Identification of Key Genes and Pathways Associated With Pathogenesis of Intervertebral Disc Degeneration by Integrated miRNA-mRNA Network Analysis

Tao Lan
  Shenzhen Second People's Hospital

Ning-dao Li
  The Third Affiliated Hospital of Shenzhen University: Shenzhen Luohu Hospital Group Luohu People's Hospital

Zhe Shen
  Shenzhen Second People's Hospital

Xiao-sheng Chen
  Shenzhen Second People's Hospital

Shi-yu Hu
  Shenzhen Second People's Hospital Department of Neurology

Bin Yan (✉ 08timmy@163.com)
  Shenzhen Second People's Hospital

Abstract

Background: Intervertebral disc degeneration (IDD) is one of the most common cause of low back pain. Previous studies have suggested that miRNAs are associated with the pathogenesis of IDD. However, the underlying mechanisms remain unclear based on inconsistent results of available literatures. In addition, integrated miRNA-mRNA comprehensive analysis is limited.

Material/Methods: In this study, we investigated the profiles of differentially expressed miRNAs (DEMIs) and mRNAs (DEGs) and constructed a miRNA-mRNA regulatory network. First, transcription factors and target genes of DEMIs were predicted. Then, an intersection between DEMIs predicted genes and DEGs were performed to screen out the most significant differential expressed common genes.

Results: A total of 65 DEMIs and 61 common target genes were identified from datasets. Functional enrichment analysis showed that most genes were mainly involved in extracellular matrix organization and extracellular structure organization. Furthermore, DEGs were primarily enriched in PI3K–Akt signaling pathway, ECM–receptor interaction, focal adhesion and p53 signaling pathway, indicating that these pathways may be the critical pathways.

Conclusions: In summary, several important miRNAs, as well as their related target genes and transcription factors in the pathogenesis of IDD were identified from our bioinformatic analysis, which may provide insights into underlying mechanisms and offer potential target genes for the treatment of IDD.

1 Introduction

Intervertebral disc degeneration (IDD) is considered as a main source of discogenic low back pain (LBP), which may cause disabilities in adults along with an enormous socioeconomic burden[1–3]. The incidence of LBP has risen dramatically with the changing of lifestyle[4]. Nowadays, LBP is highly recognized as a global health problem and it is estimated that over two-thirds of the population experience at least one episode of LBP, with roughly 10% turning to be chronically disabled[5]. Despite years of effort and intensive investigation, the currently available treatment options remain unsatisfactory. Several major theories have been put forward to explain the biology of IDD, including apoptosis[6], inflammation[7], aging[8, 9] and biomechanical loading[10, 11]. However, the genetic factors are regarded as the most significant contributor. Therefore, it is necessary to elucidate the possible molecular mechanism underlying IDD for developing effective treatments.

MicroRNAs are a class of non-coding single-stranded RNA of approximately 22 nucleotides in length encoded by endogenous genes. Increasing evidence has shown that miRNAs participate in RNA silencing and post-transcriptional regulation of gene expression. Recent reports have demonstrated that miRNAs are closely associated with pathogenesis of IDD and even act as a new therapeutic target or clinical biomarker for early diagnosis. For example, miR-24-3p leads to IDD by targeting insulin-like growth factor binding protein 5 and the ERK signaling pathway[12]. Inhibition of miR-129-5p results in IDD by promoting the apoptosis of nucleus pulposus cells via targeting BMP2[13].

To date, integrated miRNA- mRNA expression analysis regarding underlying mechanisms of IDD is still limited. The aim of this study is to investigate reliable miRNAs and their target genes related to IDD. Meanwhile, the study focuses on the functions of the target genes regulated by the differentially expressed miRNAs (DEMIs) during the pathogenesis of IDD, which may provide new insights into pathogenesis of IDD and potential target candidates for new therapy.

2 Materials And Methods

2.1 Microarray data collection. Both miRNA (GSE116726) and mRNA (GSE70362) were downloaded from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database. GSE116726 is a miRNA microarray containing 3 nucleus pulposus (NP) tissues from IDD patients and 3 normal NP tissues from fresh traumatic lumbar fracture patients. Meanwhile, mRNA expression profile of NP tissue was extracted from GSE70362, including 14 non-degenerative NP samples and 10 degenerative NP samples. The whole analysis process is summarized in Fig. 1.

2.2 Identification of differential expressed miRNAs (DEMI) and mRNAs (DEG) Limma package version 3.28.21 of Bioconductor 3.5 (http://www.bioconductor.org/packages/3.5/bioc/html/limma.html) was used to identify both DEMs and DEGs between the IDD samples and normal controls. Genes with Log |(fold change)| >4 and P < 0.01 were considered as DEMI. Log |(fold change)| >0.6 and P < 0.05 were set as the cut-offs to screen out DEG. Both heatmap and volcano plots were constructed to present expression profiles of differentially expressed genes, which was performed using R software.

2.3 Prediction of miRNA-targeted gene and DEMI-DEG regulatory network Both transcription factor (TF) and target genes of differentially expressed miRNAs were predicted using FunRich software (http://www.targetscan.org/), which is a powerful functional enrichment analysis tool that not only predicts TF and target genes of miRNAs but also provides GO enrichment analysis. The intersection of predicted target genes of DEMIs and DEGs were regarded as significantly differential expression target genes, which was performed by R software. These significant differential expression target genes and corresponding miRNAs were used to construct the miRNA-mRNA regulatory network using the Cytoscape software.

2.4 Functional enrichment analysis of target 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)[14]. 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[15]. 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.

3 Results

3.1 Differential Expression Analysis. For the expression profiles of miRNAs in IDD, a total of 414 DEMIs were identified from intervertebral disc samples in GSE116726. Among them, 189 were downregulated and 225 were upregulated within the P value < 0.01 and |log2FC| >4 criterion. Meanwhile, for the expression profiles of genes in IDD, A total of 207 genes were identified to be significantly differently expressed (P < 0.05), including 116 downregulated and 91 upregulated DEGs. Heatmap plots and volcano plots of both DEMIs and DEGs are shown in Fig. 2 (A and B) and Fig. 2 (C and D) respectively, in which the red represents upregulated genes and the green represents downregulated genes.

