Identification of Potential Therapeutic Targets For Rheumatoid Arthritis By Bioinformatics

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Research Article

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Abstract

Objective: Based on GEO database, we performed bioinformatics analysis on rheumatoid arthritis (RA)-related gene chips to obtain key genes and signaling pathways of RA, understand the molecular mechanism of RA occurrence and development, and provide candidate targets for the diagnosis and treatment of RA.

Methods: The chip GSE77298 related to rheumatoid arthritis in GEO database was retrieved, and the R Programming Language analyzed the differential genes. Subsequently, the differential gene protein-protein interaction (PPI) relationship was constructed. The hub gene was screened, and the DAVID database was used for GO enrichment analysis and KEGG pathway analysis of key differential genes. The miRNAs were then subjected to target gene prediction, and then a miRNA-mRNA visualization network map was constructed using Cytoscape. Finally, transcription factors were predicted by the AnimalTFDB database.

Results: The chip with the serial number of GSE77298 was retrieved from GEO database, and 1539 differential genes were screened out using the R Programming language analysis, including 1156 up-regulated genes and 383 down-regulated genes. By DAVID online functional enrichment analysis of differential genes, it was shown that the signaling pathways were mainly rheumatoid arthritis, Staphylococcus aureus infection, chemokine signaling pathway, viral myocarditis, cytokine-cytokine receptor interaction, etc. PPI was constructed through String database. Its hub genes were CXCL12, CD44 and CDH2. The top 10 key differential genes in Degree were CXCL8, PTPRC, MMP9, TLR2, FN1, ITGB2, CXCL1, CCL5, CXCR4 and CXCL10. Ten important miRNAs such as hsa-miR-30a-3p, hsa-miR-34a-5p, hsa-miR-30d-3p were predicted. Transcription factors such as GTF3C2, GLYR1, TRIM24, YY1 were predicted.

Conclusion: PI3K-AKT signaling pathway and many other pathways are involved in the occurrence and development of RA, and CXCL8, SOCS3, and TLR2 genes may be the key genes in RA. Ten important miRNAs such as has-miR-340-5p may participate in the pathogenesis of RA. Many transcription factors such as YY1 may be involved in RA's disease process, which will provide directions for further research on RA diagnosis and treatment targets of RA.

Introduction

Rheumatoid arthritis (RA) is a common progressive and systemic inflammatory autoimmune disease, which may cause joint destruction, deformity, and disability, and seriously affect the quality of life of patients[1]. RA's pathogenesis is complex, and RA is mainly characterized by synovitis, cartilage, and bone destruction[2, 3]. It harms the human body and has high group morbidity and mortality[4]. Therefore, early diagnosis and prognosis of rheumatoid arthritis treatment are essential. However, RA's exact pathogenesis is not clear. It is currently considered a multi-cause disease involving genotype, environmental triggers, and chance[5]. With the development and popularization of high-throughput

sequencing technology and genomics research, bioinformatics analysis based on gene chips provides a convenient and effective method for finding molecular markers of disease and signaling pathways of related genes[6]. In this study, bioinformatics was used to analyze the gene chips of the synovial tissues of RA patients and healthy people, to find the differentially expressed genes and possible signaling pathways, and to predict their possible connections, which will provide an experimental basis for the early diagnosis of RA and the development of targeted drugs.

Materials And Methods

2.1 Obtain gene expression microarray data from RA synovial tissue

Gene expression microarray data for RA synovial tissue were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/GEO/)[7]. Inclusion criteria: (1) Subjects including patients with RA and normal controls; (2) Samples should be collected from humans; (3) Each group should have at least two samples. Exclusion criteria: (1) Duplicate data sets; (2) If the study examined other types of arthritis, such as osteoarthritis, the study was excluded; (3) Data sets lacking RA sample group; (4) Data sets lacking control group; (5) Researches or data on animals or cell lines. A gene expression data set for RA synovial tissue, GSE77298, comprising 23 synovial biopsy samples (16 individuals were terminal RA by synovial biopsies and 7 without joint disease).

DEGs were screened with the limma package in Bioconductor (screening conditions were set to $P < 0.05$, $|\log2FC| > 1$) to obtain DEGs between RA and control samples. The chip's original volcanic map was drawn with the plot package, and the heat map was drawn with the heatmap2 package.(Figure 9)

2.2 GO Functional Analysis and KEGG Pathway Enrichment Analysis of DEGs

After identifying DEGs, the annotation of cellular components (CCs), biological processes (BPs) and molecular functions (MFs) of DEGs were determined by Gene Ontology (GO) enrichment analysis. The Kyoto Encyclopedia of Genes and Genome (KEGG) pathway (http://www.genome.jp/) analysis was used to determine the gene clusters and related functions pathway. This study used the Database for Annotation, Visualization, and Integrated Discovery database (DAV6.8) (https://david.ncifcrf.gov)[8] to perform KEGG pathway and GO enrichment analysis. P values < 0.05 were considered statistically significant. The result was used to draw the bubble diagram by the the R Programming Language ggplot2 installation package.

2.3 PPI network and module analysis

Using the online tool STRING (https:/string-db.org/) to construct protein interaction networks (PPIs) of DEGs. Then we analyzed the PPI network graph.The screening condition was set as an interaction score > 0.4 to identify the proteins at the key nodes, the related proteins, and their corresponding relationships.

The PPI network was visualized using Cytoscape software (Cytoscape 3.7.2) (an open-source bioinformatics software platform for visualizing molecular interaction networks[9]), and the CytoHubba

plugin was used to calculate the degree of freedom of the network. And the nodes, which were the top 10 genes with degree ≥1 considered as central genes.

2.4 Predicting related miRNA

With targetScan (http://www.targetscan.org/vert_71/) and miRDB (http://www.mirdb.org), the database performs target gene prediction for miRNAs from DEGs. Venny was used to generate intersection genes which were considered as target genes. The predicted target genes were then intersected with differentially expressed mRNAs from the GSE77298 dataset to sort out miRNA-mRNA relationship pairs. Finally, a miRNA-mRNA visualization network diagram was constructed using Cytoscape software (Cytoscape 3.7.2) (Figure 8).

2.5 Transcription factor prediction

After obtaining the promoter sequence, using the UCSC Genome Browser (http://genome.ucsc.edu/index.html), then use the AnimalTFDB database (http://bioinfo.life.hust.edu.cn/AnimalTFDB/) to predict the corresponding transcription factors.

Results

3.1 DEGs comparison between RA and normal synovium

A total of 1,539 differential genes were obtained from the synovial membrane of terminal RA and normal synovial membrane by limma package analysis in the R Programming Language, including 1,156 up-regulated genes and 383 down-regulated genes, as shown in Figure 1.

