Analysis of differentially expressed genes between paroxysmal and persistent atrial fibrillation

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Abstract

Background This study aims to analyze the differentially expressed long noncoding RNAs and messenger RNAs (DELncRNAs and DEmRNAs) in patients with paroxysmal and persistent atrial fibrillation and explore the novel AF-related molecular mechanisms.

Methods Two target datasets about paroxysmal and persistent atrial fibrillation patients (GSE75092 and GSE113013) were downloaded and further analyzed from the Gene Expression Omnibus database by R software. Upregulated and downregulated differentially expressed genes (DEGs) and the co-expressed differentially expressed genes (co-DEGs) of the two datasets were identified, of which enrichment analyses and protein-protein interaction network construction were performed.

Results A total of 127 DELncRNAs and 321 DEmRNAs were screened in GSE75092; a total of 46 DELncRNAs and 64 DEmRNAs were screened in GSE113013. 8 co-DEGs were further identified in the overlap between the two datasets on Venn Diagram, which comprises 3 LncRNA and 5 mRNA. GO and KEGG analysis were successfully performed on the DEGs and co-DEGs mentioned above and the protein-protein interaction network of DEGs was constructed.

Conclusions Multiple DEGs were found in patients with either paroxysmal or persistent atrial fibrillation and their functions were mainly enriched in metabolism and inflammation-related signaling pathways. The antiviral defense mechanism of PAF is an interesting difference we found.

Introduction

Atrial fibrillation (AF), which is one of the most common arrhythmias in adults, has complex molecular mechanisms of occurrence and maintenance[1]. Paroxysmal atrial fibrillation (PAF, conversion to sinus rhythm within 7 days) and persistent atrial fibrillation (PsAF, lasting more than 7 days and needing drugs or electric shock to convert to sinus rhythm) are of greater interest to clinicians in clinical decision-making phase. Because they can benefit more from conversion to sinus rhythm compared to patients with permanent atrial fibrillation, those with failed sinus rhythm conversion or maintenance of less than 24 hours[1, 2]. However, there is a large difference in the success rate and maintenance time before recurrence between the two[3].

This difference may be related to the different degree of pathophysiological changes in the two types of AF. Although controversial, it is generally accepted that patients with PsAF may have a more extensive electrical and structural remodeling[4]. A subset of patients with PAF may naturally progress to PsAF or even permanent AF. It is a progressive disease? However, some studies have found that PsAF might spontaneously transform into the paroxysmal subtype during follow-up[5]. This highlights both the complex natural history of AF and the fact that much is unclear about the underlying molecular mechanisms and potential regulatory factors of AF.

Long noncoding RNAs (LncRNAs) play a regulatory role in a variety of diseases, including cardiovascular disease[6]. The presence of DELncRNAs in AF patients have been confirmed and some have been experimentally confirmed to regulate AF progression[7]. However, comparative studies of differentially expressed genes (DEGs) between PAF and PsAF patients are scarce. Yang et al.[8] and Sun et al.[9, 10] performed RNA sequencing of cardiac tissues from several patients with paroxysmal, persistent AF and controls, respectively, but with widely divergent results under the same screening criteria. While the information obtained from silico analysis was complicated and difficult to extract what we needed.

This study was designed to explore the DELncRNAs and differentially expressed messenger RNAs (DEmRNAs) in patients with PAF and PsAF by combining and analyzing independent experiments in datasets. The aim of this work was to unveil some of the potential regulatory factors or pathways that affect the progression of AF at genetic level.

Methods

1. Access to the Data

The Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) was searched by two reviewers with the keyword "atrial fibrillation" and 70 series were independently found. Subsequently, we excluded the series that the organisms were not homo sapiens, the subtype of AF was not mentioned and the results did not contain LncRNAs. 3 datasets were found but GSE135445 was excluded as an empty set. Finally, two target gene datasets, GSE75092[11] and GSE113013[12], about patients with PAF and PsAF were assessed until August 2021.

2. DEGs screening and Identification

The series matrix was downloaded through the "getGEO" function in the R package "GEOquery" (version 2.58.0). The annotation document of the platform was used to annotate the gene expression profile. If a gene symbol corresponded to multiple probe IDs, the maximum value of the row average was taken as its gene expression value. The raw data was first transformed by log2 respectively and then normalized through the "normalizeBetweenArray" function in the R package "LIMMA" (version 3.46.0). Then, use the "LIMMA" package to identify DEGs. The cut-off criteria were set as adjust *P* value < 0.05 and |log2 fold change| >1 (log2FC). Finally, the co-expressed DEGs (co-DEGs) between the two gene sets were identified in the overlap of Venn diagram (http://bioinformatics.psb.ugent).

3. Functional and Pathway Enrichment Analyses

The "enrichGO" function from R package “clusterProfiler” (Version 3.18.1) was used for the Gene Ontology (GO) analysis and the "enrichKEGG" function from the package was used for the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The R packages "GOplot" and "clusterProfiler"

(version 1.0.2) were used to visualize the results. An adjusted *P* value < 0.05 was the cut-off criterion of above.

4. Protein-protein Interaction (PPI) network construction

STRING (http://string-db.org) was used to construct the PPI network of DEGs. The combined confidence score of ≥ 0.4 was the cut-off value. The Cytoscape software (version 3.8.2) was used to visualize the intra-node connectivity and interaction between DEGs, and plug-in "cytoHubba" of the software was used to calculate the degree between DEGs and display the top 10 hub genes in the PPI network.

Results

1. DEGs screening and co-DEGs identification between PAF and PsAF patients

The datasets GSE75092 and GSE113013 were both collected from GPL16956 platform, using Agilent-045997 Arraystar human LncRNA microarray. The GSE75092 dataset contained peripheral blood samples and coronary sinus blood samples from three PAF patients and peripheral blood samples from three controls[13]. While the GSE113013 dataset included atrial tissue samples from 5 PsAF patients (lasting more than 2 months) with valvular heart disease, which has been checked with the author Dr. Rao by email, and 5 controls (sinus rhythm patients with valvular heart disease). The information of the above two datasets is shown in Table 1.

Table 1

Basic information of datasets used in the study

GEO accession	GSE75092	GSE113013
Platform	GPL16956	GPL16956
Diseases	PAF	PsAF
Samples	Blood leukocytes	Atrial tissue
Number of subjects	3 patients + 3 controls	5 patients + 5 controls

PAF, paroxysmal atrial fibrillation; PsAF, persistent atrial fibrillation.

