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Analysis of genes associated with prognosis of lung adenocarcinoma based on GEO and TCGA databases

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Abstract

Backgrounds: Lung adenocarcinoma (LUAD) is one of the most common malignancies, and is a serious threat to human health. The aim of the present study was to assess potential biomarkers for the prognosis of LUAD through the analysis of gene expression microarrays.

Methods: The gene expression data for GSE118370 was downloaded from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) between normal lung and LUAD samples were screened using the R language. The DAVID database was used to analyze the functions and pathways of DEGs. The STRING database was used to the map protein–protein interaction (PPI) networks, and these were visualized with the Cytoscape software. Finally, the prognostic analysis of the hub gene in the PPI network was performed using the Kaplan–Meier tool.

Results: A total of 406 downregulated and 203 upregulated DEGs were identified. The GO analysis results revealed that downregulated DEGs were significantly enriched in angiogenesis, calcium ion binding and cell adhesion. The upregulated DEGs were significantly enriched in the extracellular matrix disassembly, collagen catabolic process, chemokine-mediated signaling pathway and endopeptidase inhibitor activity. The KEGG pathway analysis revealed that downregulated DEGs were enriched in neuroactive ligand-receptor interaction, hematopoietic cell lineage and vascular smooth muscle contraction, while upregulated DEGs were enriched in phototransduction. In addition, the top 10 hub genes and the most closely interacting modules of the top 3 proteins in the PPI network were screened. Finally, the independent prognostic value of each hub gene in LUAD patients was analyzed through the Kaplan–Meier plotter. Seven hub genes (*ADCY4, S1PR1, FPR2, PPBP, NMU, PF4*, and *GCG*) were closely correlated to overall survival time.

Conclusion: The discovery of these candidate genes and pathways reveals the etiology and molecular mechanisms of LUAD, providing ideas and guidance for the development of new therapeutic approaches to LUAD.

Abbreviations: BP = biological process, CC = cellular component, DAVID = the database for annotation, visualization and integrated discovery, DEGs = differentially expressed genes, EGA = European Genome-phenome Archive, EMT = epithelial-to-mesenchymal transition, GCBI = Gene Cloud Biotechnology Information, GEO = Expression Omnibus database, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, LSQ = lung squamous cell carcinoma, LUAD = lung adenocarcinoma, MF = molecular function, NSCLC = non-small cell lung cancer, OS = overall survival, PPI = protein-protein interaction, PPI = protein–protein interaction, SCLC = small cell lung cancer, STRING = The Search Tool for the Retrieval of Interacting Genes, TCGA = The Cancer Genome Atlas.

Keywords: differentially expressed genes, gene ontology, kyoto encyclopedia of genes and genomes, lung adenocarcinoma, prognosis, protein–protein interaction

1. Introduction

Lung cancer is a highly differentiated malignant tumor, and the leading cause of cancer-related death in the world.[1] This is majorly classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC, among which lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LSQ) are the most common subtypes, accounts for nearly 85%

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

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The datasets generated during and/or analyzed during the current study are publicly available.

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of lung cancers.[2] At the same time, LUAD is the most familiar histological subtype of lung cancer, accounting for almost 50% of all lung cancers in most countries.[3] It has been reported that LUAD is associated with abnormalities, such as epidermal growth factor receptor (*EGFR*) kinase domain mutations and anaplastic lymphoma kinase (*ALK)* fusion or rearrangement.[4–7] In recent years, many targeted treatment drugs have been developed for aberrant gene products. Among these, drugs such as gefitinib, erlotinib, and crizotinib, have been widely used in clinical treatment. Unfortunately, merely few patients with LUAD are ideal candidates for targeted therapies.[8] In addition, due to the on-target genetic resistance mutations or off-target mechanisms of resistance, such as the upregulation of bypass signaling pathways, patients receiving these medications may develop resistance and render the treatment ineffective.[9,10] Therefore, revealing the intrinsic mechanisms of LUAD and finding new potential targets is needed for developing effective diagnostic and therapeutic strategies. With the advancement of gene chips and high-throughput second-generation sequencing technologies, more and more genetic data is stored in public databases for researchers to mine. Therefore, the combination of gene expression data with bioinformatics methods can be used to judge the expression of differentially expressed genes (DEGs) in the development and progression of LUAD, and discover potential targets for the treatment of LUAD. For example, Xiao et al used the Gene Cloud Biotechnology Information (GCBI) bioinformatics platform to identify DEGs that eliminated gender differences in LUAD and normal lung tissue. Then, these screened for transcription factor 21 (*TCF21*) by constructing a gene co-expression network on the GCBI platform. In addition, these used Kaplan–Meier plotters and PrognoScan to assess the prognostic value of *TCF 21* in patients with LUAD. It was concluded that the decreased mRNA expression of *TCF 21* is a predictor of poor prognosis in patients with LUAD.[11]*TCF* 21 is inactivated in many cancers due to DNA methylation, which was reported by Jiang et al 1 year later. It was suggested that the development of drugs that target the DNA methylation of *TCF21* may have important clinical significance for the treatment or prevention of LUAD.[12]

In the present study, microarray data GSE118370 was downloaded from the Gene Expression Omnibus database (GEO database, http://www.ncbi.nlm.nih.gov/geo). Bioinformatics methods were used to screen for relevant DEGs between disease and normal samples in patients with LUAD. Subsequently, Gene Ontology (GO) terminology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and protein–protein interaction (PPI) analysis were performed to screen for key genes and biological pathways that are closely correlated to LUAD patients. Finally, the effect of hub gene expression level on overall survival (OS) was explored. These analysis results can provide new targets in further studies on LUAD.

2. Materials and methods

2.1. Microarray data

The gene expression profiles of the GSE118370 dataset were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). The GSE118370 dataset was based on the GPL570 platform, [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array, which was submitted by Xu et al[13] The GSE118370 dataset contained 12 samples, including 6 LUAD samples and 6 normal lung tissue samples (Table 1).

Table 1

Information for patients that was used in performing microarray.

Clinical Characteristics	No. (microarray)
Gender	
Male	3
Female	3
Age (years)	
<60	3
≥60	3
Smoking history	
No	3
Yes	3
CEA (IU/ml)	
<5.0	3
≥5.0	3
Size (cm)	
≤1.0	0
>1.0-≤2.0	3
>2.0-≤3.0	3
>3.0	0
Pathological diagnosis	
AIS	0
MIA	0
IAC	6
Pleural invasion	
No	6
Yes	0
Lymph node metastasis	
No	5
Yes	1
TNM stage	
0	0
I	4
II	2
III	0
Tumor stage	
Tis	0
T1	4
T2	2
T3	0

A "T" score is based upon the size and/or extent of invasion. The "N" score indicates the extent of lymph node involvement. The "M" score indicates whether distant metastases are present. We made this classification based on the 8th edition of UICC.

AIS = adenocarcinoma in situ, CEA = carcino-embryonic antigen, IAC = invasive adenocarcinoma, MIA = minimally invasive adenocarcinoma.