3.2 Functional enrichment and pathway analysis of key differential genes

To reveal more specific functional patterns of these genes, we used the DAVID database to analyze the 1,539 DEGs by GO and KEGG enrichment analysis.GO analysis showed that the changes of cytological processes (BPs) were mainly 148 functional clusters such as immune response, phagocytosis, and recognition, inflammatory response, innate immune response, and chemotaxis, as shown in Figure 2; the molecular functions (MFs) changes were mainly 37 functional clusters such as immunoglobulin receptor binding, chemokine activation, antigen binding, muscle structural composition and MHC II receptor activation, as shown in Figure 3; and the cellular components (CCs) changes were mainly 47 functional clusters such as extracellular space, extracellular region, Z-disc, lateral plasma membrane, and plasma membrane, as shown in Figure 4. KEGG pathway analysis showed that genes were enriched in a total of 44 signaling pathways such as rheumatoid arthritis, Staphylococcus aureus infection, chemokine signaling pathway, viral myocarditis, cytokine-cytokine receptor interaction, and PI3K-Akt signaling pathway, as shown in Figure 5.

3.3 Build PPI network

Using the String database, 1,539 DEGs were built to PPI network maps, and then we screened the key genes, as shown in Figure 6. The CytoHubba plugin calculated the degree of freedom of target genes to obtain 10 key differential genes, including CXCL8, PTPRC, MMP9, TLR2, FN1, ITGB2, CXCL1, CCL5, CXCR4, and CXCL10, as shown in Figure 7.

3.4 Predicting significant miRNAs and mRNAs

With the miRDB database (http://www.mirdb.org), we generated the miRDB_v6.0_prediction_result. Ten significant miRNAs were predicted from the above results: hsa-miR-153-5p, hsa-miR-3163, hsa-miR-340-5p, hsa-miR-3613-3p, hsa-miR-607, hsa-miR-5692a, hsa-miR-1250-3p, hsa-miR-4282, hsa-miR-12123 and hsa-miR-1277-5p.

According to the MCC method, we ranked the mRNA in miRNA-mRNA.network.links.txt and found that the top 10 mRNAs were: PRKAA2, SPCS3, PDK4, SLC2A3, CAP2, CXCL8, CXCL14, KLHL6, RGS1, and MMP13.

3.5 Predicting significant transcription factors

Use AnimalTFDB database website to generate AnimalTFDB_tfbs_predict_result.Some important transcription factors were predicted from the above results: GTF3C2, GLYR1,TRIM24, YY1, etc.

Discussion

In the past decade, with the early combined application of slow-acting antirheumatic drugs, the treatment of extra-articular lesions, and the emergence of new treatments, the prognosis of rheumatoid arthritis has been significantly improved. However, people do not know enough about the deep pathological mechanism of RA. In this study, the bioinformatics method was used to explore rheumatoid arthritis synovium's gene expression profile in GSE77298 data set. The results showed that compared with the normal control group, there were 1539 DEGs in the synovium of RA. Functional annotation and KEGG pathway enrichment analysis showed that these differential genes were mainly involved in the chemokine signal pathway, PI3K-Akt signal pathway, and so on. The key differences of Degree in the top 10 are CXCL8, TLR2, and so on. The degrees of freedom of these genes are all more than 30, which play an important role in the whole network. These are expected to become RA's biomarkers and reflect the /disease's onset and / or progression.

MiRNAs also plays an important role in the development of RA[7, 10], especially the changes of miRNAs expression in patients with RA are related to the proliferation, migration, and apoptosis of fibroblast-like synoviocytes in RA[11, 12]. MiR-340-5p in our prediction is one of them. The expression of miR-340-5p in patients with rheumatoid arthritis decreases, and its overexpression in fibroblast-like synoviocytes of rheumatoid arthritis can significantly inhibit synovial cell proliferation promote synovial cell apoptosis, and inhibit the expression of inflammatory factors, to improve the disease. In our predicted results, the primary genes related to miR-340-5p are SOCS3, PRKAA2, etc., and STAT3 is the target of miR-340-5p[13], in which SOCS3 has always been considered to be the main negative regulatory factor of STAT3[14] and

is highly expressed in peripheral blood monocytes of patients with rheumatoid arthritis[15]. SOCS3 is a potential inhibitor of JAK tyrosine kinase activity, mainly involved in the negative regulation of the JAK-STAT pathway in inflammatory T cells, neutrophils, and macrophages[16, 17].

Meanwhile, it has been reported that SOCS3 can regulate the phosphorylation activation of Akt through JNK/STAT3 signal pathway[18]. The activation of Akt under the action of growth factors and cytokines is essential for monocyte survival[19]. The activation of Akt can also promote cell proliferation, inhibit apoptosis, induce cytokines' expression, and induce specific cytokines' expression. Inhibition of PI3K/Akt/mTOR signal transduction can delay abnormal immune cells' survival, induce apoptosis of a large number of cells, and inhibit apoptosis of articular chondrocytes in inflammatory, proliferative synovium, and enhance the repair of articular cartilage[20]. In the follow-up study, we can regulate the expression of SOCS3 and target AKT to treat diseases, which provides a new idea for the clinical development of new anti-RA drugs whether the pathogenicity of promoting synovial cell proliferation and inhibiting synovial cell apoptosis due to the decreased expression of miR-340-5p in patients with rheumatoid arthritis is related to the increased expression of SOCS3 to activate Akt and activate PI3K/Akt/mTOR signal pathway, which needs to be further studied.

Akt plays a role in the PI3K-Akt signaling pathway. As one of the top 10 DEGs of degree, TLR2 (Toll-like receptor 2) is the pathway's target gene. TLR2 is highly expressed in the synovium of patients with RA and participates in RA inflammatory process [21]. It has been proved that TLR2 mediates the activation of nuclear factor (NF)-β and mitogen-activated protein kinase (MAPK)[22]. The activation of NF-κB depends on the phosphorylation of the IκB kinase (IKK) complex and the degradation of IκB (the inhibitor of NF-κB). Akt can, directly and indirectly, regulate the activity of IKK and affect the activation of NF-κB[23]. In a study of allergic inflammatory disease, the researchers found that TLR2 mainly regulates autophagy associated with PI3K/Akt signal pathway in mice. When the TLR2 gene was knocked out, airway inflammation, cytokine imbalance, and protein expression decreased in mice[24]. However, as far as the current research results are concerned, there is no research to show the direct role of TLR2 and PI3K/Akt signal pathway in rheumatoid arthritis, which needs to be further explored. What is certain is that TLR2 and PI3K/Akt signaling pathways play an essential role in RA pathogenesis.