3.2 The transcription factor analysis of the differential expressed miRNAs in IDD. We also analyzed the TF genes corresponding to the differential expressed miRNAs in IDD and compared the similarities and differences. The top 10 most significantly differential TFs included EGR1, SP1, SP4, POU2F1, MEF2A, NFIC, ZFP161, NKX6-1, FOXA1 and TCF3. All TFs were mainly responsible for transcription factor activity, protein serine/threonine kinase activity, GTPase activity, ubiquitin-specific protease activity and transcription regulator activity in terms of molecular function (MF). The TFs mostly enriched in regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism, signal transduction, cell communication, transport and regulation of gene expression with respect to biological process (BP). The TFs primarily participated in nucleus, cytoplasm, golgi apparatus, lysosome and early endosome in terms of cellular component (CC). The functional enrichment analysis of TFs was summarized in Fig. 3.

3.3 Differential expression miRNA predicted target genes (DEMIGs) and miRNA−mRNA regulatory network. Target genes of miRNA were predicted by FunRich software. In order to find out the most significant differential expressed common genes, an intersection between DEMI predicted genes of GSE116726 and DEGs of GSE70362 were performed by R software and subsequently screened out 61 common genes, including 13 downregulated and 48 upregulated. These 61common DEGs and DEMIs were used to constructed miRNA−mRNA network, which consisted of 126 nodes and 218 edges, including 65 miRNAs (11 up-regulated and 54 down-regulated) (Fig. 4). The top 10 hub genes were identified by CytoHubba plugin using the Maximal Clique Centrality (MCC) method, including EDEM3, ITGB8, JARID2, GHR, THBS1, GATA6, hsa-miR-19b-3p, COL4A2, hsa-let-7f-5p, and CHUK. To be mentioned, hub DEGs were all up-regulated while both hsa-miR-19b-3p and hsa-let-7f-5p were downregulated in the network. The relationship between miRNA and mRNA of the regulatory network was summarized in Table 1.

3.4 Functional analysis of common target genes of miRNA-mRNA network. 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 extracellular matrix organization, extracellular structure organization, response to acid chemical, endoderm development, and cellular response to acid chemical in terms of biological process (BP). The DEGs mostly enriched in external side of plasma membrane, endoplasmic reticulum lumen, collagen – containing extracellular matrix, and protein complex involved in cell adhesion regarding cellular component (CC). The DEGs primarily participate in growth factor binding, cytokine binding, integrin binding, cell adhesion molecule binding with respect to molecular function (MF). The top 5 BP, CC and MF enrichment analysis of DEGs are summarized in Table 2. KEGG enrichment analysis showed that DEGs were enriched in 'PI3K – Akt signaling pathway', 'Human papillomavirus infection', 'ECM – receptor interaction', 'Focal adhesion' and 'p53 signaling pathway'. The GO and KEGG enrichment related bubble charts are presented in Fig. 5. The chord diagrams plot of GO and KEGG enrichment were illustrated in Figs. 6 and 7.

Table 1
Top 10 up/down-regulated miRNA and target mRNA of network.

Group	miRNA	Target mRNA	mirnaLogFC	mrnaLogFC
Up-regulated	hsa-miR-29c-3p	OSTC	21.75736119	-0.774850666
	hsa-miR-21-5p	TGFBI	20.52959811	-1.243326463
	hsa-miR-146a-5p	FAM169A	15.13262492	-0.776932799
	hsa-miR-139-5p	SLC25A3	8.551048025	-1.613530327
	hsa-miR-141-3p	SLC25A3	8.357302106	-1.613530327
	hsa-miR-338-3p	FAM169A	8.106111031	-0.776932799
	hsa-miR-296-5p	GMPR	7.405091511	-0.687825296
	hsa-miR-520b	DKK1	7.067523881	-0.852100332
	hsa-miR-520b	FOXF2	7.067523881	-0.828666373
	hsa-miR-520b	PKD2	7.067523881	-0.979895874
Down-regulated	hsa-miR-125b-5p	ARSI	-14.79236013	0.643525601
	hsa-miR-125b-5p	LIFR	-14.79236013	0.82878294
	hsa-miR-125b-5p	THEMIS2	-14.79236013	0.650826702
	hsa-let-7b-5p	CHUK	-28.24178662	0.8186234
	hsa-let-7b-5p	COL4A2	-28.24178662	0.996699315
	hsa-let-7b-5p	EDEM3	-28.24178662	0.668762938
	hsa-let-7b-5p	GHR	-28.24178662	0.680642041
	hsa-let-7b-5p	GLRX	-28.24178662	0.778291162
	hsa-let-7b-5p	ITGB8	-28.24178662	0.829814446
	hsa-let-7b-5p	THBS1	-28.24178662	0.627148366

Table 2
GO and KEGG enrichment analysis of target genes.