2.2. Data preprocessing and DEG screening

The raw data were preprocessed and normalized using the affy package (http://www.bioconductor.org/packages/release/bioc/html/affy.html)[14] under the R environment (version3.5.3, https://www.r-project.org/). Then, the limma package (available at http://www.bioconductor.org/packages/release/bioc/html/limma.html)[15] in R was used to identify DEGs between LUAD samples and normal lung samples. The *t*-test method was utilized to calculate the *P* values of genes. Merely genes with $|\log2\text{fold change}| > 2$ and $P < .05$ were considered as DEGs.

2.3. Gene ontology and functional enrichment analysis

The biological function of DEGS was determined by GO enrichment analysis (http://www.geneontology.org).[16] The GO analysis was performed to annotate genes and classify upregulated and downregulated DEGs. The GO terms consisted of 3 parts: biological process (BP), cellular component (CC), and

molecular function (MF). The KEGG database (www.genome.jp/kegg/)[17] includes the systematic analysis, annotation, and visualization of gene functions. The database for annotation, visualization and integrated discovery (DAVID, http://david.abcc.ncifcrf.gov/)[18] is a comprehensive functional annotation tool that understands the biological implications behind a large number of genes, and this can be used to analyze the functions of upregulated and downregulated DEGs. The GO enrichment and KEGG pathway analysis were performed using the DAVID online tool. $P < .05$ was considered statistically significant.

2.4. Construction of the protein–protein interaction (PPI) network and module analysis

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, www.string-db.org)[19] is a database that searches for known protein interactions online. The confidence score was set at >0.9 for the PPI analysis. Then, the protein interaction data was imported into the Cytoscape software (version 3.7.1, www.cytoscape.org)[20] to obtain a network map. Afterwards, the most connected modules were analyzed, and the hub gene was identified. $P < .05$ was considered statistically significant.

2.5. Prognosis analysis using the Kaplan–Meier plotter database

The present study performed an OS analysis of 866 patients with LUAD using the Kaplan–Meier plotter database to assess the prognostic value of the hub gene in patients with LUAD. Gene expression data, and relapse free and OS information were downloaded from the Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov), the GEO database (http://www.ncbi.nlm.nih.gov/geo/), and the European Genome-phenome Archive (EGA, https://www.ebi.ac.uk/ega/).[21] These databases contained gene expression data and survival information from 1928 lung cancer patients, including 866 LUAD patients.

3. Results

3.1. Identification of DEGs in LUAD

The GSE118370 dataset was downloaded from the GEO database. Six LUAD samples and 6 normal lung samples were analyzed. A total of 609 DEGs were identified (|log2fold change | > 2, $P < .05$), which included 203 upregulated and 406 downregulated genes (Fig. 1A). Subsequently, the expression values of DEGs were hierarchically clustered, and the results were presented in the form of heat maps (Fig. 1B). As shown in Figure 1B, the heat map clearly distinguishes the LUAD samples from normal lung samples.

3.2. Functional enrichment analysis

In order to further analyze the function of the identified DEGs, the online software DAVID was used for the GO analysis of DEGs. As mentioned earlier, the GO analysis results consisted of 3 parts: BP, CC, and MF. The results indicated that the downregulated DEGs were significantly enriched in BP-associated angiogenesis, immune response and cell adhesion (Fig. 2). In addition, the upregulated DEGs were mainly enriched in the collagen catabolic process, extracellular matrix disassembly, and chemokine-mediated signaling pathway (Fig. 3). For the CC, the downregulated DEGs were mainly enriched in the integral component of the plasma membrane, plasma membrane, and integral component of the membrane (Fig. 2), while the upregulated DEGs were mainly enriched in the extracellular space, extracellular region and extracellular exosome (Fig. 3). Furthermore, through the MF analysis, it was found that the downregulated DEGs were notably enriched in calcium ion binding, chemorepellent activity and heparin binding (Fig. 2), while the upregulated DEGs had an evidently enriched endopeptidase inhibitor activity, serine-type endopeptidase activity, and serine-type endopeptidase inhibitor activity (Fig. 3).

3.3. KEGG pathway analysis

KEGG was used to analyze the signaling pathways that downregulate DEGs and upregulate DEG enrichment. The downregulated DEGs were enriched in neuroactive ligand-receptor interaction, hematopoietic cell lineage, and vascular smooth muscle contraction (Fig. 2), while the upregulated DEGs were enriched in phototransduction (Fig. 3).

3.4. PPI network construction and modules selection

The PPI network visualized by Cytoscape included 174 nodes and 417 edges. Based on the STRING database, a degree of >10 was set as the cutoff criterion, and the top 10 degree scores were considered as the hub genes (Table 2). The hub genes were the following: guanine nucleotide-binding protein G(T) subunit gamma-T1 (*GNGT1*), adenylate cyclase 4 (*ADCY4*), formyl peptide receptor 2 (*FPR2*), pro-platelet basic protein (*PPBP*), neuromedin U (*NMU*), platelet factor 4 (*PF4*), glucagon (*GCG*), sphingosine 1 phosphate receptor 1 (*S1PR1*), G protein-coupled receptor 37 (*GPR37*), and sphingosine-1-phosphate receptor 5 (*S1PR5*). These genes may play an important role in the development and progression of LUAD. Subsequently, by using the plug-in MODE to detect potential modules, the first 3 most connected modules were obtained, and the proteins of the same module had strong interactions (Fig. 4).

3.5. Prognostic value of the hub gene

The expression and prognosis of 10 hub genes in LUAD were evaluated by Kaplan–Meier analysis. It was found that the expression levels of 7 hub genes in LUAD patients were closely correlated to OS, and the difference was statistically significant ($P < .05$, Fig. 5). In Figure 5, the high expression of *ADCY4* and *S1PR1* was associated with longer OS, while the high expression of *FPR2, PPBP, NMU, PF4,* and *GCG* was associated with lower OS. Furthermore, the expression levels of *GNGT1, GPR37,* and *S1PR5* were not significantly correlated with the OS of patients with LUAD.

4. Discussion

Regardless of the tremendous progress in the past few years, LUAD still represents as a tumor with poor prognosis when detected at the advanced clinical stage.[22] Therefore, uncovering the etiological and molecular mechanisms of LUAD is of vital importance for therapy and prevention. With the continuous development of gene chip technology and second-generation sequencing technology, gene data has tremendously increased. Hence, determining how to use these data to help humans clarify the relationship between genes and tumors has become a hot research topic.[23]