TLR2 can also mediate the expression of angiogenic factors, vascular endothelial growth factor, and CXCL8 in rheumatoid synovial fibroblasts[25]. CXCL8 is one of the hub genes we predicted, binding to G protein-coupled receptors (CXCR1 and CXCR2)[26]. The synovium and circulation levels of CXCL8, CXCL12, and CXCL13 in RA patients were higher than those in healthy subjects. Overexpression of CXCL8, CXCL12, and CXCL13 promoted the migration of immune cells to the joints, resulting in joint destruction. Among the transcription factors we predicted, YY1 plays an important role in the pathogenesis of rheumatoid arthritis, which is a widely distributed transcription factor with the function of activating and inhibiting gene transcription[27]. It was found that the levels of CXCL8 and YY1 in peripheral blood of patients with RA were significantly increased, and there was a positive correlation between YY1 and CXCL8. CXCL8 is also related to PI3K/Akt signal pathway. YY1 can induce the production of CXCL8 through the PI3K-AKT-mTOR signal pathway. Inhibition of YY1 can reduce the

production of CXCL8 and reduce neutrophil infiltration in RA[28]. This can reduce the up-regulating effect of TLR2 on CXCL8, and it can be seen that YY1 is also an important therapeutic target for RA. In a study of angiogenesis in lung cancer, YY1-mediated MCM3AP-AS1 overexpression promotes angiogenesis and progression in lung cancer by targeting the miR-340-5p/KPNA4 axis[29].

Meanwhile, some studies have shown that MCM3AP-AS1 can significantly improve p-Akt, and the potential downstream signal pathway of MCM3AP-AS1-mediated tumor metabolism may be PTEN/Akt[30]. Overexpression of microRNA-340-5p can significantly inhibit the expression of CXCL8 in fibroblast-like synoviocytes of RA[13]. We speculate that the YY1-mediated PI3K-AKT-mTOR signaling pathway may also be affected by miR-340-5p in RA, but further study is needed.

Generally speaking, the study of SOCS3, TLR2, CXCL8, PI3K-AKT-mTOR signal pathway, microRNA-340-5p, and transcription factor YY1 is of great significance in the diagnosis, treatment, and drug screening of RA. Our predicted results are of great value in verifying the pathogenesis of RA. Our findings provide a research direction for further understanding the pathogenesis of RA and a candidate target for diagnosing and treatingRA's diagnosis and treatment.

Abbreviations

rheumatoid arthritis (RA), cellular components (CCs), biological processes (BPs), molecular functions (MFs), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genome (KEGG), TLR2 (Toll-like receptor 2), mitogen-activated protein kinase (MAPK), IκB kinase (IKK), protein-protein interaction (PPI);

Declarations

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used during the present study are available from the corresponding author upon reasonable request.

Competing interest

No competing interests.

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Authors' contributions

Erbai Ye and Xiaoling Xiao contributed significantly to the analysis and wrote the manuscript;

Senpeng Zhang contributed to acquiring data;

Xinni Ye and Lulu He helped review the manuscript;

Chun Yang and Yi-kuan Du helped perform the analysis with constructive discussions;

All authors read and approved the final manuscript.

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Figures

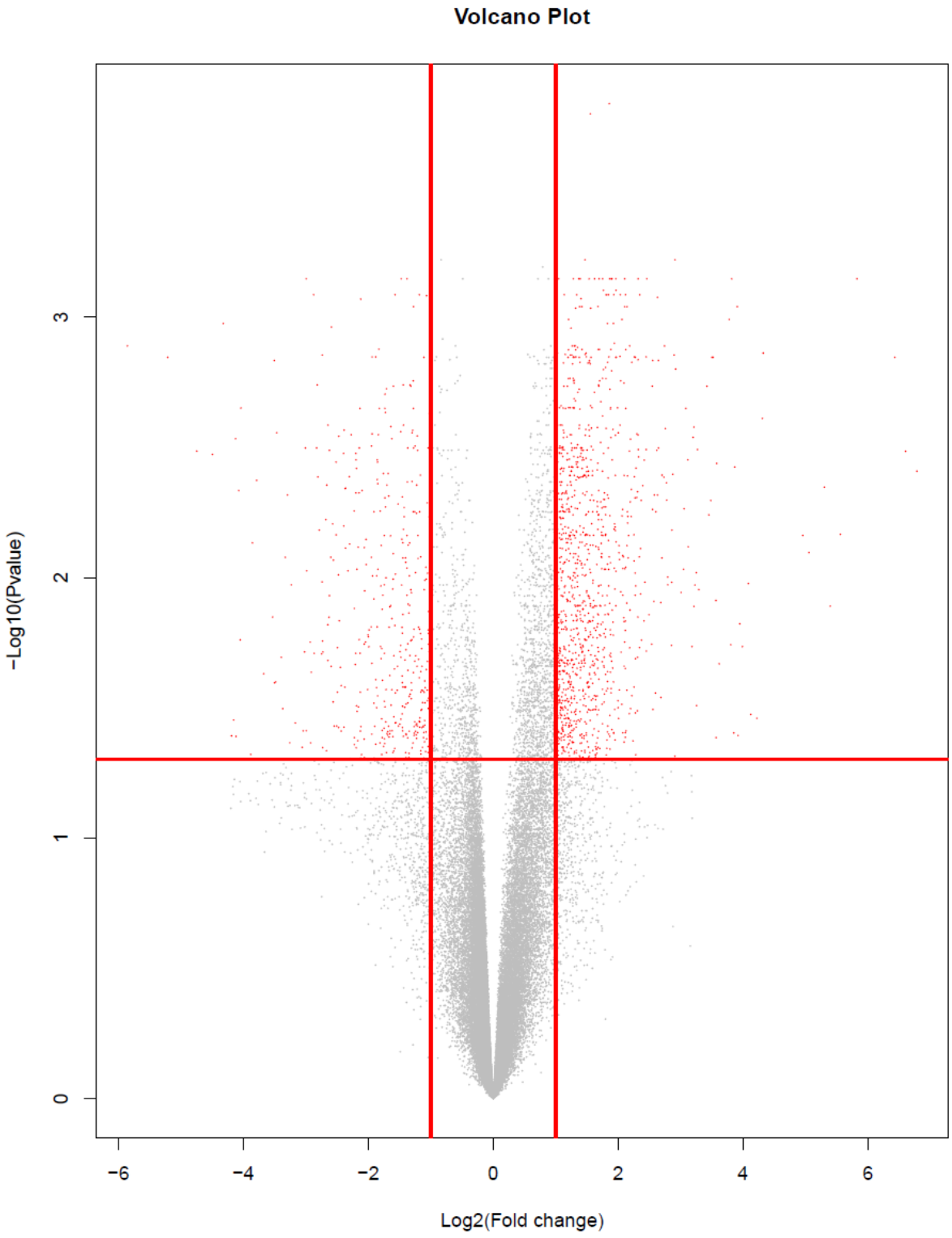

Figure 1

Volcano diagram of differential genes. A dot on the right represents a gene. The 383 red dots on the left indicates significantly down-regulated differential expression genes, and the 1,156 red dots on the right

indicate significantly up-regulated differential expression genes. Log2 (fold change)≥1 indicates up-regulation, and Log2 (Fold change)≤-1 indicates down-regulation