ONTOLOGY	ID	Description	Gene Ratio	P value	p.adjust	Gene ID	Count
BP	GO:0007492	endoderm development	7/57	3.14E-09	4.45E-06	COL4A2/DKK1/GATA6/ZFP36L1/ ITGA5/ITGAV/MMP2	7
	GO:0035987	endodermal cell differentiation	6/57	4.59E-09	4.45E-06	COL4A2/DKK1/GATA6/ITGA5/ITGAV/MMP2	6
	GO:0030198	extracellular matrix organization	10/57	1.48E-07	7.20E-05	FOXF2/COL4A1/COL4A2/ITGB8/THBS1/TGFBI/ TNFRSF11B/ITGA5/ITGAV/MMP2	10
	GO:0043062	extracellular structure organization	10/57	5.23E-07	0.0002	FOXF2/COL4A1/COL4A2/ITGB8/THBS1/TGFBI/ TNFRSF11B/ITGA5/ITGAV/MMP2	10
	GO:0001101	response to acid chemical	9/57	8.89E-07	0.0002	LDLR/CHUK/COL4A1/DKK1/PKD2/MTOR/SESN3/ MMP2/GCLM	9
CC	GO:0009897	external side of plasma membrane	8/61	2.71E-05	0.003	LDLR/GHR/THBS1/LIFR/CALR/ITGA5/ITGAV/IL1R1	8
	GO:0005788	endoplasmic reticulum lumen	7/61	4.58E-05	0.003	COL4A1/COL4A2/EDEM3/THBS1/ARSI/CALR/IGFBP3	7
	GO:0008305	integrin complex	3/61	0.0001	0.006	ITGB8/ITGA5/ITGAV	3
	GO:0098636	protein complex involved in cell adhesion	3/61	0.0001	0.006	ITGB8/ITGA5/ITGAV	3
	GO:0062023	collagen-containing extracellular matrix	6/61	0.001	0.029	COL4A1/COL4A2/THBS1/TGFBI/CALR/MMP2	6
MF	GO:0019838	growth factor binding	7/57	2.74E-07	5.99E-05	COL4A1/GHR/THBS1/LIFR/ITGAV/IGFBP3/IL1R1	7
	GO:0019955	cytokine binding	6/57	3.41E-06	0.0002	GHR/THBS1/LIFR/ITGAV/NBL1/IL1R1	6
	GO:0005178	integrin binding	6/57	4.08E-06	0.0002	ITGB8/THBS1/TGFBI/CALR/ITGA5/ITGAV	6
	GO:0050840	extracellular matrix binding	3/57	0.0008	0.0234	THBS1/TGFBI/ITGAV	3
	GO:0050839	cell adhesion molecule binding	7/57	0.001	0.023	ITGB8/THBS1/TGFBI/CALR/CDH11/ITGA5/ITGAV	7
KEGG	hsa04151	PI3K-Akt signaling pathway	9/46	0.0001	0.01	CHUK/COL4A1/COL4A2/GHR/ITGB8/THBS1/MTOR/ ITGA5/ITGAV	9
	hsa04512	ECM-receptor interaction	6/46	9.74E-06	0.001	COL4A1/COL4A2/ITGB8/THBS1/ITGA5/ITGAV	6
	hsa04510	Focal adhesion	6/46	0.0008	0.025	COL4A1/COL4A2/ITGB8/THBS1/ITGA5/ITGAV	6
	hsa04145	Phagosome	5/46	0.001	0.037	THBS1/CALR/ITGA5/ITGAV/C1R	5
	hsa04115	p53 signaling pathway	4/46	0.0007	0.025	THBS1/SESN3/EI24/IGFBP3	4

Discussion

IDD is a complex and multifactorial pathophysiological process which is characterized by excessive apoptosis of nucleus pulposus (NP) cells and over-degradation of extracellular matrix (ECM) components, leading to reduced hydration, loss of disc height, and decreased ability to absorb load[16]. To date, genetic factors have been considered as main contributor of IDD while the exact underlying mechanism has not been fully elucidated.

In the present study, we used mRNA and miRNA expression profiles to identify differential expression mRNAs (DEGs) and miRNAs (DEMIs). A total of 414 DEMIs and 207 DEGs were identified to be significantly differently expressed. In addition, the top 10 most significantly differential TFs included EGR1, SP1, SP4, POU2F1, MEF2A, NFIC, ZFP161, NKX6-1, FOXA1 and TCF3. Furthermore, 61 common genes via an intersection between DEMIs predicted genes and DEGs were used to build miRNA-mRNA regulatory network. Functional and pathway enrichment analysis of common DEGs were associated with extracellular matrix organization and PI3K–Akt signaling pathway.

Increasing evidence has shown that many cellular processes, including cell proliferation, apoptosis, and cytokine release, are regulated by miRNAs. Recent studies further demonstrated that miRNA functioned by suppressing protein production from targeted genes which was closely associated with development of IDD[17]. Hsa-let-7b-5p is the most significant downregulated miRNA, which belongs to the let-7 family and plays an important regulatory role in the cell cycle and differentiation. Inhibition of let-7b-5p is associated with elevated expression of TNF-α, IL-1β, and IL-6, leading to activation of inflammation cascade[18]. Hsa-miR-29c-3p is the most significant upregulated miRNA. Recent study showed that overexpression of miR-29c-3p might inhibit proliferation and promote apoptosis and differentiation by inhibiting the expression of Akt3[19]. We found that hsa-miR-106b-5p was downregulated while target gene MMP-2 was upregulated in our network. MMP-2 is a member of MMP family and MMP-2 mediates local degradation and remodeling of collagen by annulus fibrosus cells of the intervertebral disc, indicating that both hsa-miR-106b-5p and MMP-2 may serve as potential therapeutic target of IDD. Hsa-miR-98-5p, a downregulated miRNA which targets CHUK, COL4A1, COL4A2, EDEM3, GHR, GLRX, ITGB8, and THBS1 in the network, has been demonstrated that contributes to extracellular matrix degradation by targeting IL-6/STAT3 signaling pathway in human intervertebral disc degeneration[20]. A preclinical study showed that miR-141 promoted IDD progression by interacting with SIRT1/NF-κB pathway and inhibition of miR-141 in vivo may serve as a potential therapeutic approach in the treatment of IDD[21]. miR-181a-5p was downregulated in IDD mice while upregulation of miR-181a protected against inflammatory response by inactivating the ERK pathway via suppression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in IDD mice[22]. This result is consistent with finding of our miRNA-miRNA network. miR-29a has been demonstrated with protective effect by silencing the expression of MMP-2, inhibiting the fibrosis process, and reversing IDD in animal models via blocking the β-catenin translocation pathway from the cytoplasm to the nucleus[23].

Transcription factors (TFs) regulate gene expression by activating or repressing target genes at the transcriptional level. Hence, we explore some important TFs related to miRNA target genes expression. SP1 is a ubiquitous zinc finger transcription factor that binds to GC-rich regions in the promoters of genes to activate transcription. Inhibition of Sp1 and Sp1 knockdown has been previously demonstrated to decrease expression of MMP3, ADAMTS4 and ADAMTS5 and subsequently suppress TNF-α-induced catabolic activity in nucleus pulposus cells[24]. EGR1 belongs to the early growth response family, with a wide range of activities as transcription factor ranging from regulation of cell growth to differentiation. EGR1 plays an important role that allows NP cells to adapt to anabolic or catabolic stimuli[25]. Both FOXA1 and FOXA2 are necessary for the formation of intervertebral disc and inhibition of FOXA1 or FOXA2 leads to aberrant differentiation of NP cells from notochord cells[26].