In GSE75092, a total of 127 DELncRNAs were screened between PAF patients and controls, of which 88 were upregulated and 39 were downregulated (**Figure 1A-1B**); and 321 DEmRNAs were screened, of which 155 were upregulated and 206 were downregulated (**Figure 1C-1D**).

In GSE113013, a total of 46 DELncRNAs were screened between PsAF patients and controls, of which 17 were upregulated and 29 were downregulated (**Figure 2A-2B**); and 64 DEmRNAs were screened, of which 22 were upregulated and 42 were downregulated (**Figure 2C-2D**).

8 co-DEGs were further identified in the overlap between the two datasets on Venn Diagram, which comprises 3 LncRNA and 5 mRNA (**Figure 3** and Table 2). Of which 5 co-DEGs (RHOXF1, MC1R, LPL, AC009509.2, LINC02446) were consistently downregulated in both datasets, while 2 co-DEGs (TMEM44-AS1, SERPINB2) were upregulated in GSE75092 and downregulated in GSE113013, and 1 co-DEGs (MX1) was the opposite (Table 2).

Table 2

the co-DEGs between the two datasets

Gene symbol	GSE75092		GSE113013		Gene type
	logFC	*P*.adjust	logFC	*P*.adjust	
RHOXF1	-3.8949	0.0000	-1.3104	0.0361	mRNA
MC1R	-3.0642	0.0002	-3.4786	0.0000	mRNA
AC009509.2	-4.4094	0.0023	-5.3268	0.0002	LncRNA
LINC02446	-1.6999	0.0164	-1.4054	0.0029	LncRNA
LPL	-1.8276	0.0189	-1.9628	0.0128	mRNA
TMEM44-AS1	1.0474	0.0251	-1.1192	0.0377	LncRNA
SERPINB2	1.3521	0.0433	-1.7661	0.0299	mRNA
MX1	-2.4066	0.0482	2.9639	0.0348	mRNA

2. Enrichment analyses of DEGs between PAF and PsAF patients

The GO analyses of the above DEGs were performed on three critical categories, biological process (BP), molecular function (MF), and cellular component (CC). The results of GSE75092-DEGs were shown in **Figure 4**. In the BP category, the top 5 were mainly enriched in response to virus ($P < 0.001$), positive regulation of cytokine production ($P < 0.001$), defense response to virus ($P < 0.001$), nuclear transport ($P = 0.004$) and nucleocytoplasmic transport ($P = 0.009$). The CC category was mainly enriched in focal adhesion ($P = 0.042$) and cell-substrate junction ($P = 0.042$). The GO analysis of GSE113013-DEGs showed that no significant functions were enriched in any category.

KEGG analysis was performed for the GSE75092-DEGs, which were mainly enriched in hepatitis C (P = 0.003), influenza A (P = 0.005), NOD-like receptor signaling pathway (P = 0.008), Epstein-Barr virus infection (P = 0.014), measles (P = 0.016), and fluid shear stress and atherosclerosis (P = 0.016). The results were shown in **Figure 5A**. And the results of GSE113013 were mainly enriched in dopaminergic synapse (P = 0.003), antigen processing and presentation (P = 0.005), circadian entrainment (P = 0.008), melanogenesis (P = 0.014), vascular smooth muscle contraction (P = 0.016), and relaxin signaling pathway (P = 0.016). The results were shown in **Figure 5B**. There were two identical pathways, influenza A and adrenergic signaling in cardiomyocytes, in KEGG analysis of the DEGs between the two datasets.

The GO analysis was also performed on the 8 co-DEGs. It was found that the co-DEGs were mainly enriched in triglyceride biosynthetic process (P = 0.046), interleukin-12-mediated signaling pathway (P = 0.046), negative regulation of blood coagulation (P = 0.046), positive regulation of interleukin-1 beta production (P = 0.046), negative regulation of coagulation (P = 0.046) and positive regulation of interleukin-1 production (P = 0.047) for the BP category. For the CC category, they were mainly enriched in very-low-density lipoprotein particle (P = 0.018), triglyceride-rich plasma lipoprotein particle (P = 0.018), plasma lipoprotein particle (P = 0.022), lipoprotein particle (P = 0.022) and protein-lipid complex (P = 0.022). And for the MF category, it was found to be mainly enriched in phospholipase A1 activity (P = 0.035), apolipoprotein binding (P = 0.035), triglyceride lipase activity (P = 0.037), lipoprotein particle binding (P = 0.037) and protein-lipid complex binding (P = 0.037). KEGG analysis of the 8 co-DEGs was enriched in cholesterol metabolism (P = 0.024), glycerolipid metabolism (P = 0.030), peroxisome proliferators-activated receptor (PPAR) signaling pathway (P = 0.037), complement and coagulation cascades (P = 0.041) and melanogenesis (P = 0.049). The details are shown in **Figure 6**.

3. PPI network of the DEGs

The PPI analysis of the DEGs among two datasets was constructed using STRING database. Finally, an interaction network of GSE75092-DEGs with 292 nodes and 757 edges, and an interaction network of GSE113013-DEGs with 30 nodes and 32 edges were established in Cytoscape, as shown in **Figure 7**.

According to the node degree calculated by the cytoHubba, the top 10 hub genes of GSE75092-DEGs and GSE113013-DEGs were screened out and visualized in **Figure 8**, respectively. These top down-regulated hub nodes (proteins) called interferon-induced helicase C domain-containing protein 1 (IFIH1, degree = 30), ISG15 ubiquitin-like modifier (ISG15, degree = 29), interferon-induced GTP-binding protein Mx1 (MX1, degree = 27), interferon-induced protein with tetratricopeptide repeats 1 (IFIT1, degree = 25), interferon-induced protein with tetratricopeptide repeats 3 (IFIT3, degree = 21), 2'-5'-oligoadenylate synthase 2 (OAS2, degree = 21), interferon alpha inducible protein 6 (IFI6, degree = 21), interferon-induced protein with tetratricopeptide repeats 2 (IFIT2, degree = 21), 2'-5'-oligoadenylate synthase 3 (OAS3, degree = 21) and Radical S-adenosyl methionine domain-containing protein 2 (RSAD2, degree = 21) were regarded as hub genes in relation to GSE75092-DEGs (**Figure 8A**). Moreover, these top down-regulated hub nodes (proteins) called cathepsin G (CTSG, degree = 4), cystatin-F (CST7, degree = 4), granzyme K (GZMK,

degree = 4), granzyme A (GZMA, degree = 4), killer cell lectin-like receptor subfamily K (KLRK1, degree = 4), protein S100-A12 (S100A12, degree = 4), calmodulin 2 (CALM2, degree = 3), and granzyme H (GZMH, degree = 3), and the top up-regulated hub node (protein) called adenylate cyclase type 2 (ADCY2, degree = 3) and guanine nucleotide-binding protein G(o) subunit alpha (GNAO1, degree = 3) were considered as top 10 hub genes related to GSE113013-DEGs (**Figure 8B**).