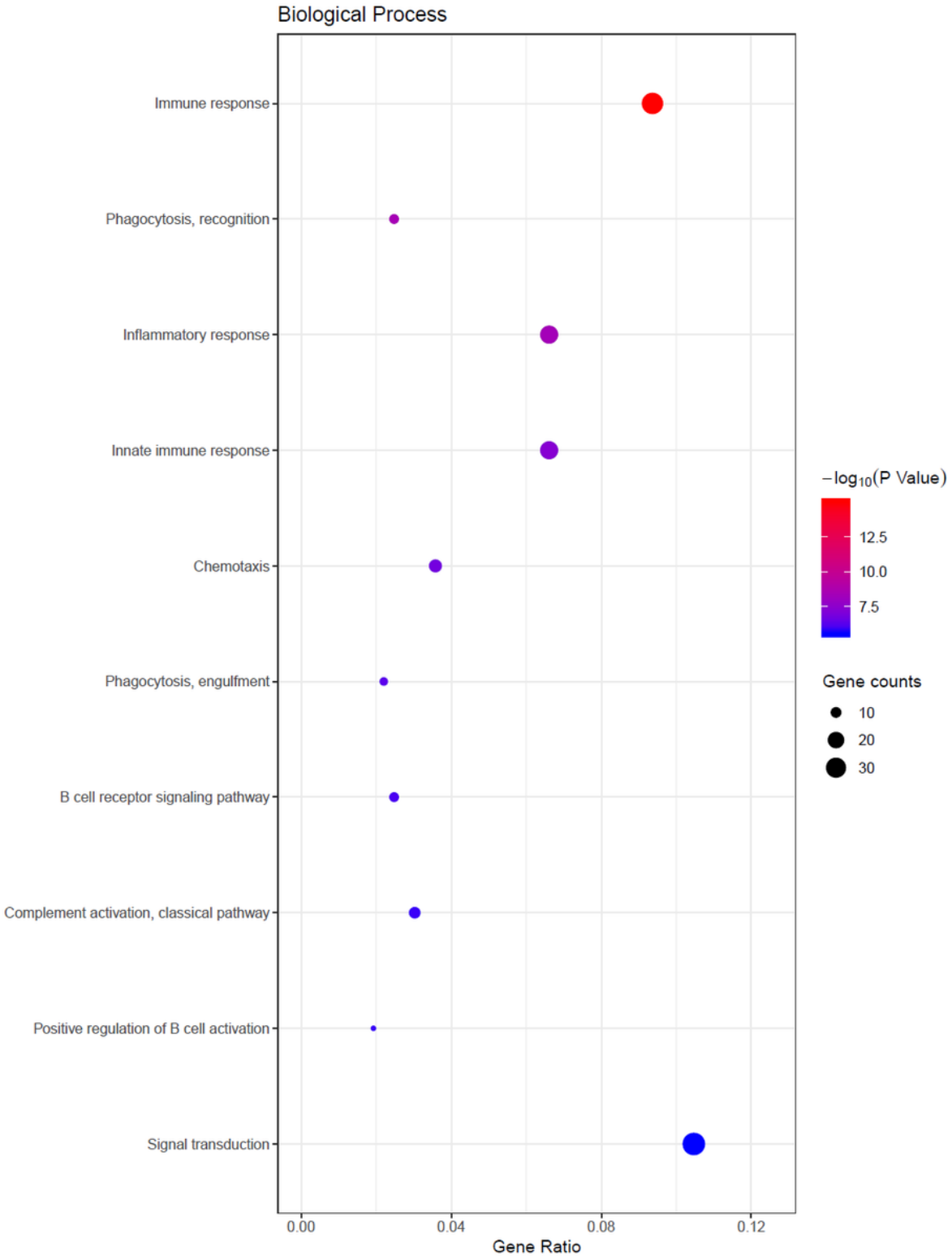

Figure 2

GO enrichment analysis of differential genes BP bubble chart. The dot size indicates the number of enriched DEGs, dot color represents the value of -log10 (P Value)

