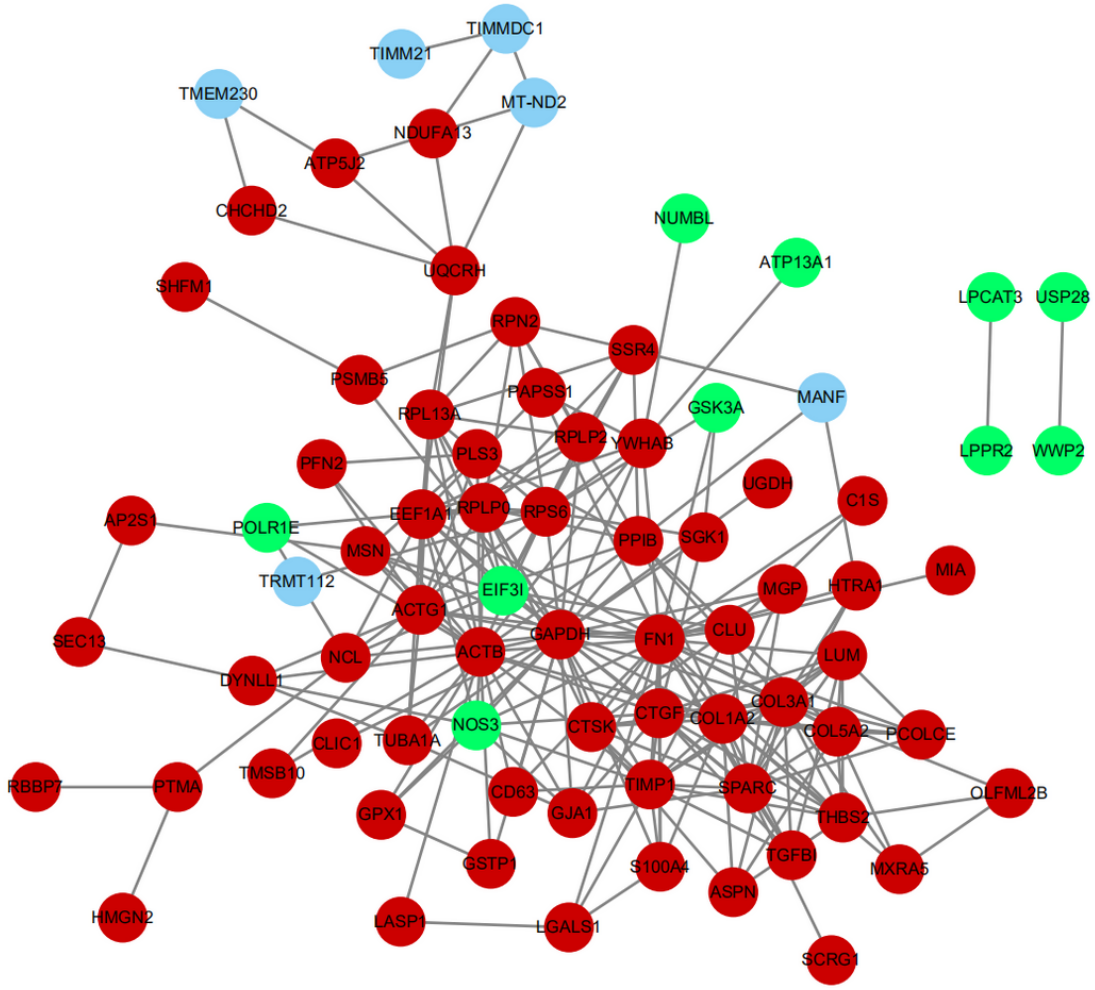

Figure 7

[No Legend]