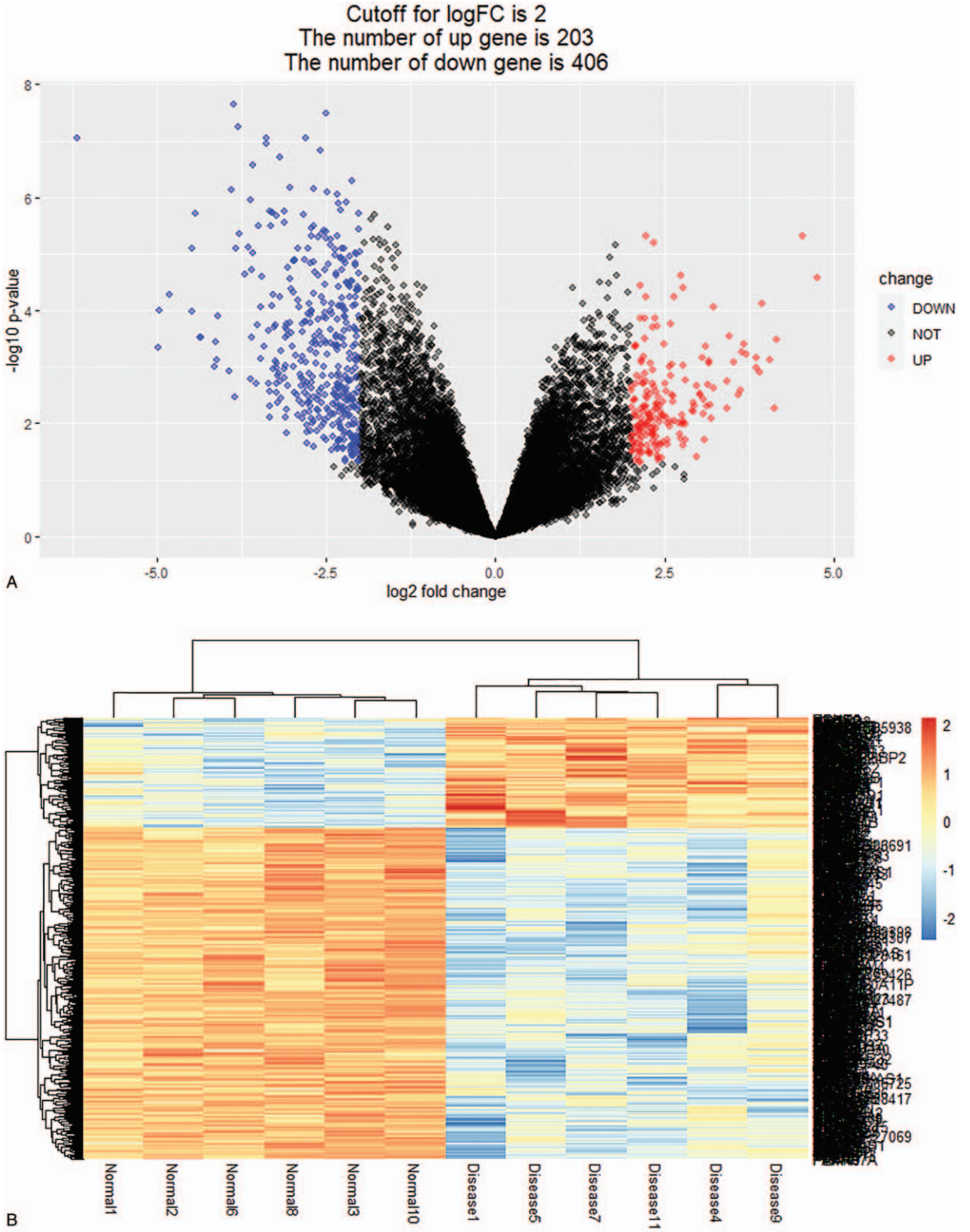


Figure 1. DEGs expression profile in the GEO dataset. (A) A volcano plot of 609 DEGs. Red: upregulation with $|\log_2\text{fold change}| > 2$, $P < .05$; blue: downregulation with $|\log_2\text{fold change}| > 2$, $P < .05$; black: unchanged genes. (B) Heatmap of the 609 DEGs with $|\log_2\text{fold change}| > 2$, $P < .05$. Red: higher expression; blue: lower expression.