Discussion

Before discussion, we must first talk about the shortcoming of the data. In this study, we analyzed the DEGs of two independent datasets of PAF and PsAF, and identified the intersection of the two datasets as co-DEGs. Before comparing the similarities and differences between the DEGs, let's pay attention to the differences of the sample sources. The samples of PAF (GSE75092) were blood and the RNAs were extracted from leukocytes in the blood. While the samples of patients with PsAF (GSE113013) were atrial tissue. Dr. Zheng had performed a similar analysis[14]. This difference makes us cautious in drawing conclusions, so the same and different results will be discussed separately.

Both types of AF have lots of DEGs (DELncRNAs and DEmRNAs) compared with the controls, which are either upregulated or downregulated. Subsequently, the gene intersection of patients with PAF and PsAF showed that there was a total of 8 co-DEGs (Table 2). Among them, 5 co-DEGs showed a consistent downward trend in both datasets, which means that the expression levels of these 5 co-DEGs were consistently downregulated in two types of AF patients whether in blood or tissue. The result is quite understandable.

And 3 co-DEGs showed opposite expression trends, which may be a bit difficult to explain. One possibility was due to the difference caused by the different types of AF. That is, these co-DEGs expressed different trends in patients with paroxysmal and persistent atrial fibrillation. The LncRNA TMEM44-AS1 (stimulating tumor cell proliferation, colony formation, migration and invasion[15]) and mRNA SERPINB2 (related to stem cell aging and toxicity[16]) were upregulated in the blood of PAF patients, and down in the tissues of PsAF patients. We assume that it may be the result or regulation factor of cell activity in different stages of atrial fibrillation.

Additionally, it was found that LncRNA GAS5 was consistently downregulated in both blood and atrial tissue in independent studies published on LncRNA expression in AF patients[17, 18], while LncRNA NEAT1 was found to be upregulated in atrial tissue of AF patients[19], but no significant difference was observed in blood[17].This provides another possible explanation that some AF-associated LncRNAs are differentially expressed in tissues and blood. This expression feature is not an individual case. Researchers[20] summarized the differentially expressed levels of microRNAs in the tissues and blood of AF patients and found that the different expression levels of microRNAs in the tissues did not necessarily lead to different levels in the blood, and vice versa. According to our assumption, the downregulation of mRNA MX1 in blood and upregulation in tissues of AF patients, which was reported to be upregulated in cardiac fibroblasts of congenital heart block[21], were assumed to be due to the active expression retained

in the cells or over-utilization from the blood. Since none of these 3 co-DEGs has been reported directly in AF, further studies are needed to confirm their exact role in AF.

Next, we further analyzed the functional enrichment analyses of the DEGs. In biological process, it was mainly enriched in response to virus, positive regulation of cytokine production and transportation of nuclear and nucleocytoplasmic. The cellular component was mainly enriched in focal adhesion and cell-substrate junction. While no significant functions were enriched in the GO analysis of the PsAF-DEGs. The common KEGG pathways of two types AF patients were Influenza A and Adrenergic signaling in cardiomyocytes, in the analysis of the DEGs between the two datasets.

Interestingly, some AF patients have been found to be caused by or related to viral infection. Higher levels of inflammatory factors were observed in cases of AF disclosed during acute viral infection compared with other patients who did not develop AF[22]. Chronic herpes simplex virus infection[23] and chronic hepatitis C virus (HCV) infection[24] may promote the occurrence of AF though the activation of inflammatory-associated pathways, which leading to the electrophysiology, structure and autonomic remodeling of the atrium. A myocardial biopsy found that 43.8% of isolated PsAF patients had persistent virus[25]. Can myocardial inflammation fully explain the enrichment of antiviral response? Not really. A case report of a novel coronavirus patient with new-onset AF combined with conduction block was found without myocardial inflammation (no increased troponin or echocardiographic damage was found)[26]. It showed that the virus can preferentially attack the cardiac conduction system. This study found that PAF patients also had an interesting functional enrichment in response to the virus. A hypothesis is proposed that PAF is directly related to the heart damage caused by viruses. It expects collaborative exploration by virologists and clinicians.

The GO analysis of the 8 co-DEGs showed that it was mainly related to the function of lipid metabolism, inflammatory factor regulation and blood coagulation, and so did the signal pathways of KEGG analysis. Their relationships with AF have been well recognized as the proinflammatory and proarrhythmic role of epicardial fat was again identified in AF[27].

No common hub genes were found between the PPI modules of the two datasets (Fig. 7 and Fig. 8). IFIH1, encoding melanoma differentiation-associated protein 5 (MDA5)[28], was the hub gene with the highest degrees in PAF-related modules. MDA5 is an important component of the innate immune response, which triggers a pro-inflammatory response including interferon after sensing viral RNAs[28]. ISG15 encodes a ubiquitin-like protein, which is described to have several biological functions including antiviral activity during viral infections[29]. The gene MX1 mentioned above has a similar antiviral effect. A guanosine triphosphate (GTP) metabolic protein is encoded by MX1 and has an interferon-induced antagonistic effect on the RNA or DNA replication process of viruses[30]. Other genes (IFIT1, IFIT2, IFIT3, IFI6, RSAD2, OAS2 and OAS3[31]) encode similar interferon-inducible proteins that have antiviral effects. The functions of these hub genes again verified the activation of antiviral defense mechanism in PAF

patients. Additionally, a reliable non-invasive diagnosis of viral infections can be seen by detecting the expression of relevant mRNA or LncRNA.

Figure 8B shows the hub genes of the PPI network in PsAF patients in two separated modules. The protease encoded by CTSG may be involved in the antimicrobial process and the remodeling process at the inflammation site[32]. The pathological remodeling of atrial structure is a prominent feature of atrial fibrillation. Cystatin F encoded by CST7 is found to be expressed in steady natural killer cells (NK) and CD8 + T cells and specific expressed in neutrophils during acute inflammation. The proteins that are encoded by GZMK, GZMA and GZMH are NK and T cell-specific serine protease, which also participate in the antibacterial process and regulate inflammation[33, 34]. The ligand-receptor interaction and calcium-dependent lectins of the KLRK1 encoding protein lead to the activation of NK and T cells[35]. S100A12 encodes a member of the S100 calcium-binding proteins, that is involved in the pro-inflammatory response of the innate immune and autoimmune systems[36]. These hub genes have common features relating to inflammation.