In terms of GO enrichment analysis, we found that most of DEGs were mainly involved in extracellular matrix organization, extracellular structure organization, response to acid chemical, endoderm development, and cellular response to acid chemical. It has been reported that the increased expression of MMPs in NPCs leads to inadequate synthesis and excessive degradation of extracellular matrix (ECM), resulting in progression of IDD. We found that MMP-2 was significantly up-regulated in the degenerative samples compared with normal samples in our analysis. Previous finding also revealed that the expression of MMP-2 was increased in intervertebral disc and MMP-2 was associated with local degradation and remodeling of collagen tissue[27].

Of the significantly enriched pathways in the KEGG pathway analysis, PI3K/AKT signaling pathway signaling was of interest as it played an important role in protective effects on human nucleus pulposus under different pathological conditions based on literatures. The activation of PI3K/AKT has been showed protective effect against IDD by increase of ECM content, prevention of cell apoptosis and induction or prevention of cell autophagy. For example, under oxidative damage, Resveratrol increased ECM synthesis of NPCs by enhancing autophagy through PI3K/AKT pathway[28]. Further study confirmed that PI3K/AKT pathway had protective effects on human nucleus pulposus-derived mesenchymal stem cells under hypoxia and nutrition deficiency[29]. Conversely, PTEN, the only known lipid phosphatase counteracting the PI3K/AKT pathway, promoted intervertebral disc degeneration by regulating nucleus pulposus cell behaviors[30]. miR-21 exosomes prevented NPCs from apoptotic process and alleviated IVD degeneration via restraining PTEN and activating PI3K/AKT pathway in apoptotic NPCs[31].

In conclusion, several important miRNAs, as well as their related target genes and transcription factors in the pathogenesis of IDD were identified from our bioinformatic analysis, which may provide insights into underlying mechanisms and offer potential target genes for the treatment of 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

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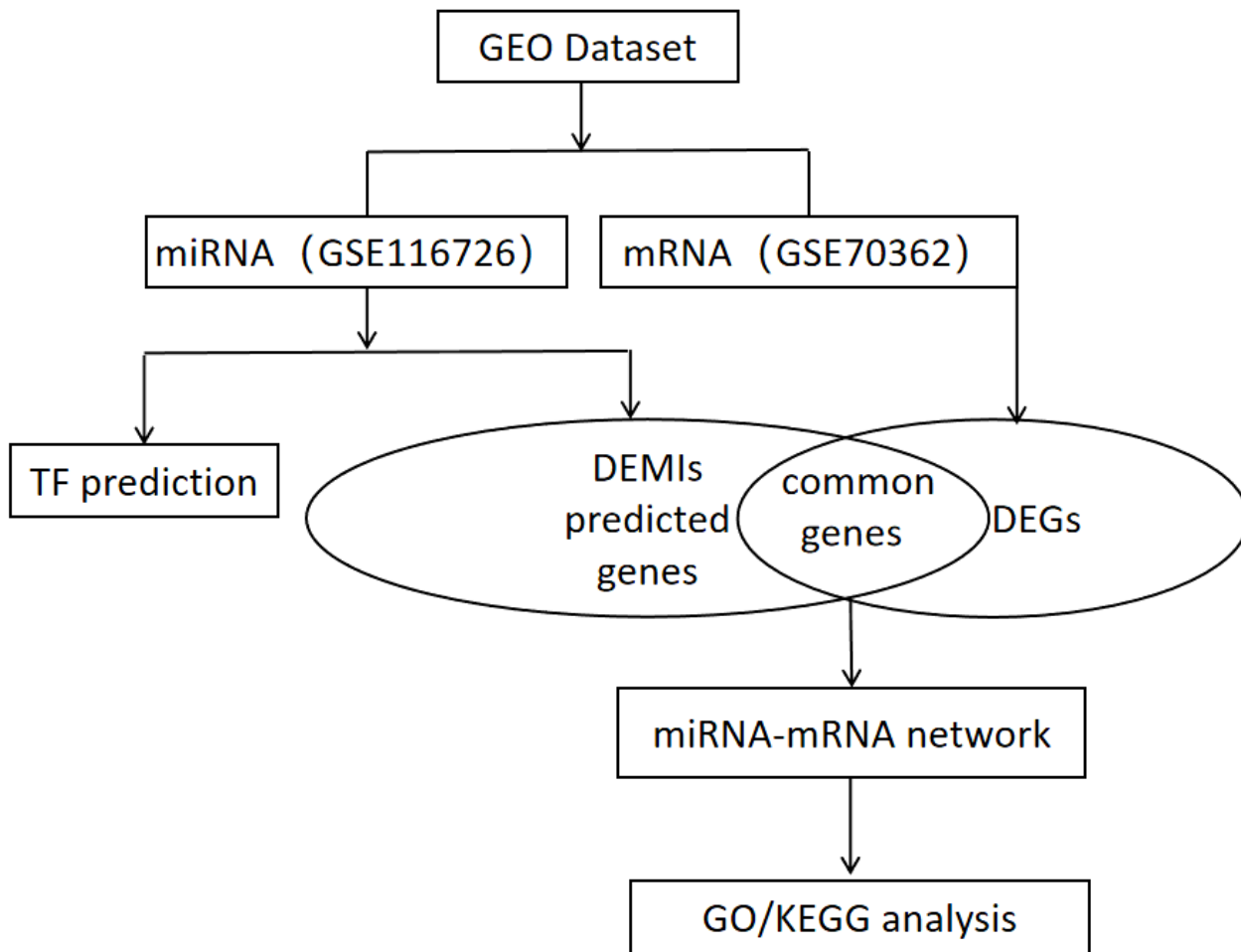
Figures

Figure 1

Microarray data collection. Both miRNA (GSE116726) and mRNA (GSE70362) were downloaded from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database. GSE116726 is a miRNA microarray containing 3 nucleus pulposus (NP) tissues from IDD patients and 3 normal NP tissues from fresh traumatic lumbar fracture patients. Meanwhile, mRNA expression profile of NP tissue was extracted from GSE70362, including 14 non-degenerative NP samples and 10 degenerative NP samples. The whole analysis process is summarized in Figure 1.