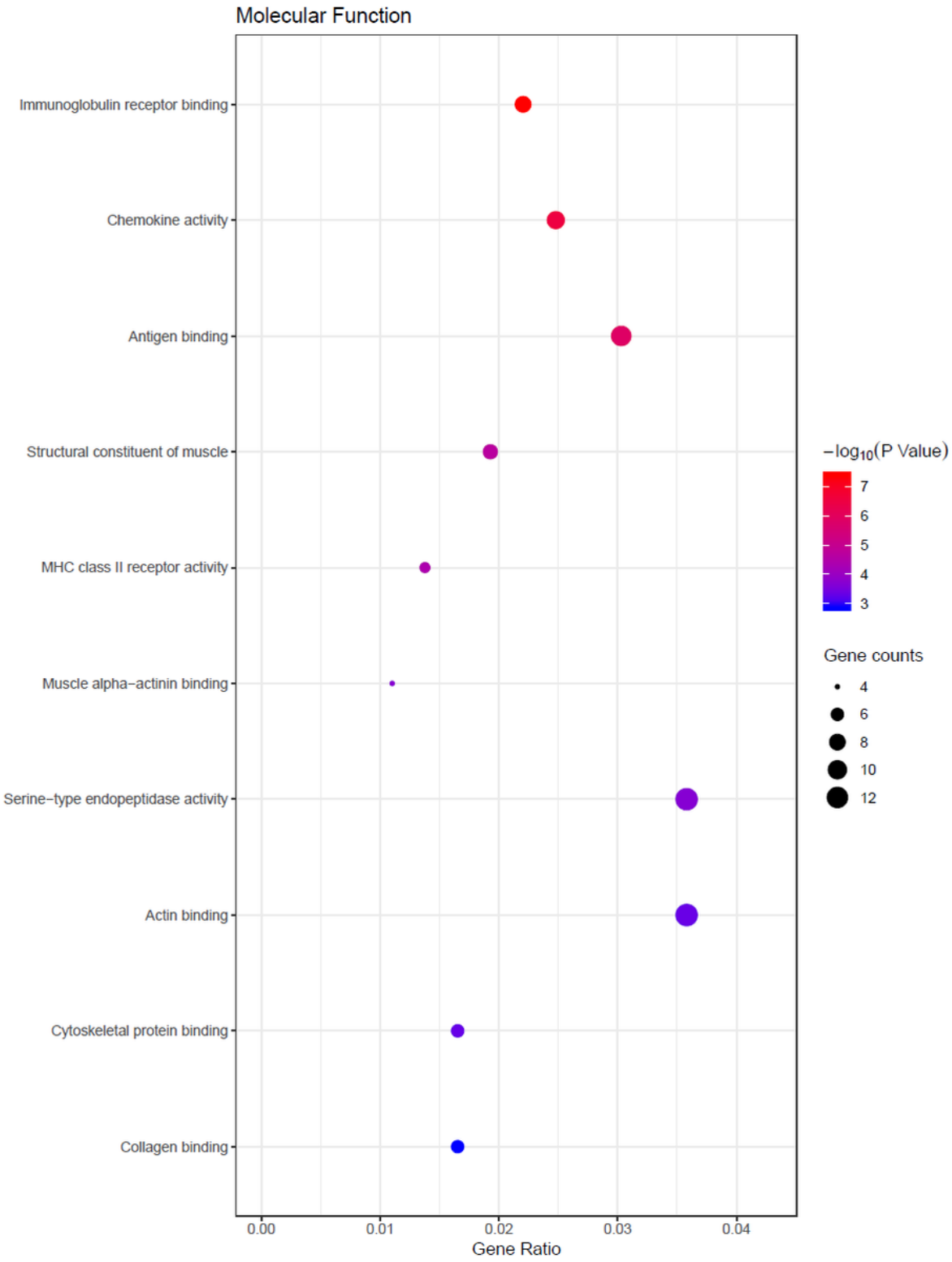

Figure 3

GO enrichment analysis of DEGs MF bubble chart. The dot size indicates the number of enriched DEGs, dot color represents the value of -log10 (P Value)

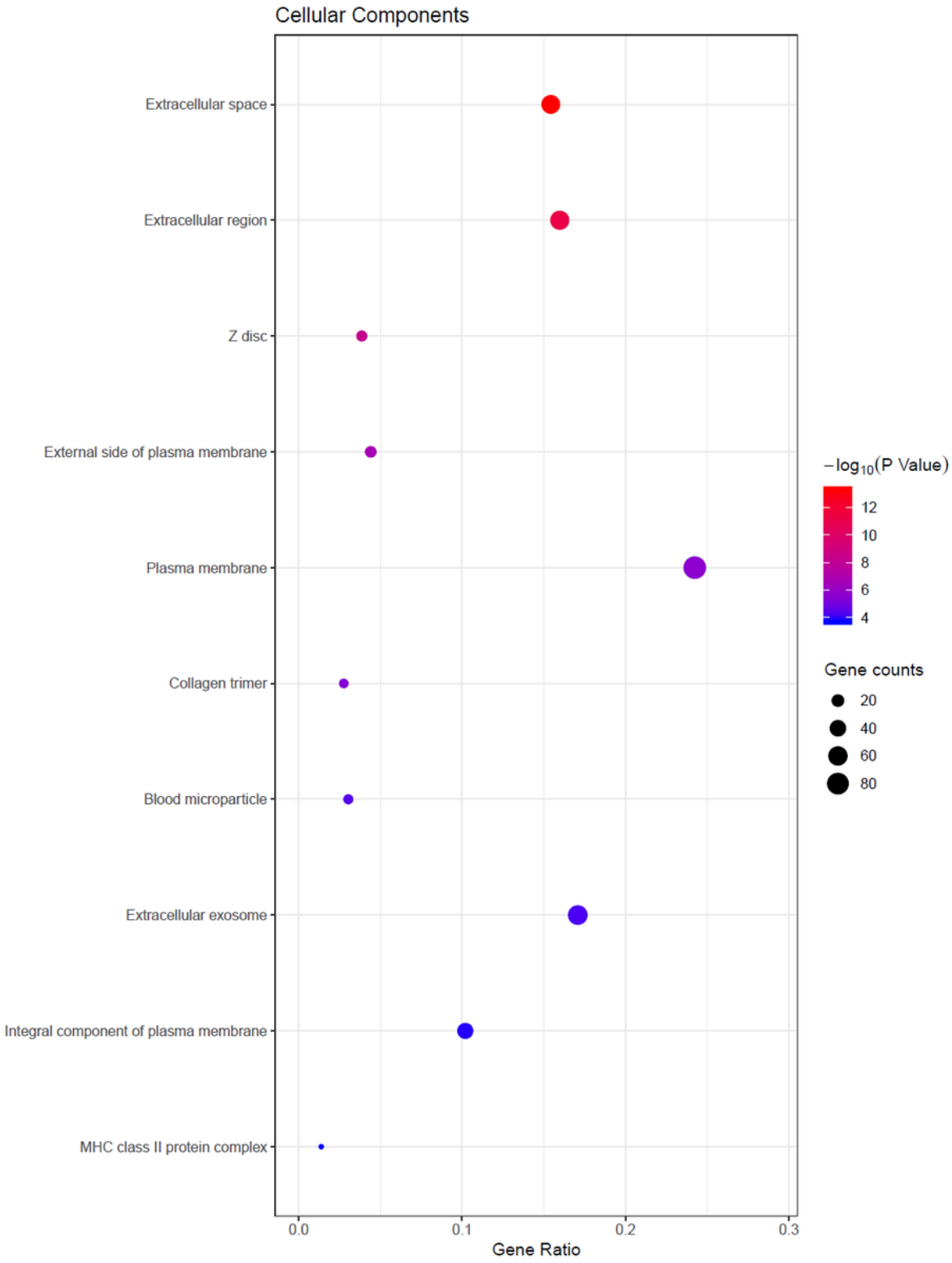

Figure 4

GO enrichment analysis of differential genes CC bubble chart. The dot size indicates the number of enriched DEGs, dot color represents the value of -log10 (P Value)