Another separated module of PsAF includes gene CALM2, ADCY2 and GNAO1, which are all calmodulin-related encoding genes[37]. The mutation of calmodulin encoding gene can cause severe arrhythmia[37].

Conclusions

Patients with either paroxysmal or persistent atrial fibrillation had common DEGs, and their functions were mainly enriched in metabolism and inflammation-related signaling pathways. The selected top hub genes of PAF patients were closely related to the antiviral process, and these of PsAF patients were related to the pro-inflammatory process. Although they were all inflammation-associated, the antiviral defense mechanism of PAF is an interesting difference we found.

Abbreviations

AF: atrial fibrillation; PAF: paroxysmal atrial fibrillation; PsAF: persistent atrial fibrillation; LncRNAs: long noncoding RNAs; DELncRNAs: differentially expressed long noncoding RNAs; DEGs: differentially expressed genes; DEmRNAs: differentially expressed messenger RNAs; GEO: Gene Expression Omnibus; Co-DEGs: Co-expressed differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; mRNA: messenger RNAs; BP: biological process; MF: molecular function; CC: cellular component; HCV: chronic hepatitis C virus; MDA5: melanoma differentiation-associated protein 5; GTP: guanosine triphosphate; NK: natural killer cells.

Declarations

Author contributions

All authors contributed to the study conception and design. WW and ZN proposed the design, WW performed the data collection and first draft of the manuscript, YL and TF analyzed the data, BT processed the images, XL review and editing the manuscript. All authors reviewed the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The website of GEO database: https://www.ncbi.nlm.nih.gov/geo/.

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Footnote

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figures

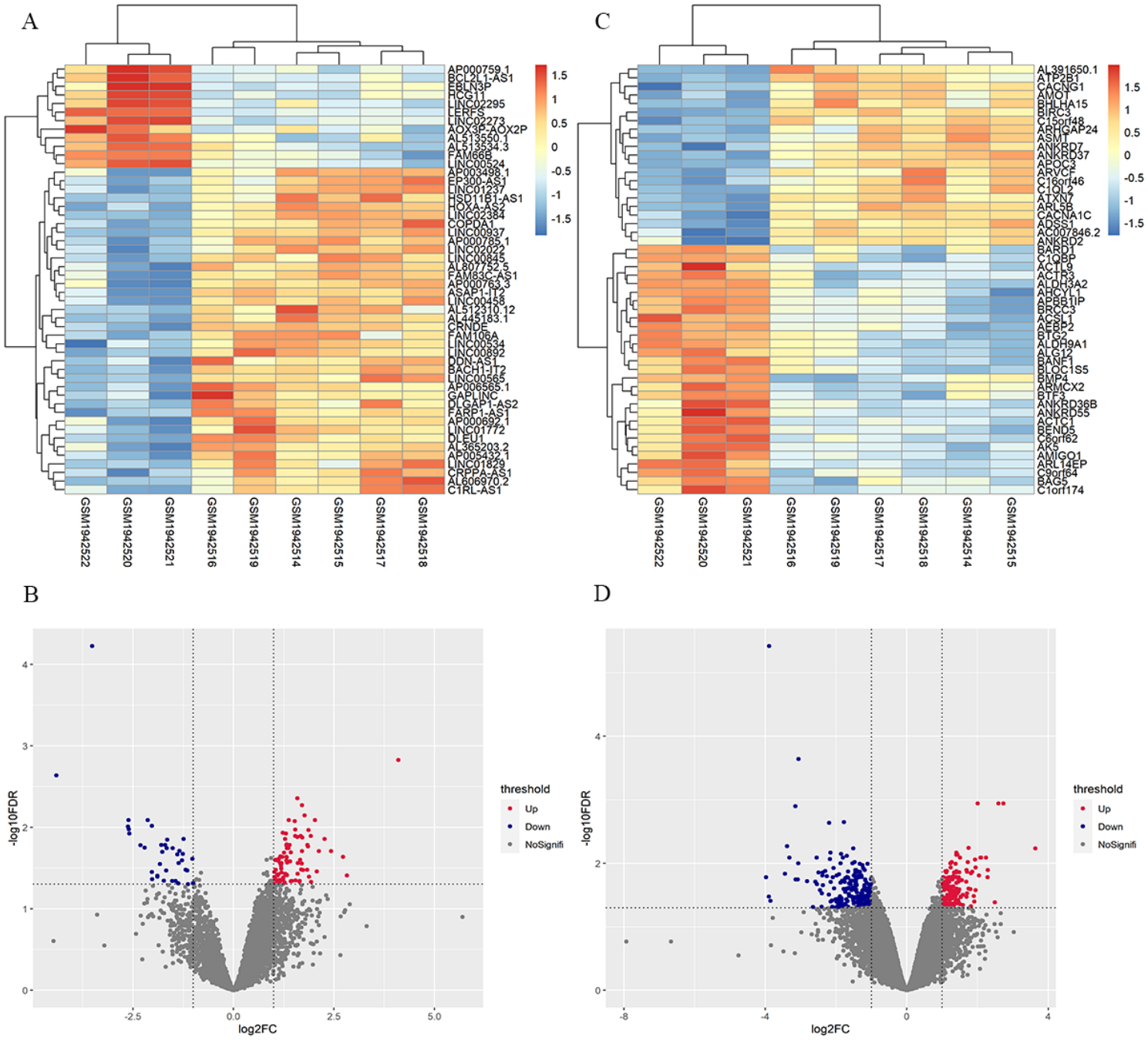

Figure 1

Heatmap and volcano plot of GSE75092-DEGs

A and B are DELncRNAs, C and D are DEmRNAs. A, heatmap of the top 50 DELncRNAs. B, volcano map of DELncRNAs. C, heatmap of the top 50 DEmRNAs. D, volcano plot of DEmRNAs. Red indicates upregulated and blue indicates downregulated.