(A)

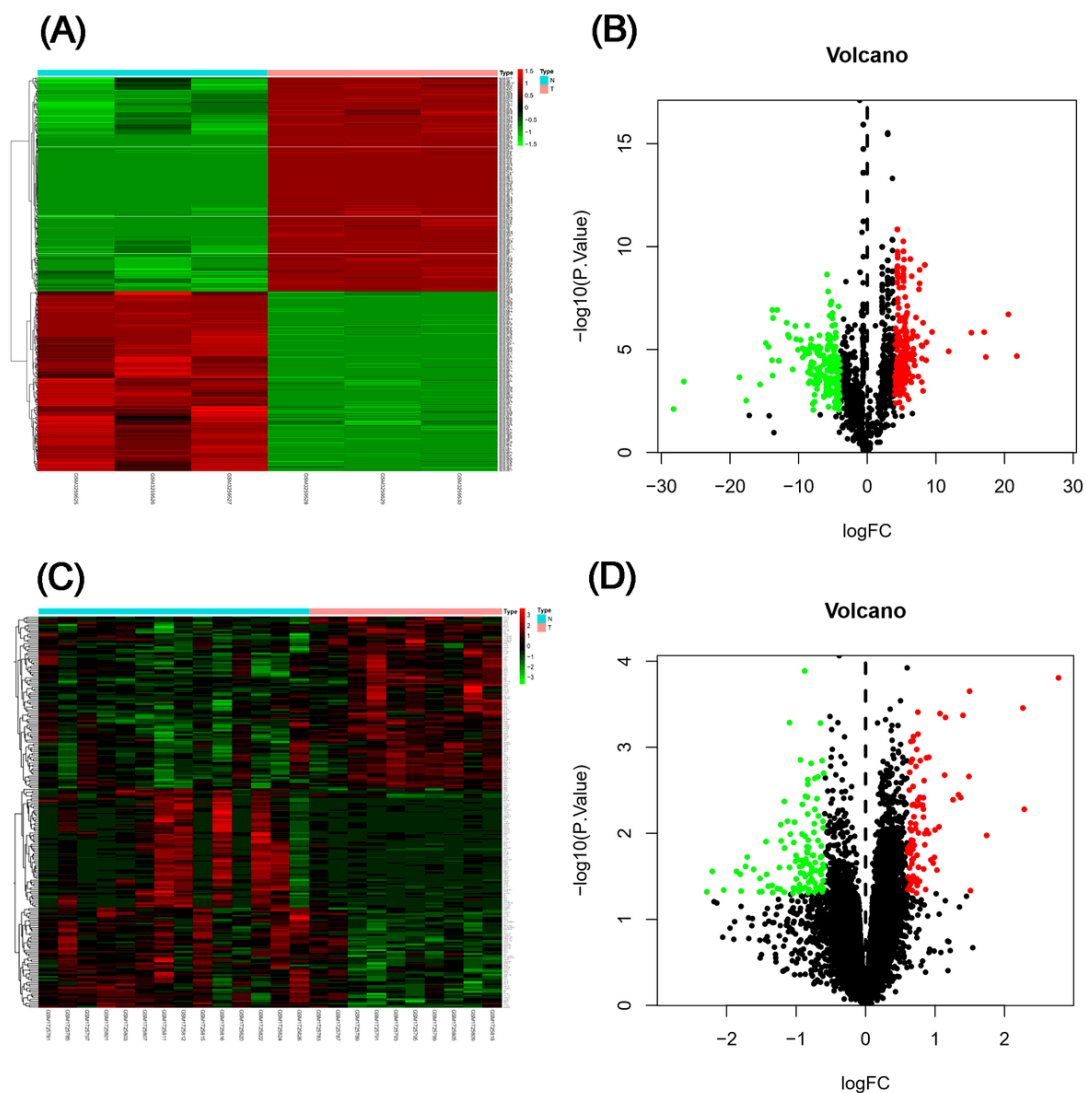**(B)** Volcano

(C)

(D) Volcano

Figure 2

Differential Expression Analysis. For the expression profiles of miRNAs in IDD, a total of 414 DEMIs were identified from intervertebral disc samples in GSE116726. Among them, 189 were downregulated and 225 were upregulated within the P value <0.01 and |log2FC| >4 criterion. Meanwhile, for the expression profiles of genes in IDD, A total of 207 genes were identified to be significantly differently expressed (P<0.05), including 116 downregulated and 91 upregulated DEGs. Heatmap plots and volcano plots of both DEMIs and DEGs are shown in Figure 2 (A and B) and figure 2 (C and D) respectively, in which the red represents upregulated genes and the green represents downregulated genes.
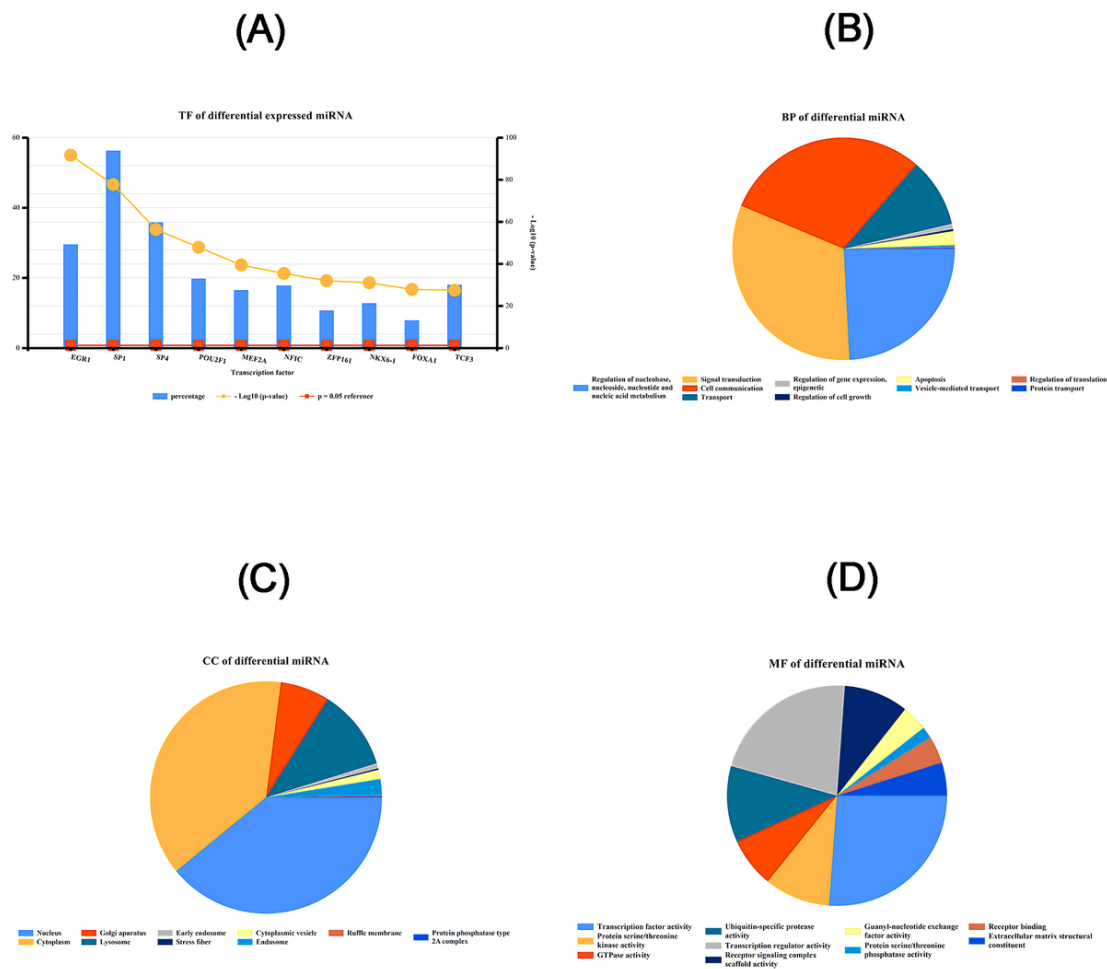
(A) TF of differential expressed miRNA