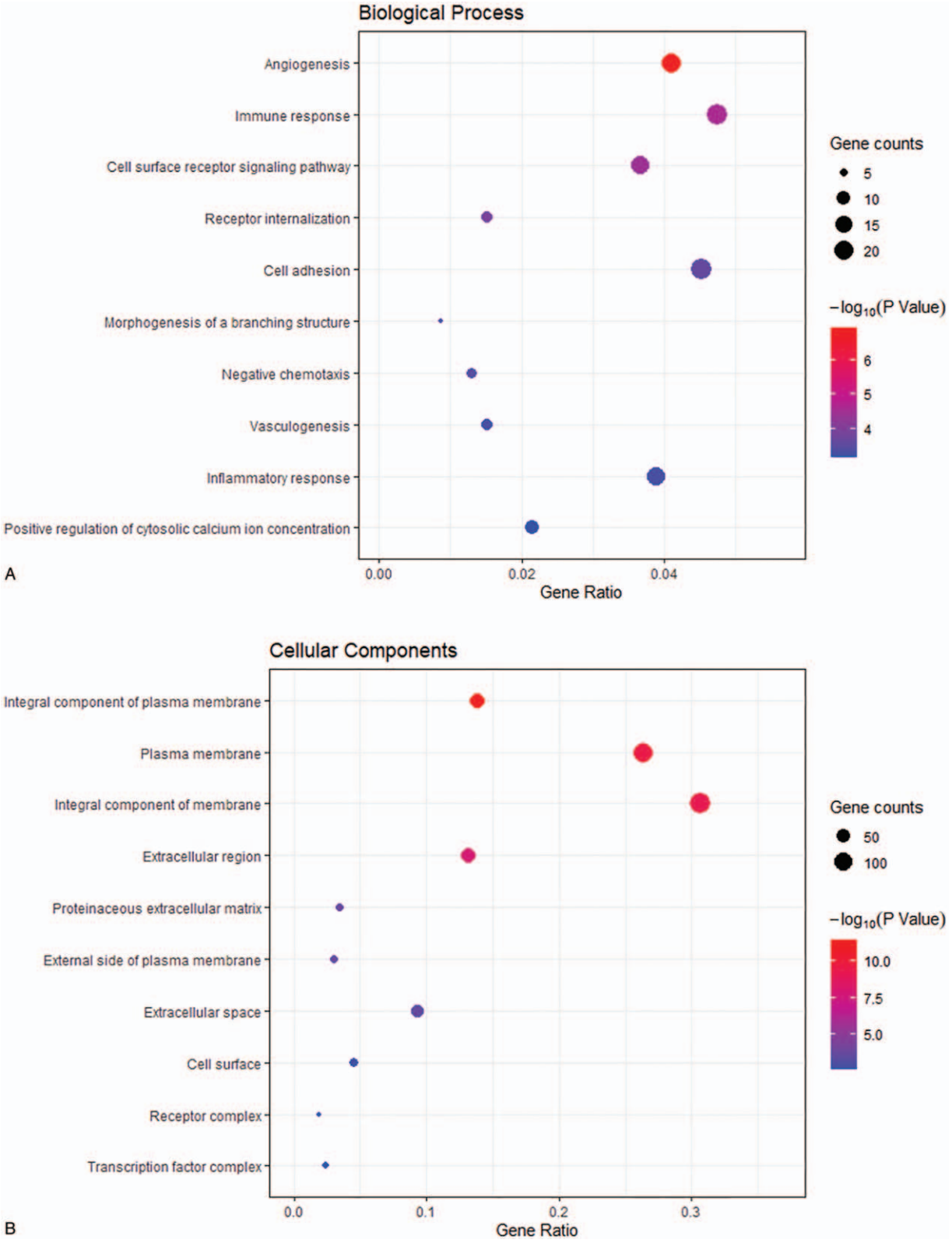
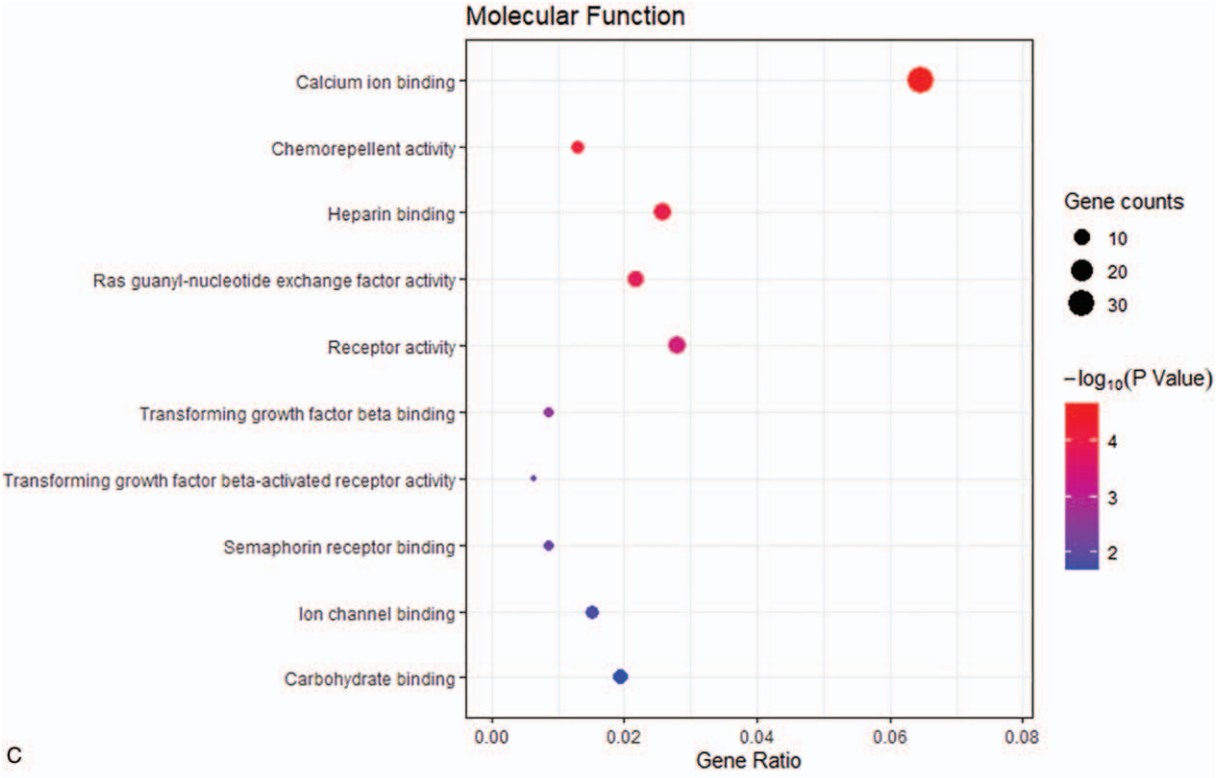
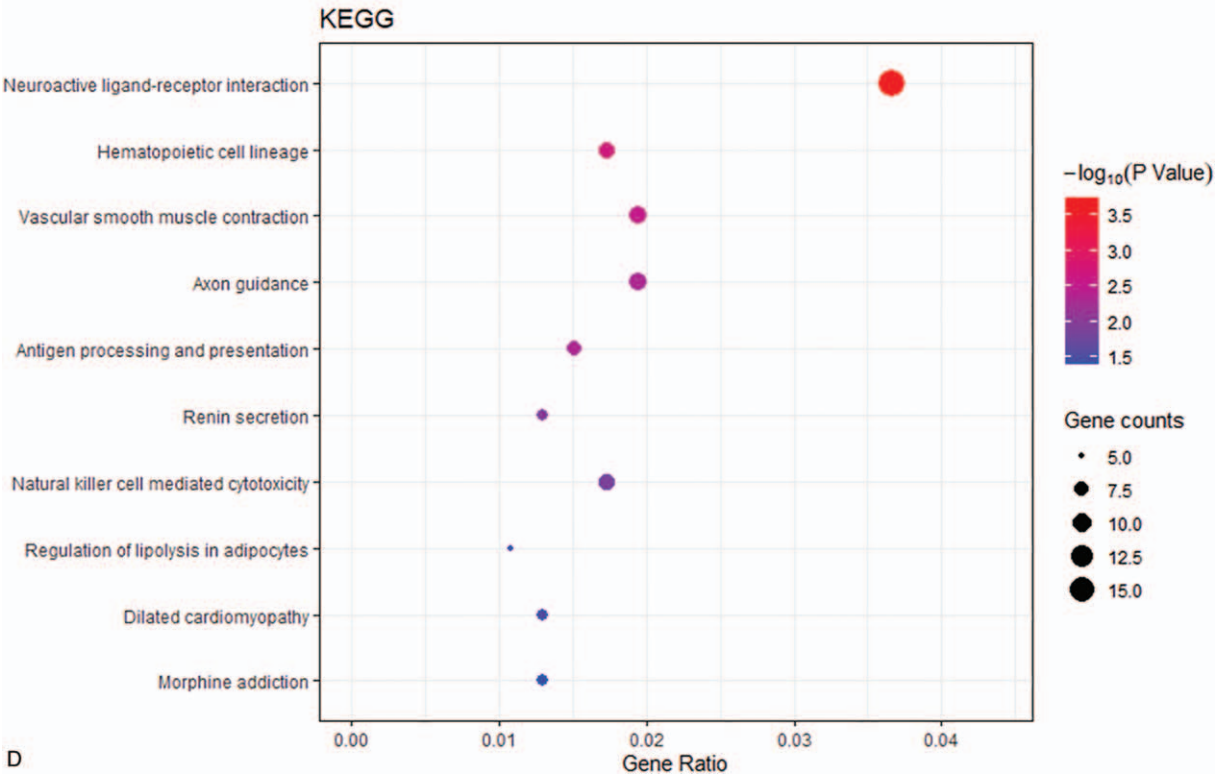


Figure 2. The Gene ontology and KEGG pathway analysis results for downregulated DEGs with $|\log_2 \text{fold change}| > 2$. (A) BP: biological process; (B) CC: cellular component; (C) MF: molecular function; (D) KEGG: Kyoto Encyclopedia of Genes and Genomes.