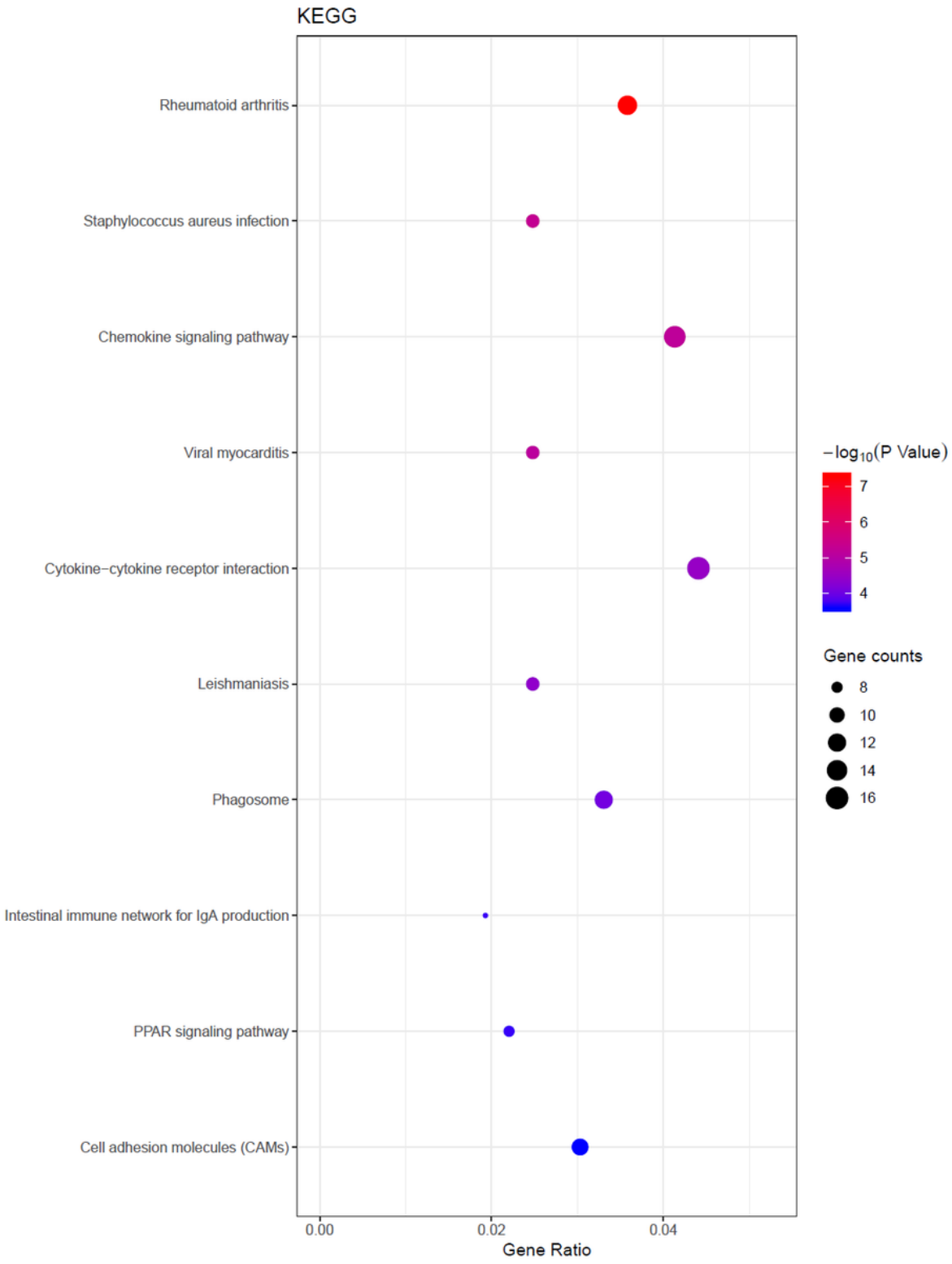

Figure 5

Bubble chart of KEGG enrichment analysis of DEGs. The dot size of the biological process indicates the number of enriched DEGs, and the dot color represents the value of-log10 (P Value)