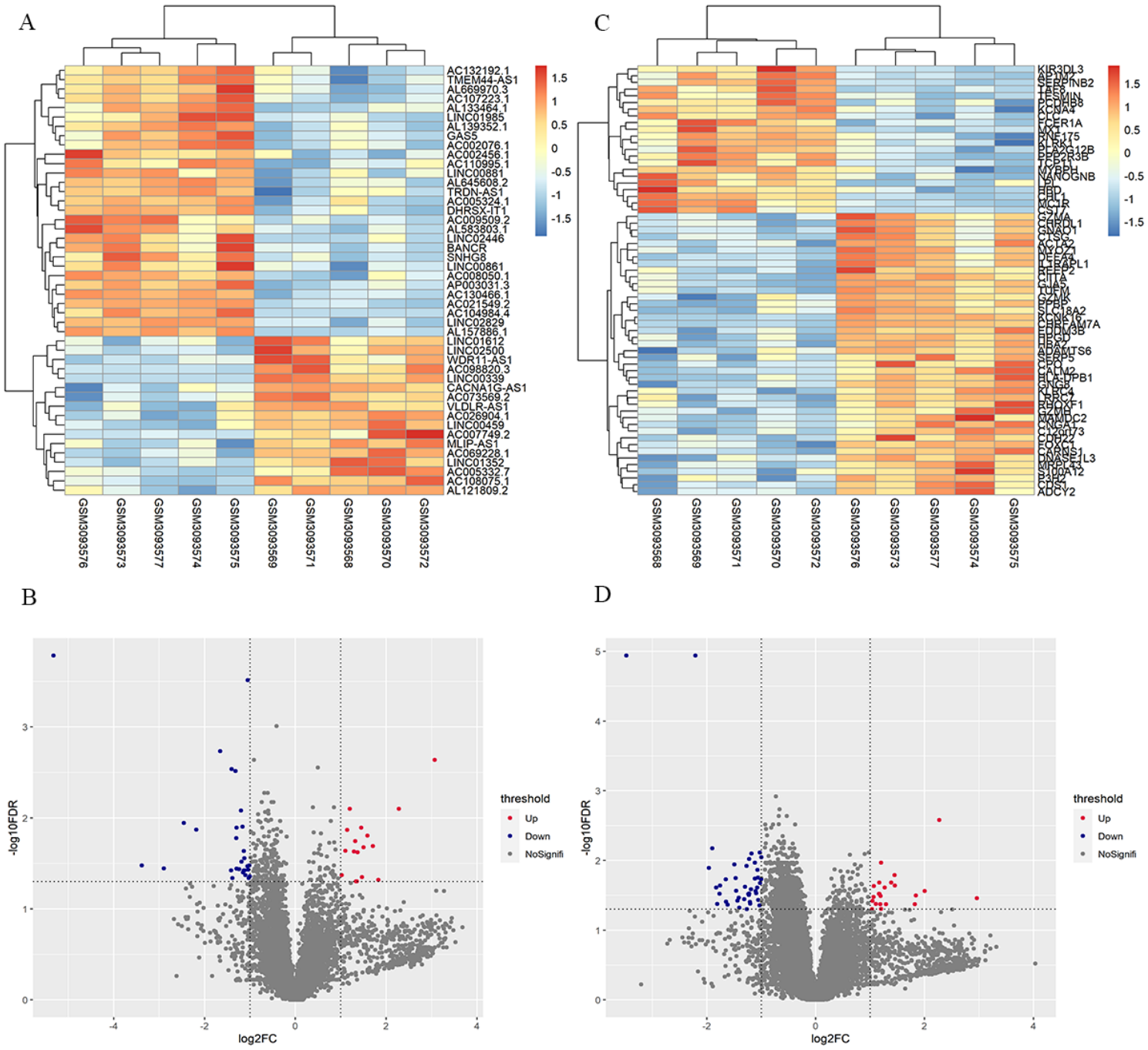

Figure 2

Heatmap and volcano plot of GSE113013-DEGs

A and B are DELncRNAs, C and D are DEmRNAs. A, heatmap of DELncRNAs. B, volcano map of DELncRNAS. C, heatmap of DEmRNAs. D, volcano plot of DEmRNAS. Red indicates upregulated and blue indicates downregulated.