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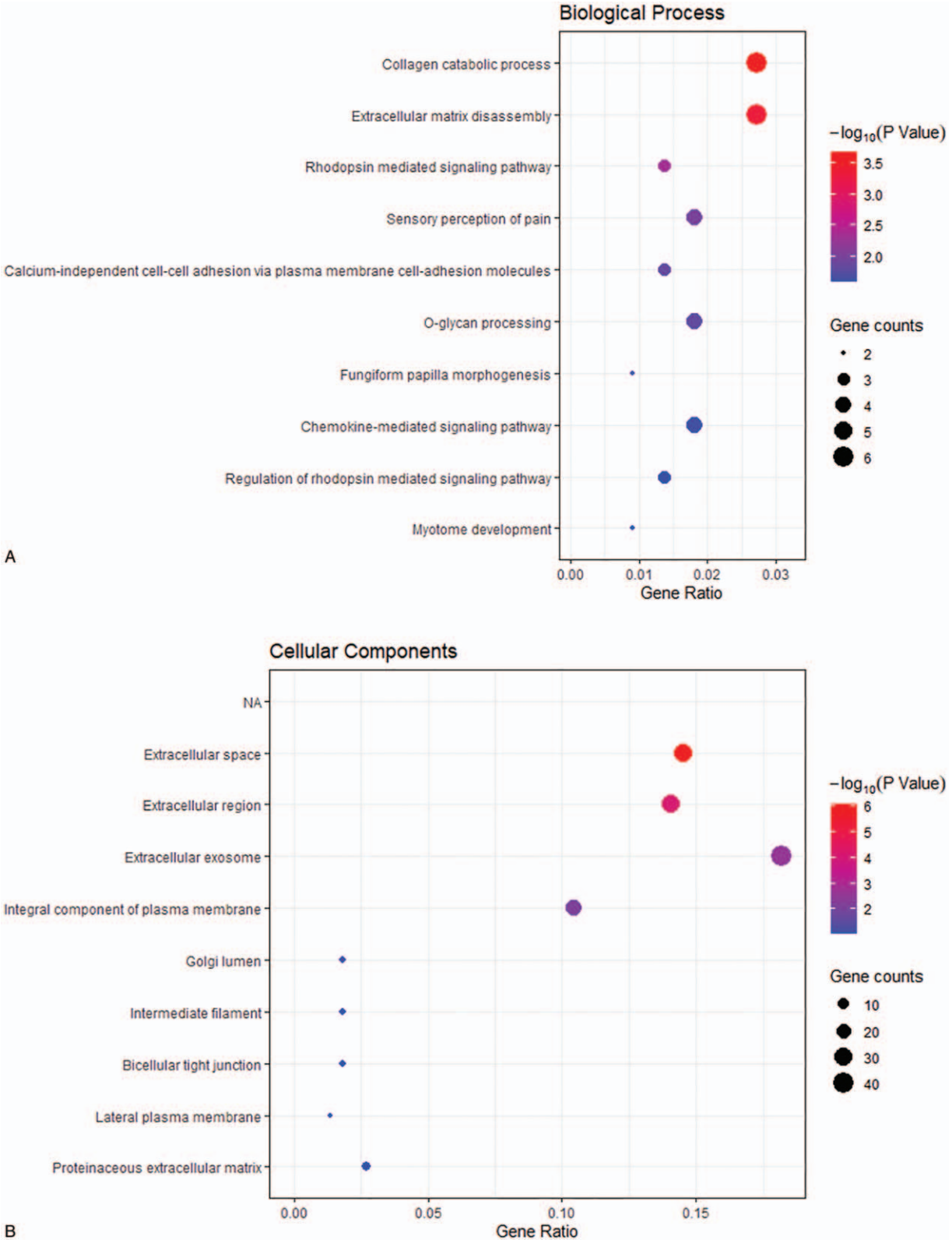


Figure 3. The Gene ontology and KEGG pathway analysis results for upregulated DEGs with $|\log_2 \text{fold change}| > 2$. (A) BP: biological process; (B) CC: cellular component; (C) MF: molecular function; (D) KEGG: Kyoto Encyclopedia of Genes and Genomes.

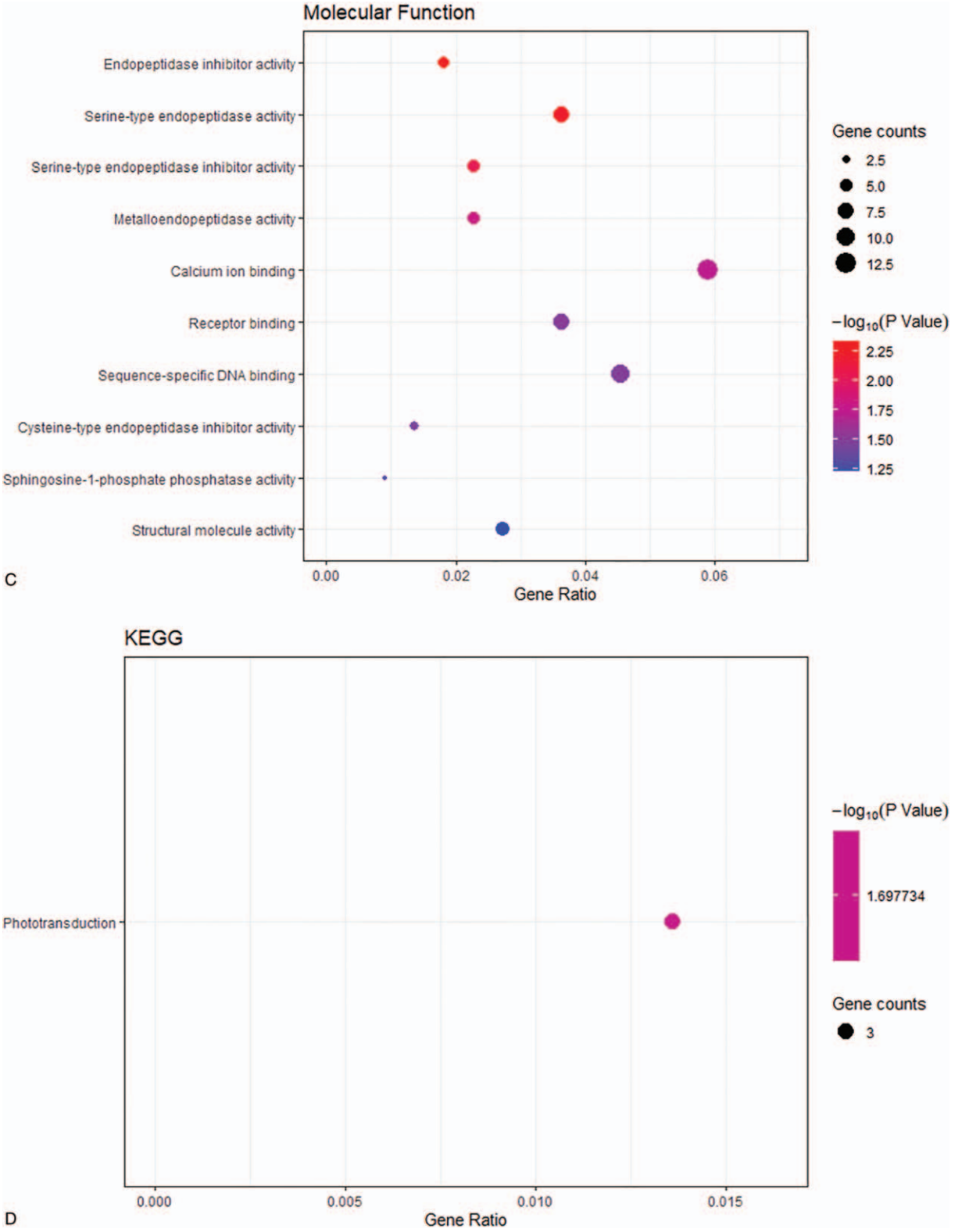


Figure 3. (Continued).