(B) BP of differential miRNA

(C) CC of differential miRNA

(D) MF of differential miRNA

Figure 3

The transcription factor analysis of the differential expressed miRNAs in IDD. We also analyzed the TF genes corresponding to the differential expressed miRNAs in IDD and compared the similarities and differences. The top 10 most significantly differential TFs included EGR1, SP1, SP4, POU2F1, MEF2A, NFIC, ZFP161, NKX6-1, FOXA1 and TCF3. All TFs were mainly responsible for transcription factor activity, protein serine/threonine kinase activity, GTPase activity, ubiquitin-specific protease activity and transcription regulator activity in terms of molecular function (MF). The TFs mostly enriched in regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism, signal transduction, cell communication, transport and regulation of gene expression with respect to biological process (BP). The TFs primarily participated in nucleus, cytoplasm, golgi apparatus, lysosome and early endosome in terms of cellular component (CC). The functional enrichment analysis of TFs was summarized in Figure 3.
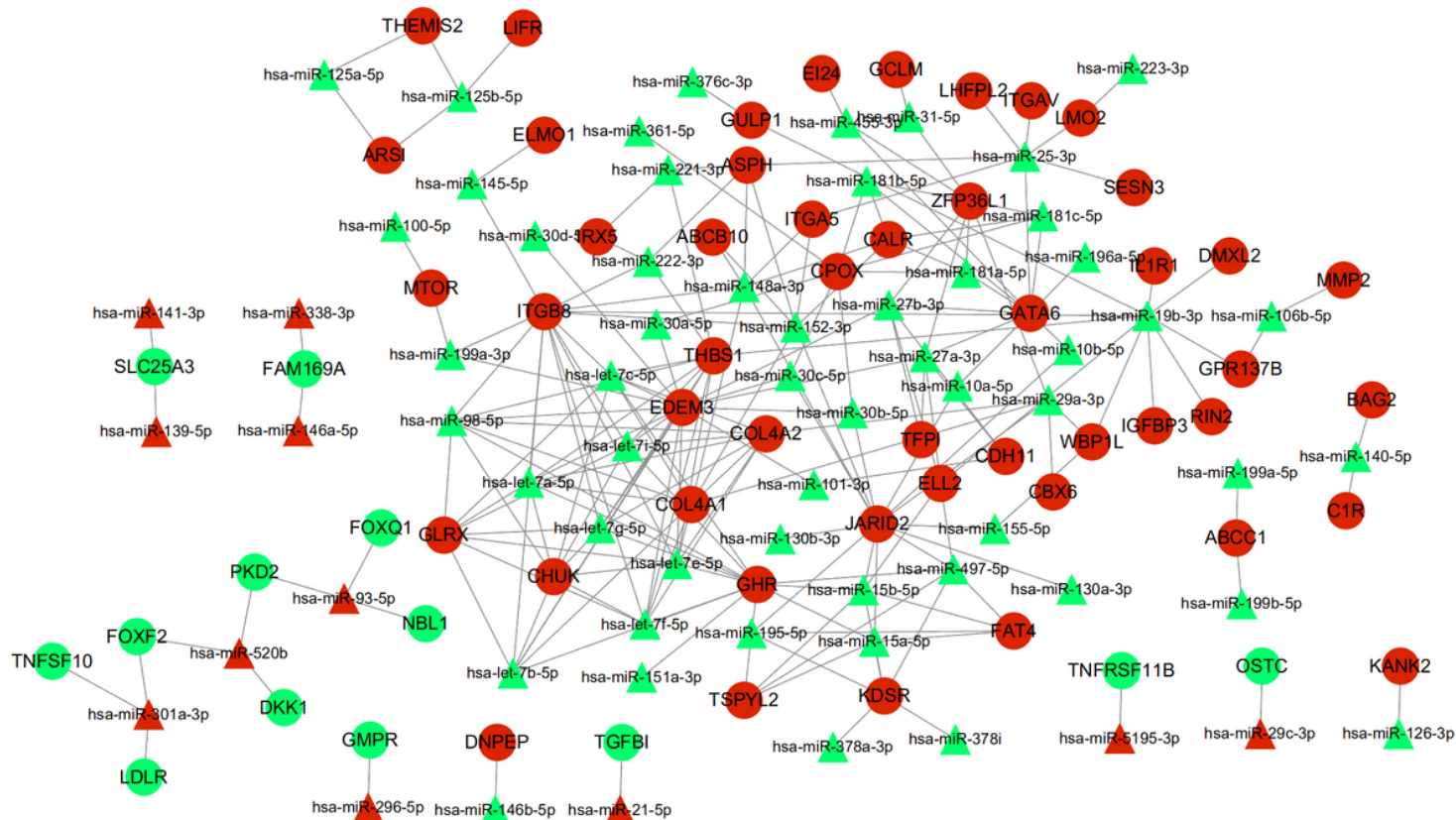
Figure 4