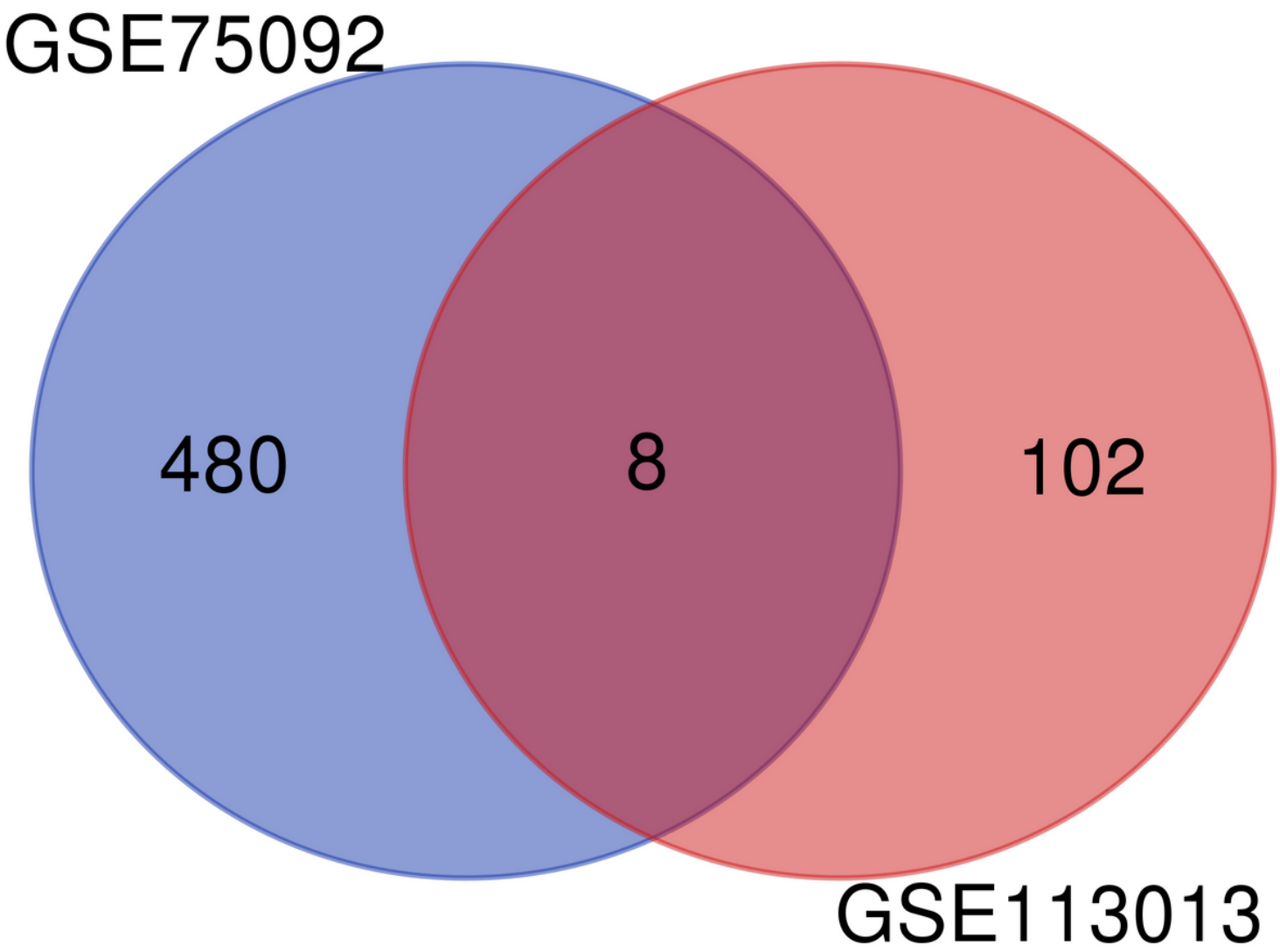

Figure 3

The Venn diagram of the co-DEGs between the two datasets

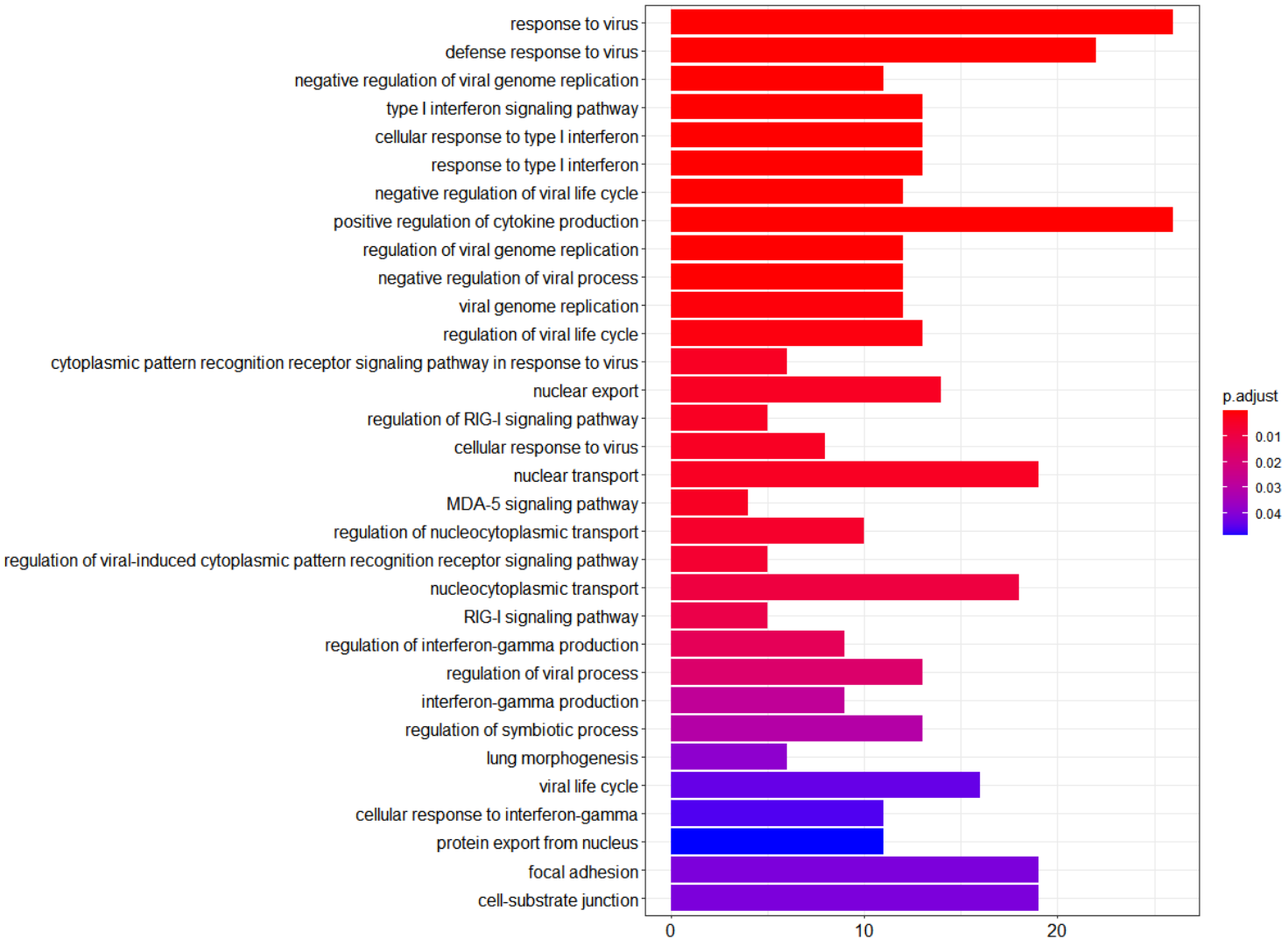

Figure 4

GO analysis of DEGs in GSE75092

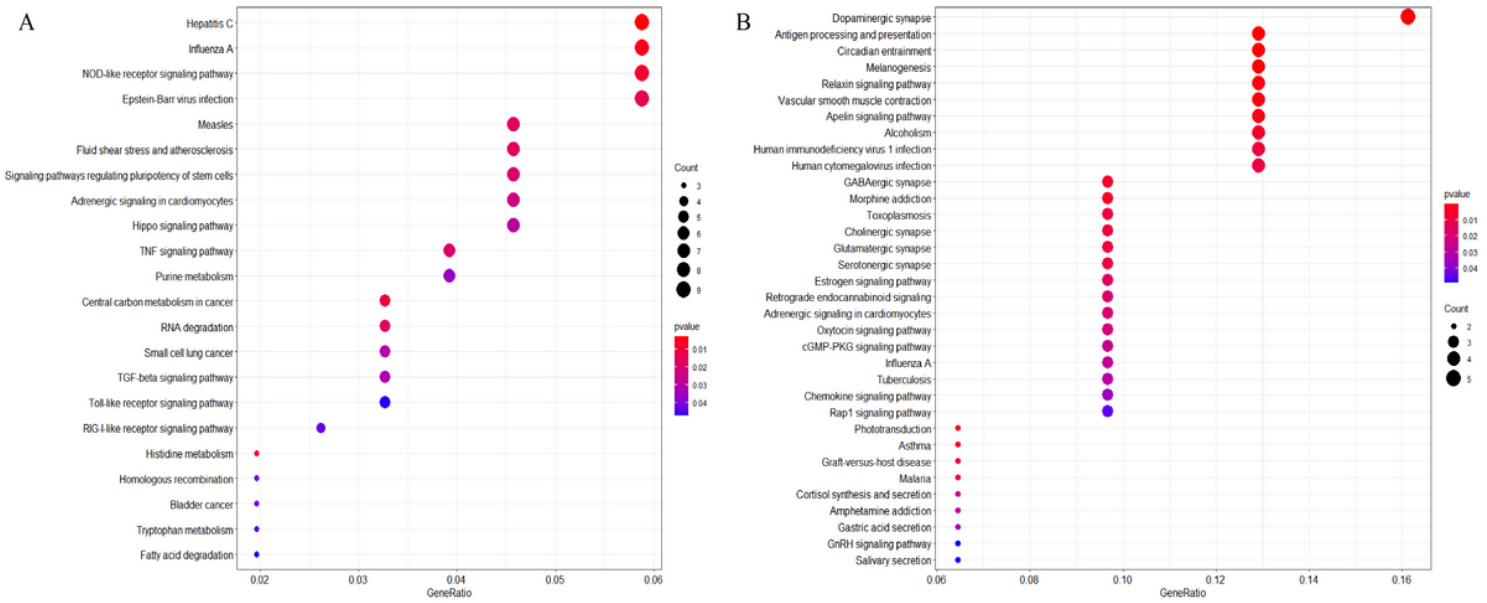

Figure 5