Table 2

The top 10 DEGs with higher degree scores.

	Degree	Gene feature	log_2fold change	*P* value
GNGT1	35.0	upregulation	2.241975	.010526
ADCY4	26.0	downregulation	−2.076325	.000010
FPR2	24.0	downregulation	−2.279682	.019484
PPBP	22.0	downregulation	−2.650435	.000850
NMU	20.0	upregulation	2.154657	.025011
PF4	19.0	downregulation	−2.861867	.001580
GCG	18.0	upregulation	2.766916	.024995
S1PR1	16.0	downregulation	−2.074276	.000016
GPR37	15.0	upregulation	3.083422	.019548
S1PR5	15.0	downregulation	−3.291861	.000082

The present study obtained gene expression data from the GSE118370 dataset, and 203 upregulated and 406 downregulated DEGs were selected between the LUAD sample and normal tissues by bioinformatics analysis.

The GO term analysis results indicated that the downregulated DEGs were mainly enriched in angiogenesis, calcium ion binding and cell adhesion, while the upregulated DEGs were involved in the extracellular matrix disassembly, collagen catabolic process, chemokine-mediated signaling pathway, and endopeptidase inhibitor activity. Angiogenesis is a complex process that plays a key role in maintaining the tumor microenvironment, tumor growth, invasion, and metastasis.[24] Previous studies have shown that the activation of intracellular Ca^{2+} may affect cancer cell metastasis and tumorigenicity.[25] Cell adhesion molecules, as a class of membrane surface glycoprotein molecules, are involved in the regulation of inflammatory responses, and in tumor spread and metastasis. Epithelial-to-mesenchymal transition (EMT) is characterized by loss of cell adhesion. As previously reported, EMT is one of the key mechanisms that induce tumor invasion and metastasis. A negative or reduced expression of some adhesion molecules correlates with distant metastasis in lung cancer.[26,27] The extracellular matrix is a complex multispatial macromolecular network, and extracellular matrix disassembly can promote tumor growth and metastasis.[28–30] Since collagen is the major component of the extracellular matrix, it is the main obstacle to the migration of cancer cells, while collagen synthesis and degradation can affect the process of cancer invasion.[31] Numerous studies have revealed that chemokines play a key role in tumor invasion, angiogenesis, and metastasis.[32] In addition, a study reported that the inhibition of endopeptidase can inhibit the degradation of the extracellular matrix and tumor invasion.[33] The KEGG pathway analysis revealed that the downregulated DEGs were observably enriched in neuroactive ligand-receptor interaction, hematopoietic cell lineage, and vascular smooth muscle contraction. The role of neuroactive steroids reveals the interaction of ligands with receptors. Neuroactive steroids affect the regulation of GABA receptors. Hence, GABA receptors have been considered to control cell proliferation. Furthermore, cell proliferation is a hallmark of cancer.[34] A study revealed that the loss of some hematopoietic cells is associated with aggressive LUADs.[35] Furthermore, a recent study on the expression of lung cancer genes revealed that the vascular smooth muscle contraction pathway is negatively regulated during tumorigenesis.[36] Therefore, studying these signaling pathways could assist in the prediction of cancer progression.

In addition, a DEG PPI network was established, and the top 10 hub genes were identified, which included *GNGT1, ADCY4, S1PR1, FPR2, PPBP, NMU, PF4, GCG, GPR37,* and *S1PR5*. The Kaplan–Meier survival analysis concluded that the high expression levels of *ADCY4* and *S1PR1* were associated with longer OS, while the high expression levels of *FPR2, PPBP, NMU, PF4*, and *GCG* were associated with lower OS in LUAD patients. *ADCY4* catalyzes the formation of the signaling molecule cAMP in response to G-protein signaling. Welldon et al reported that *ADCY4* is significantly associated with calcium signaling pathways.[37] Intracellular Ca^{2+} activation may affect the tumorigenicity and metastasis of LUAD cells.[25]*S1PR1* is a G-protein coupled receptor for the bioactive lysosphingolipid sphingosine 1-phosphate. Studies have shown that *S1PR1* can promote the invasion and proliferation of cancer cells by activating ERK signaling pathways.[38] At the same time, *S1PR1* and its downstream target VE-cadherin can inhibit the germination of angiogenesis and improve vascular stability, thereby effectively delaying tumor growth.[39] As another G-protein coupled receptor, *FPR2* usually directly or indirectly retains the immunological activity of macrophages, and effectively limits tumor progression.[40]*PPBP* belongs to the CXC chemokine subfamily, and promotes angiogenesis, tumorigenesis and metastasis.[41] Furthermore, a previous study published that the level of *PPBP* in patients with NSCLC is significantly higher than that in normal people.[42]*NMU* is a neuropeptide that is a member of the neuromedin family. A recent study revealed that *NMU* mRNA expression levels in NSCLC patients have a negative correlation with OS.[43]*PF4* is an endocrine factor. The study conducted by Ferdinando et al[44] revealed that *PF4* promotes platelet accumulation and lung cancer growth. *GCG* encodes glucagon protein. It acts on glucose metabolism and homeostasis, and regulates blood sugar by increasing gluconeogenesis and reducing glycolysis.[45] Tumors are a rare cause of hypoglycemia. Hypoglycemia manifests as paraneoplastic symptoms of a tumor with a potentially serious prognosis.[46] Thus, the level of *GCG* expression has a certain indicator of tumor production. These hub genes are directly or indirectly involved in the regulation of LUAD.

In summary, by analyzing the gene expression profiles, the present study established hub genes and important signaling pathways closely correlated to the occurrence and development of LUAD. Many genes are considered to be associated with LUAD.[47] However, it is difficult to determine which genes are the most relevant. Previous studies usually only investigated genes or signaling pathways,[48] and few studies have combined