Differential expression miRNA predicted target genes (DEMIGs) and miRNA−mRNA regulatory network. Target genes of miRNA were predicted by FunRich software. In order to find out the most significant differential expressed common genes, an intersection between DEMI predicted genes of GSE116726 and DEGs of GSE70362 were performed by R software and subsequently screened out 61 common genes, including 13 downregulated and 48 upregulated. These 61common DEGs and DEMIs were used to constructed miRNA−mRNA network, which consisted of 126 nodes and 218 edges, including 65 miRNAs (11 up-regulated and 54 down-regulated) (Figure 4). The top 10 hub genes were identified by CytoHubba plugin using the Maximal Clique Centrality (MCC) method, including EDEM3, ITGB8, JARID2, GHR, THBS1, GATA6, hsa-miR-19b-3p, COL4A2, hsa-let-7f-5p, and CHUK. To be mentioned, hub DEGs were all up-regulated while both hsa-miR-19b-3p and hsa-let-7f-5p were downregulated in the network. The relationship between miRNA and mRNA of the regulatory network was summarized in table 1.
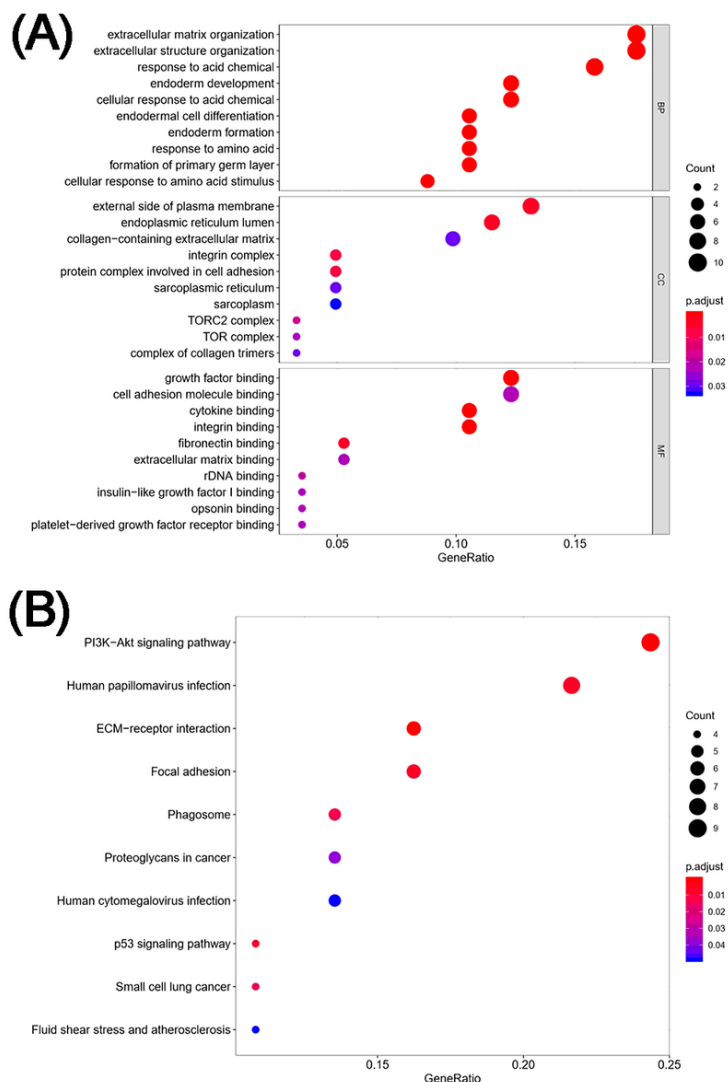
Figure 5

Functional analysis of common target genes of miRNA-mRNA network. 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 extracellular matrix organization, extracellular structure organization, response to acid chemical, endoderm development, and cellular response to acid chemical in terms of biological process (BP). The DEGs mostly enriched in external side of plasma membrane, endoplasmic reticulum lumen, collagen-containing extracellular matrix, and protein complex involved in cell adhesion regarding cellular component (CC). The DEGs primarily participate in growth factor binding, cytokine binding, integrin binding, cell adhesion molecule binding with respect to molecular function (MF). The top 5 BP, CC and MF enrichment analysis of DEGs are summarized in table 2. KEGG enrichment analysis showed that DEGs were enriched in 'Pl3K–Akt signaling pathway', 'Human papillomavirus infection', 'ECM–receptor interaction', 'Focal adhesion' and 'p53 signaling pathway'. The GO and KEGG enrichment related bubble charts are presented in Figure 5.
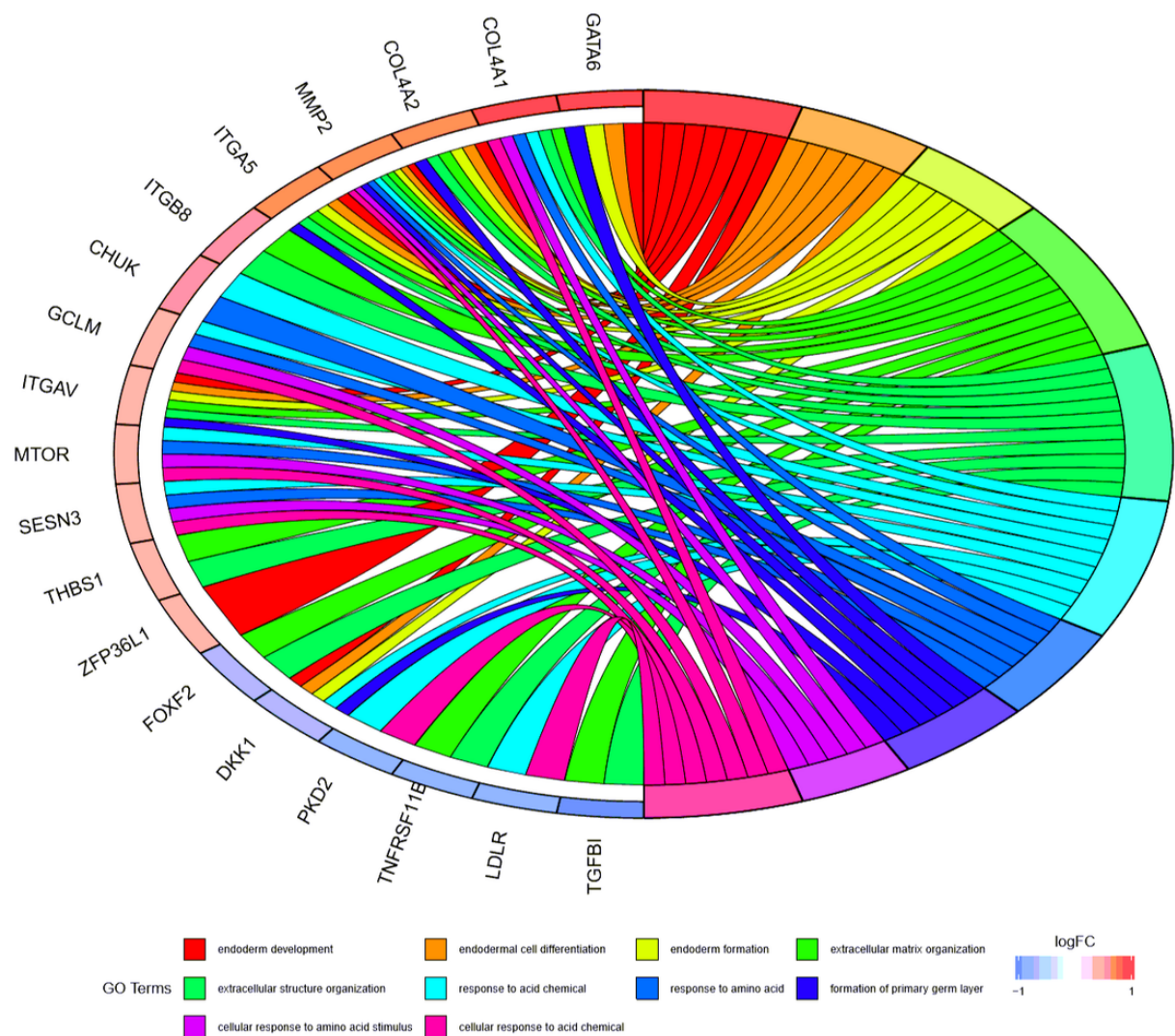
Figure 6