KEGG analysis of the DEGs between the two datasets

A, KEGG analysis of GSE75092-DEGs. B, KEGG analysis of GSE113013-DEGs.

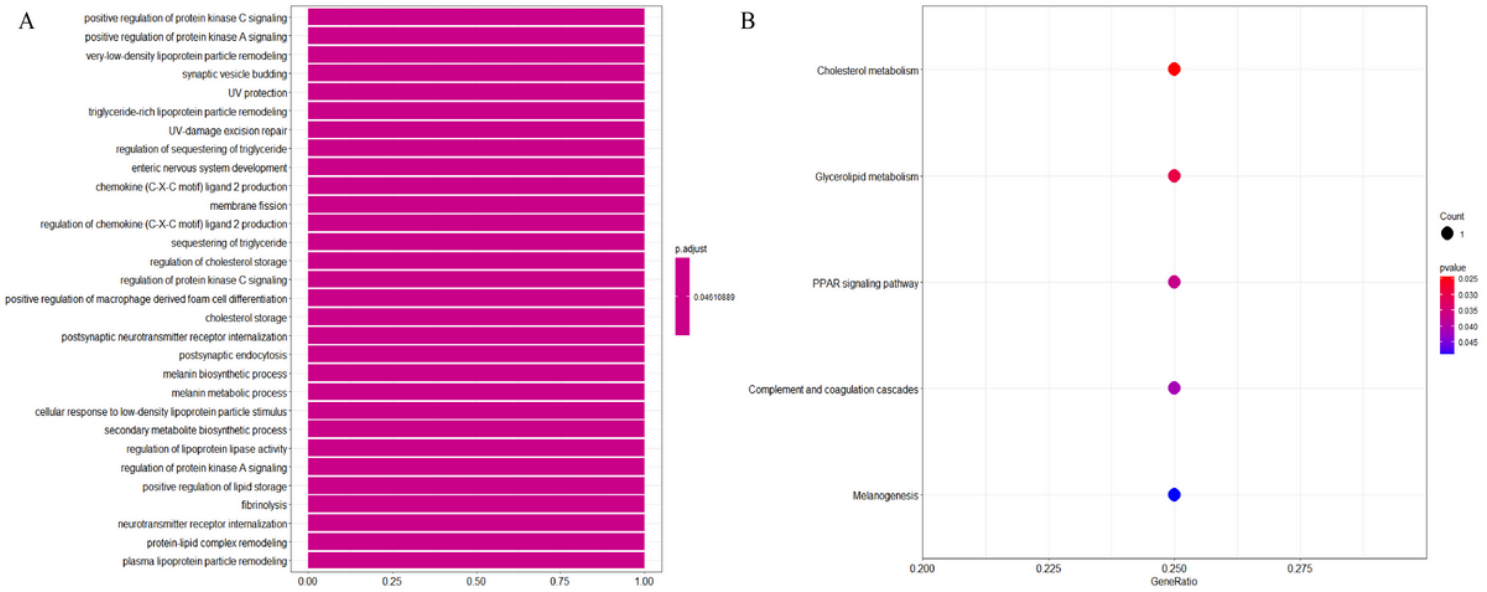

Figure 6

Enrichment analyses of the co-DEGs

A, the top 30 terms of GO analysis for the co-DEGs. B, KEGG analysis of the co-DEGs.