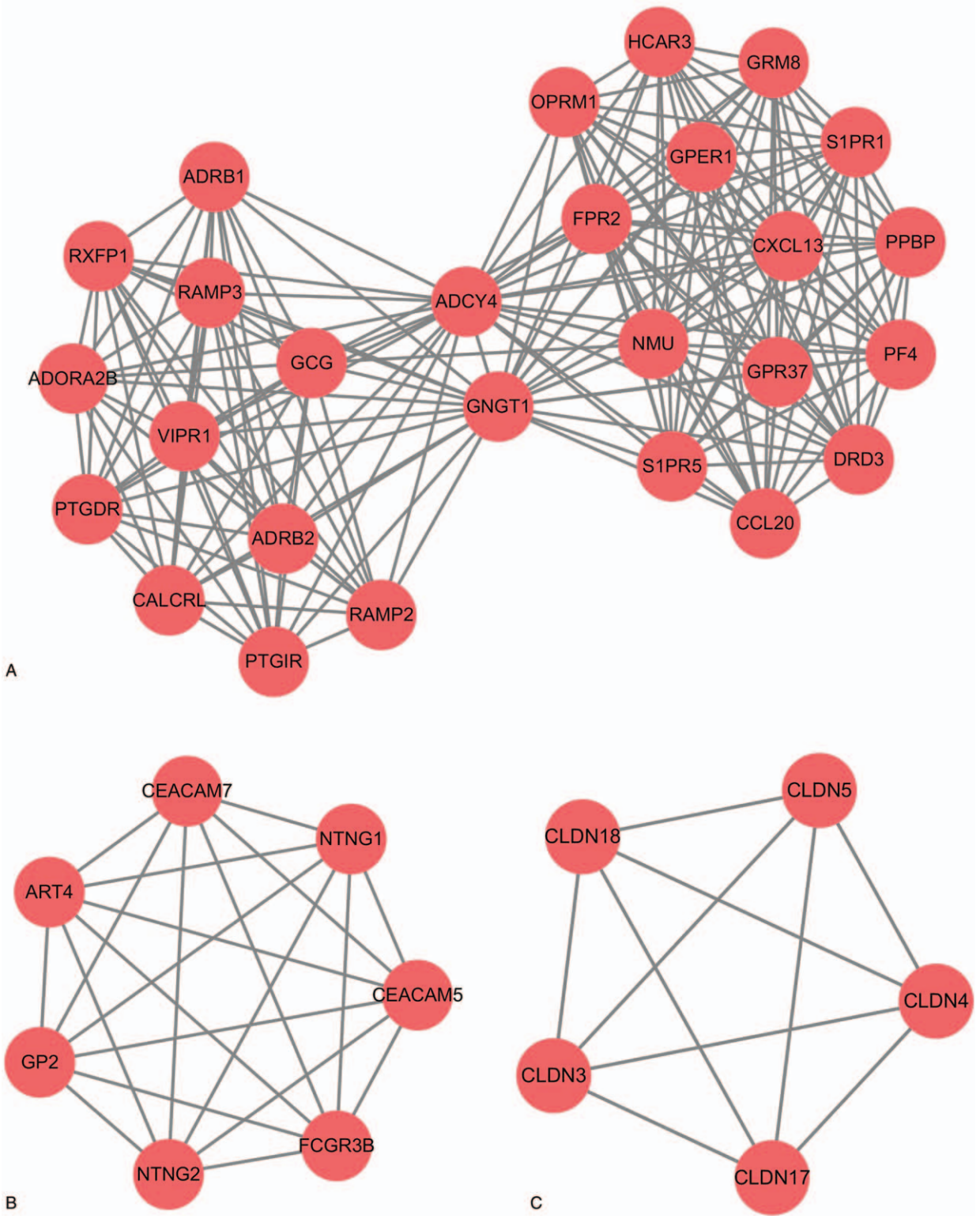


Figure 4. Module analysis of the PPI network. (A) module 1; (B) module 2; (C) module 3.

these to identify common parts. Therefore, after screening the hub genes and important signaling pathways, it was found that the calcium signaling pathway, and the angiogenesis and chemokine-mediated signaling pathway were screened in the functional enrichment analysis. Furthermore, it was found that these were correlated to *ADCY4*, *S1PR1*, and *PPBP* in the hub gene, which is worthy of further in-depth study. These findings provide novel insights on the study of the potential biomarkers and pathogenesis of LUAD. However, further molecular biology or cell experiments are needed to verify these findings.

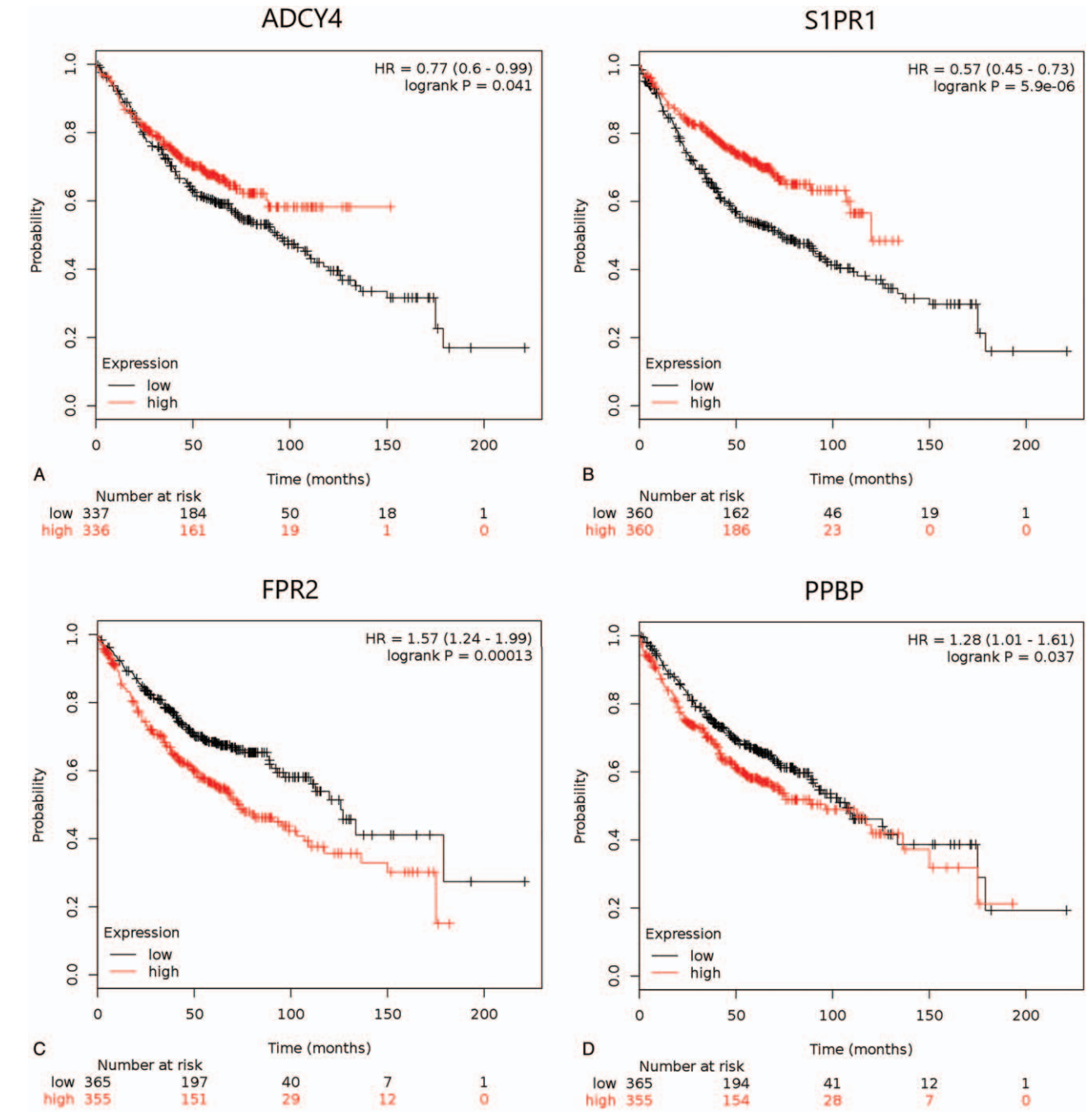
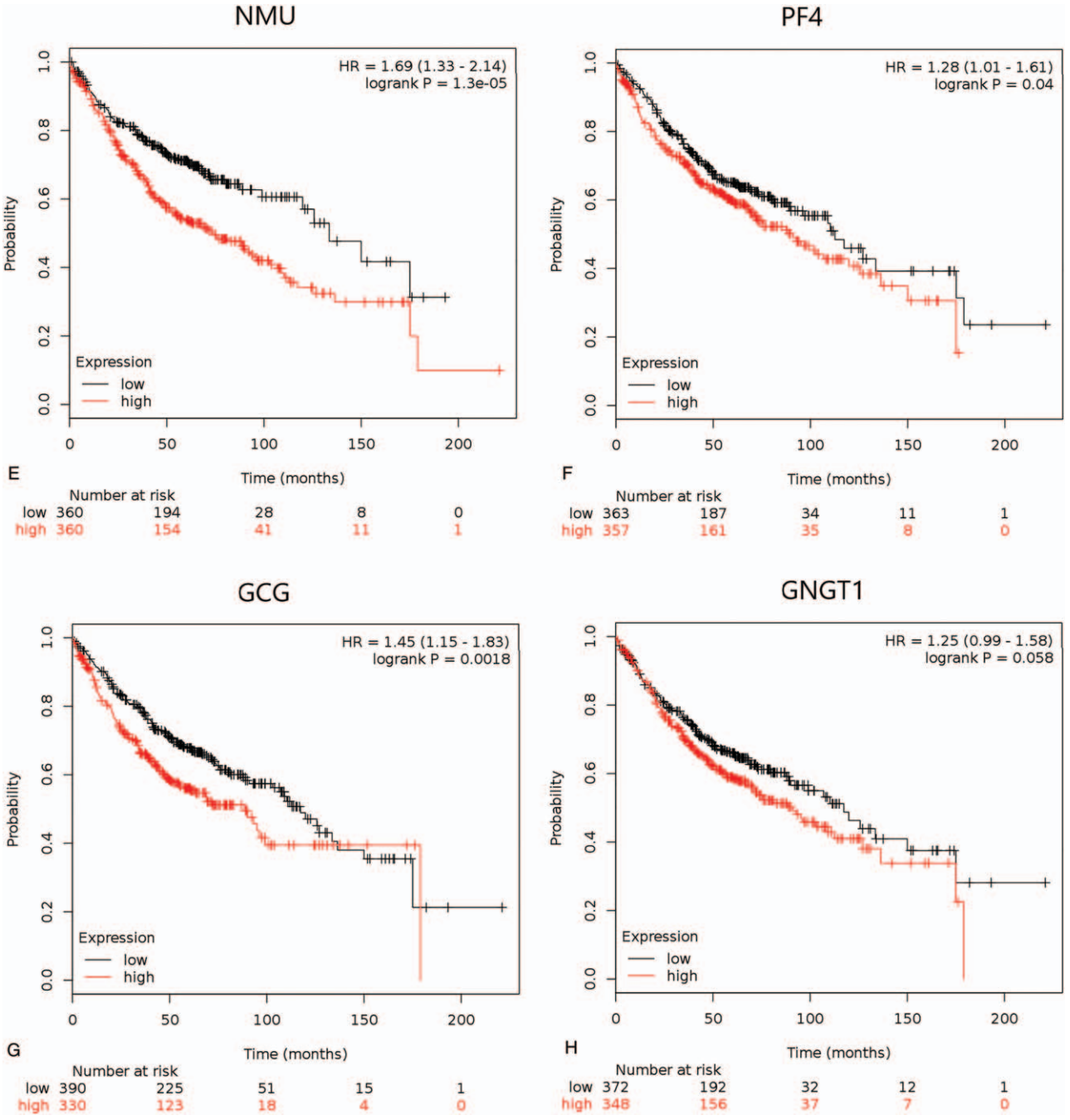