The chord diagrams plot of GO and KEGG enrichment were illustrated in Figure 6 and 7.
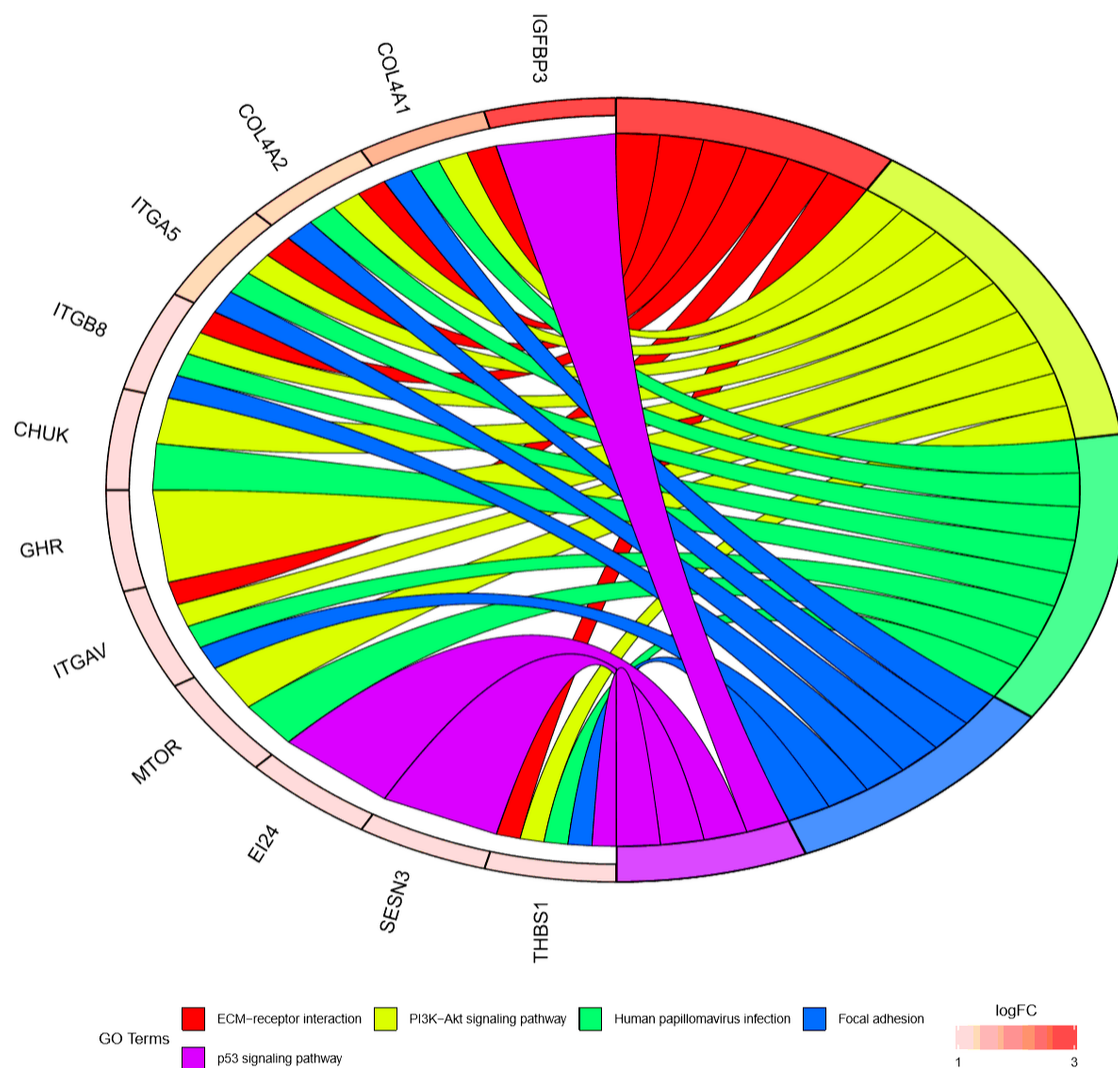
Figure 7

The chord diagrams plot of GO and KEGG enrichment were illustrated in Figure 6 and 7.