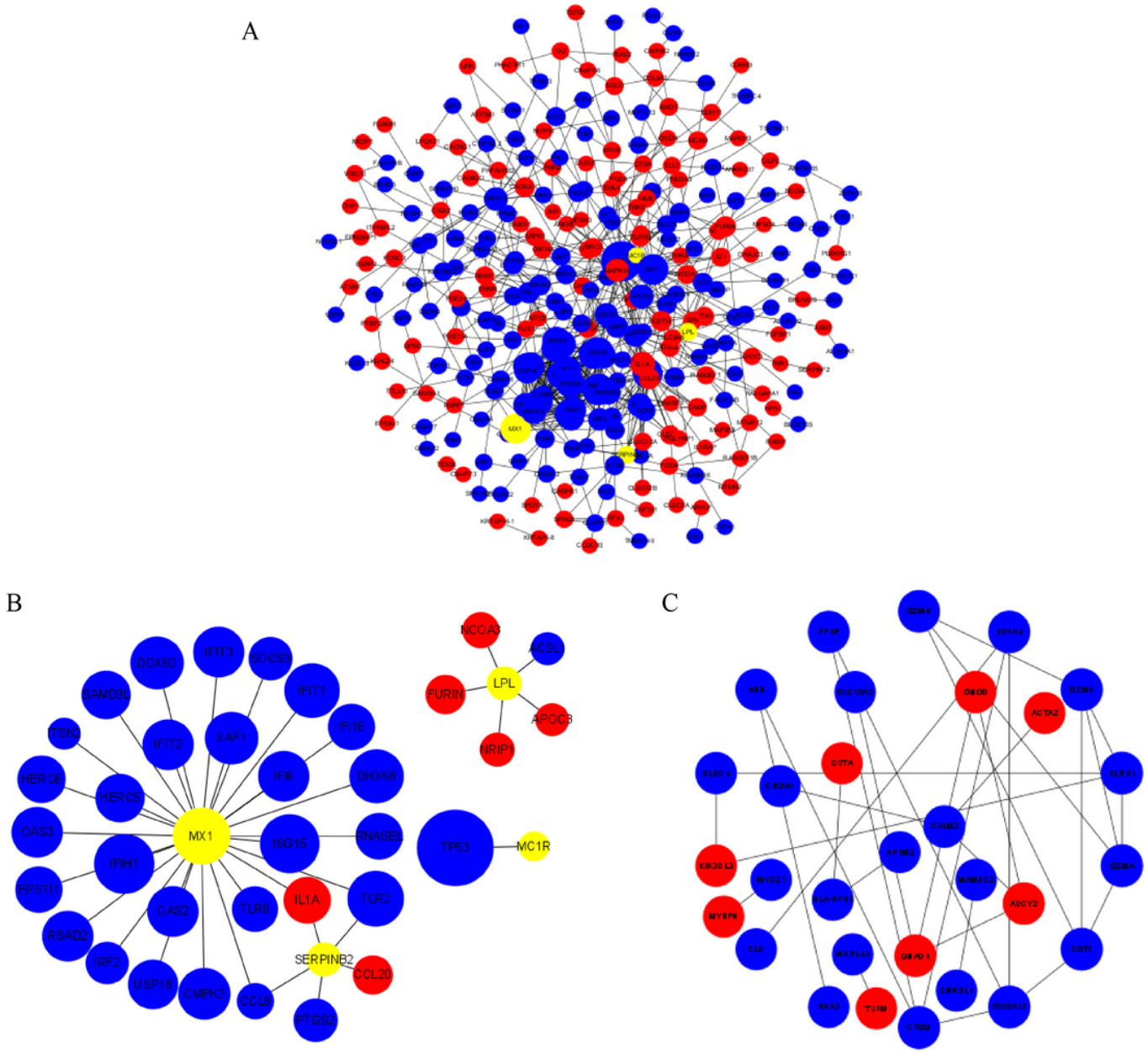

Figure 7

PPI networks for the modules among GSE75092-DEGs and GSE113013-DEGs, respectively (threshold > 0.4)

A, the overall network of the GSE75092-DEGs. B, derived modules from the overall network of the GSE75092-DEGs. C, the overall network of the GSE113013-DEGs. Red: upregulated genes, blue: downregulated genes, and yellow: 8 co-DEGs. Nodes: proteins, lines: interactions (edges) between DEGs.

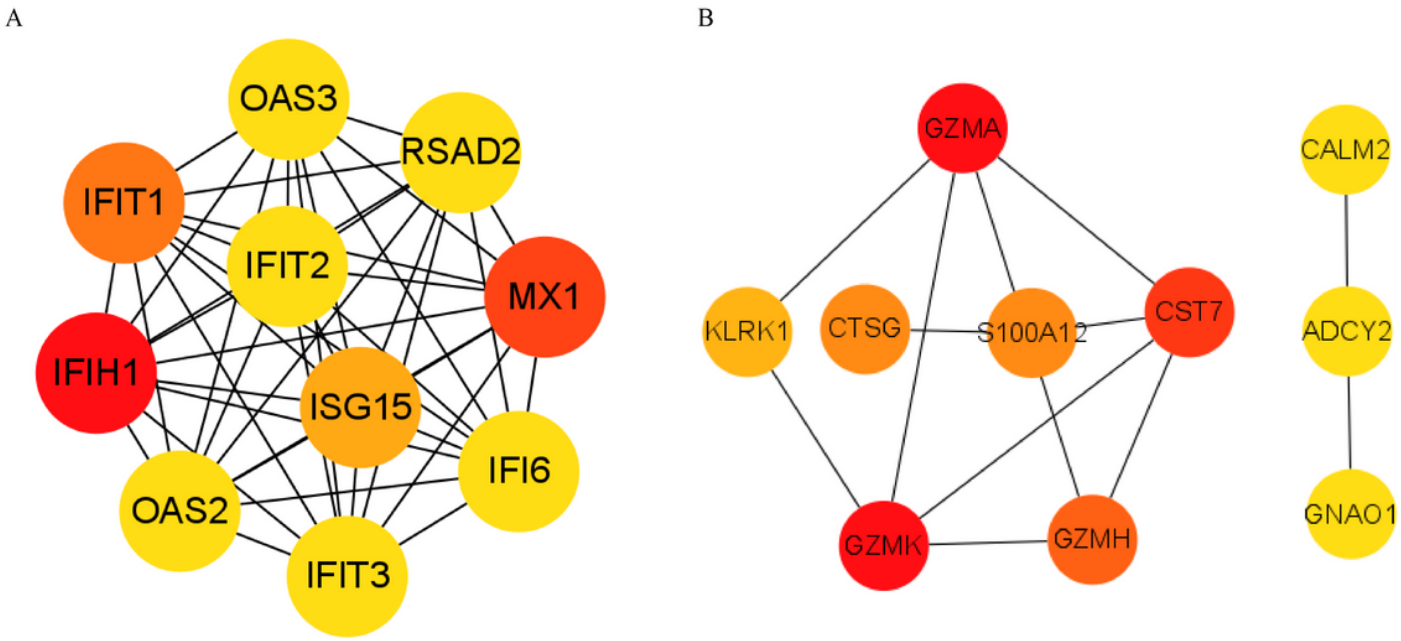

Figure 8

The top 10 hub genes of the significantly upregulated and downregulated genes for GSE75092-DEGs and GSE113013-DEGs in the PPI networks, respectively

A, The top 10 hub genes of the PPI network in GSE75092. B, The top 10 hub genes of the PPI network in GSE113013. Color depth represents degree, dark color represents higher degree.