Figure 5. The Kaplan–Meier survival curves by mRNA expression levels of 10 hub genes of 866 LUAD patients (The log-rank $P < .05$ was statistically significant). (A) *ADCY4*; (B) *S1PR1*; (C) *FPR2*; (D) *PPBP*; (E) *NMU*; (F) *PF4*; (G) *GCG*; (H) *GNGT1*; (I) *GPR37*; (J) *S1PR5*.



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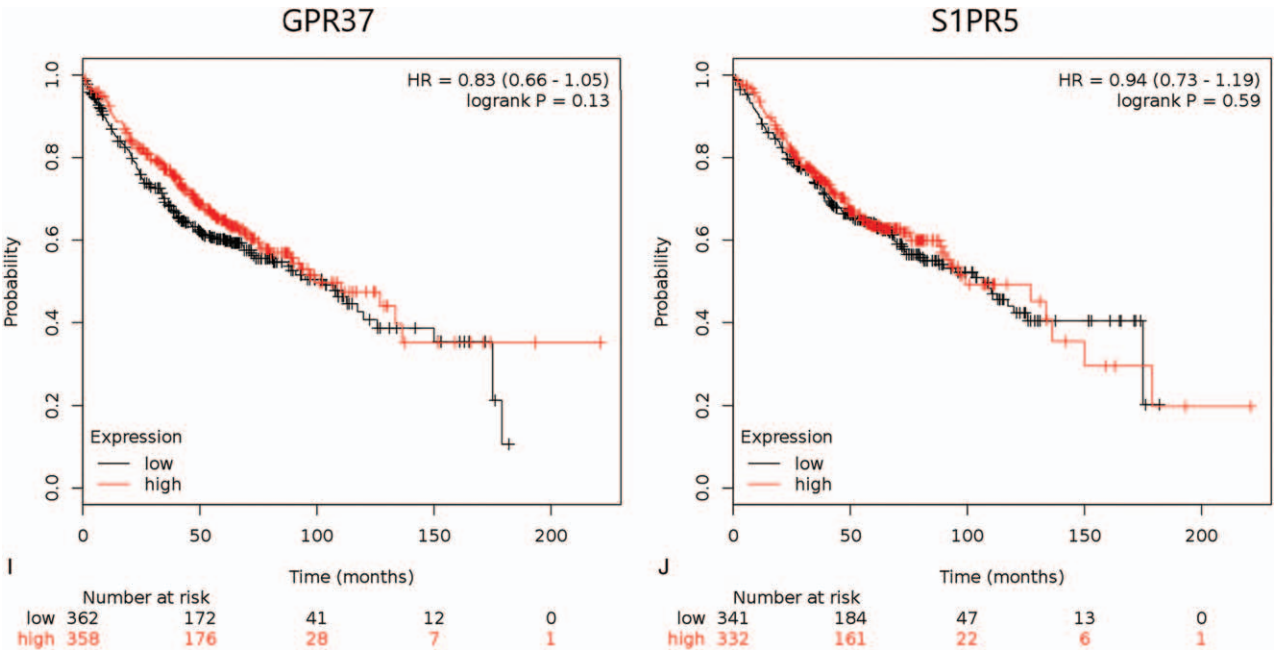

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Author contributions

Ye Yu: Methodology, Software, Formal analysis, Writing - Original Draft, Visualization.

Xuemei Tian: Conceptualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

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