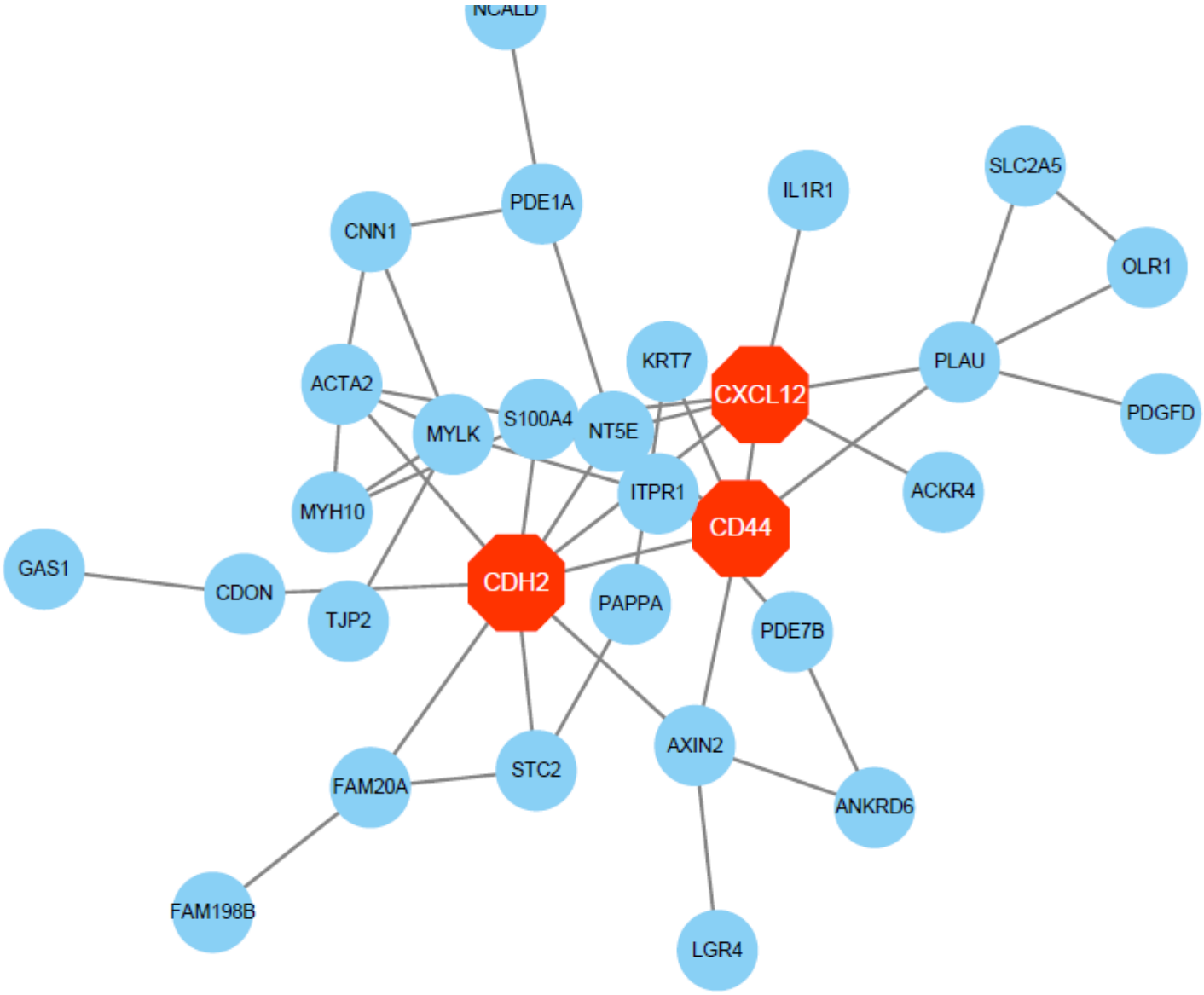

Figure 6

PPI network of Hubgene. The more connections there are, the more important the node is, and the red node is the key node