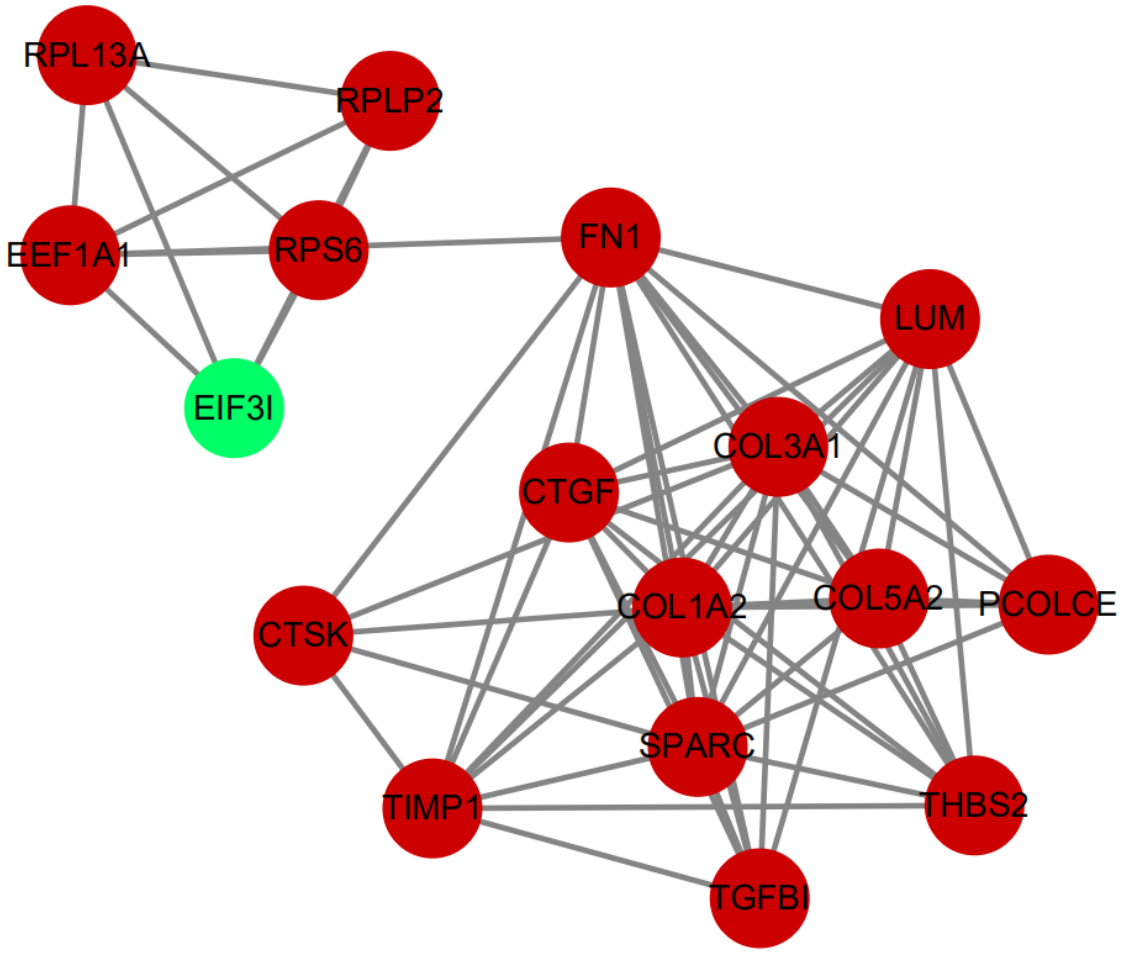

Figure 8

[No Legend]

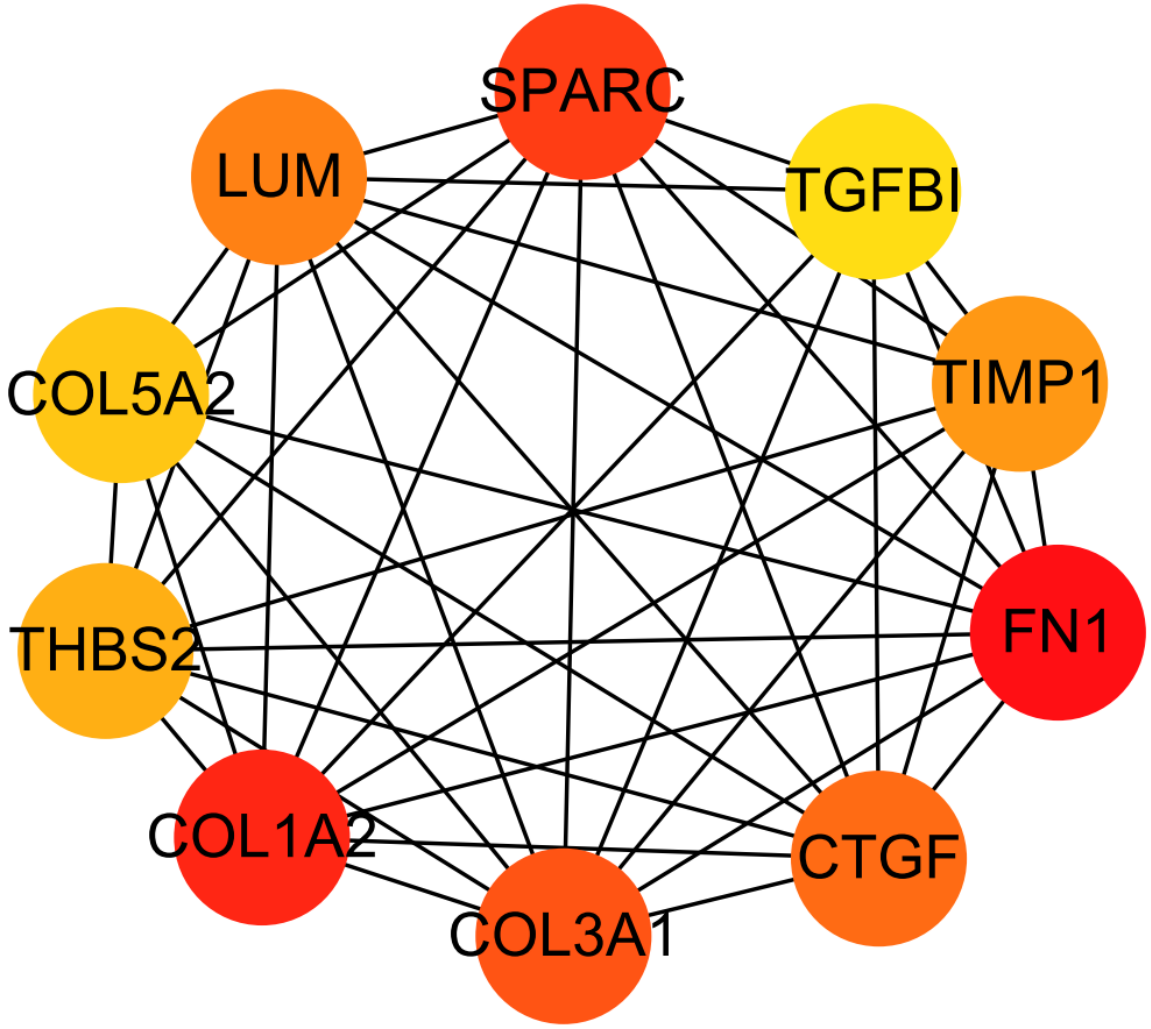

Figure 9

[No Legend]