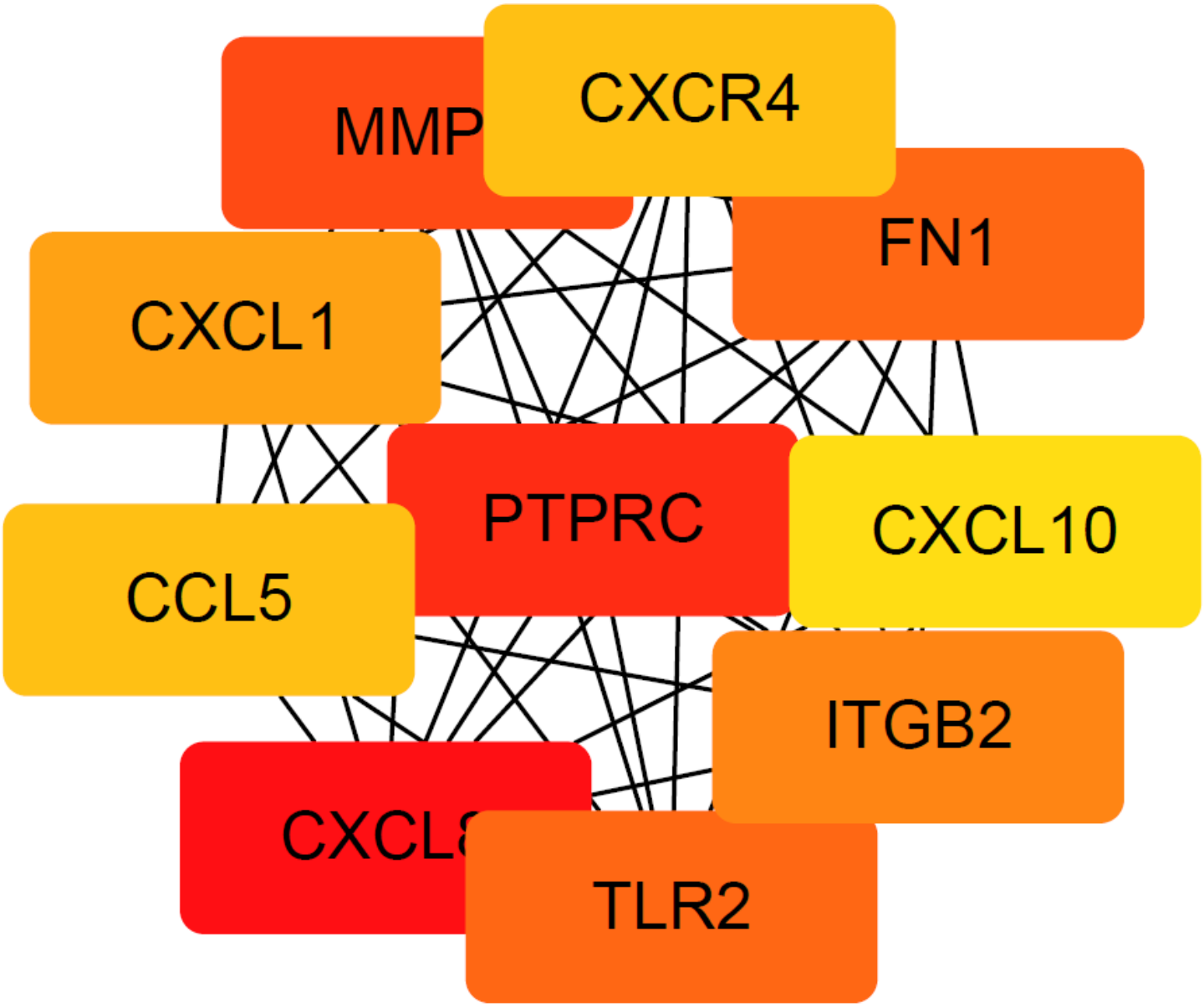

Figure 7

Interaction network of key differential genes ranked in the top 10 by Degree

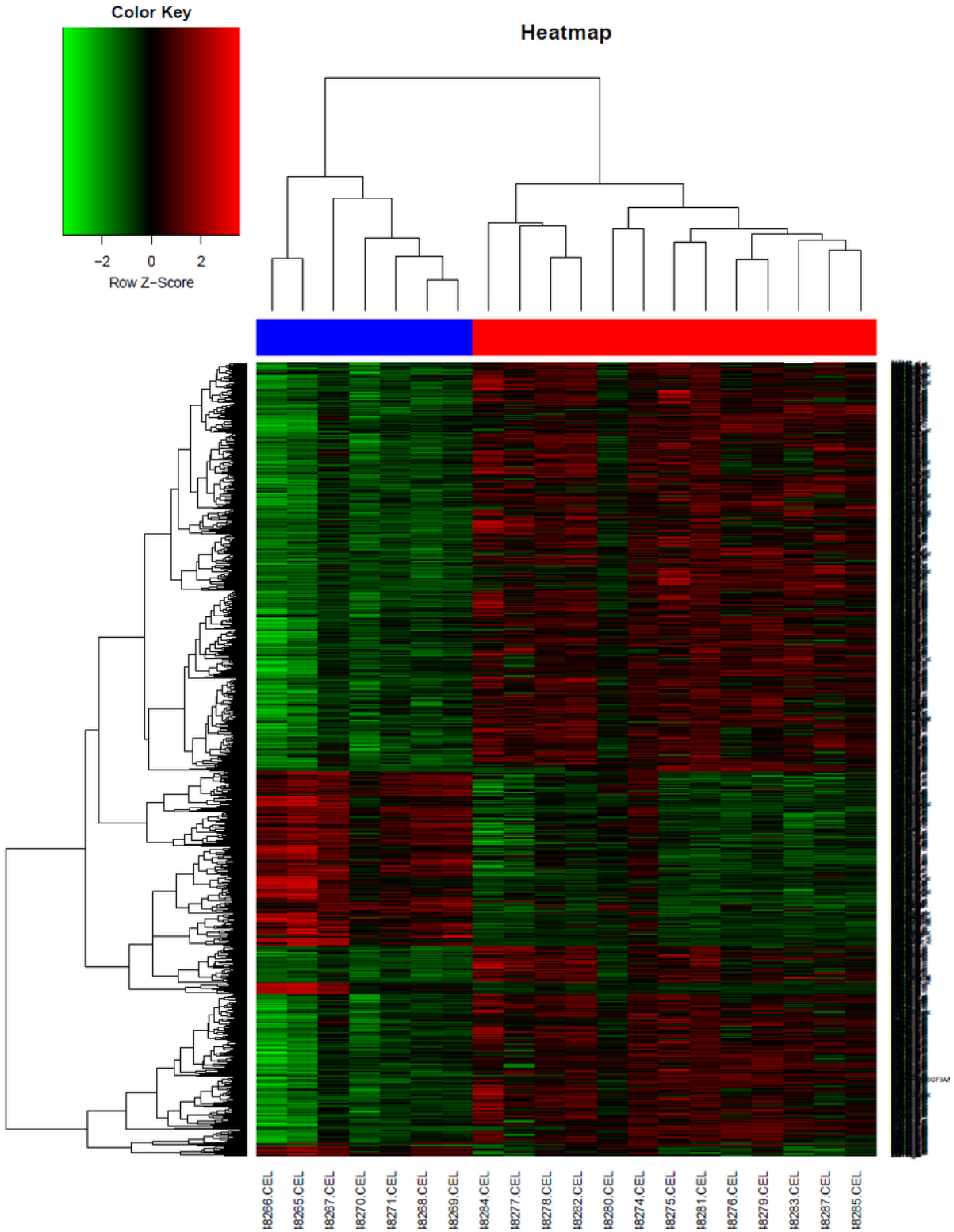

Figure 8

Heat map. Gene expression data are expressed in matrix form. Rows represent individual genes, and columns represent each sample. Green means lower expression, red means higher expression, and black means no differential expression

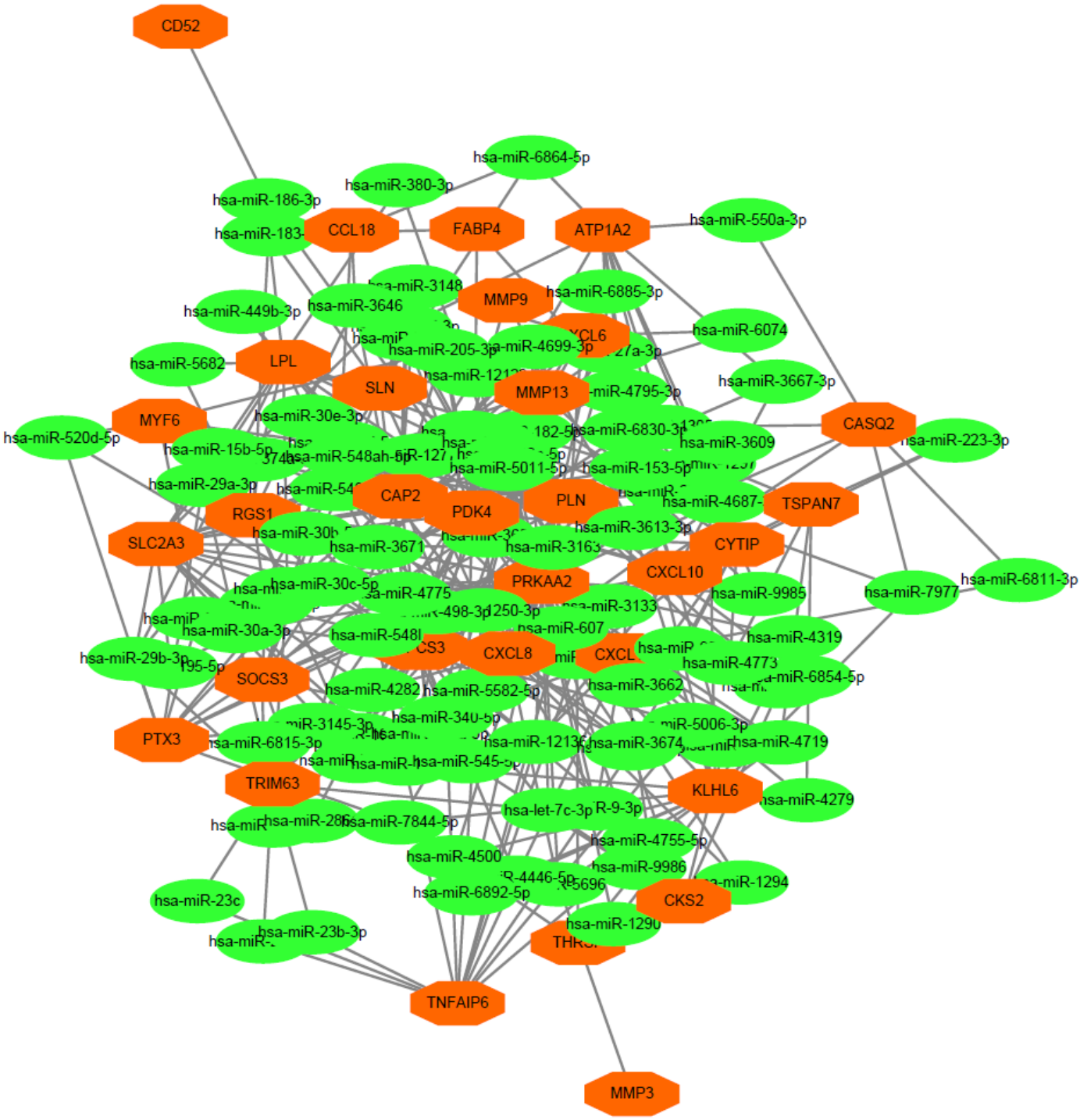

Figure 9

Mirna-mRNA regulatory network. Ellipse represents miRNA, and polygon represents mRNA