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Identification of potential biomarkers and pathways related to major depressive disorder by integrated bioinformatic analysis and experimental validation

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ABSTRACT

Objective: To identify promising biomarkers for the pathogenesis of major depressive disorder (MDD).

Methods: Microarray chips of MDD patients, including the GSE98793, GSE52790, and GSE39653 datasets, were obtained from the Gene Expression Omnibus database. The biological processes and pathways related to MDD were investigated using the GO and KEGG pathway tools. Weighted gene coexpression network analysis was conducted to identify modules related to MDD. The hub genes associated with MDD were obtained *via* protein-protein interaction analysis. Finally, the expression of hub genes in the hippocampal tissues of depression-like rats was detected by reverse transcription-polymerase chain reaction and Western blotting.

Results: A total of 658 differentially expressed genes were identified from the Gene Expression Omnibus datasets; thus, these genes and the GSE98793 dataset were used to conduct weighted gene coexpression network analysis. A total of 244 module-related genes were identified and these genes were highly correlated with MDD. These genes were involved in the Ras signaling pathway, regulation of the actin cytoskeleton, and axon guidance according to the KEGG analysis. Hub genes, including *MAPK14*, *SOCS1*, *TLR2*, *PTK2B*, and *GRB2*, were obtained *via* protein-protein interaction analysis. All these hub genes showed better diagnostic efficiency in the GSE52790, GSE39653, and GSE98793 datasets. *In vivo* experiments revealed that compared with those in control rats, *SOCS1* and *MAPK14* expression was significantly decreased; while *GRB2*, *TLR2*, and *PTK2B* expression was increased in the hippocampi of depression-like rats.

Conclusions: Our study demonstrates that *GRB2*, *TLR2*, *SOCS1*, *PTK2B*, and *MAPK14* are promising hub genes, and targeting these five genes may be an effective treatment strategy for MDD.

KEYWORDS: Major depressive disorder; Bioinformatic; Biomarkers; Microarray; Hub genes; Weighted gene coexpression network analysis

1. Introduction

Major depressive disorder (MDD) is characterized by chronic low mood, a lack of motivation, altered sleep and appetite patterns, cognitive difficulties, and, most seriously, suicidal tendencies[1]. Depression often leads to serious adverse outcomes, including disability and excessive mortality, and is a global burden[2]. In addition, clinically common antidepressant drugs have many side

Summary

Question: Are there any promising biomarkers for diagnosis of major depressive disorder?

Findings: Through weighted gene coexpression network analysis and protein-protein interaction analysis, five hub genes (*MAPK14*, *SOCS1*, *TLR2*, *PTK2B*, *GRB2*) were identified and validated in depression-like rats.

Meaning: These genes may serve as potential treatment targets for major depressive disorder.

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effects, such as gastrointestinal reactions, the emergence of other psychiatric symptoms, and other unforeseen side effects[3,4]. Thus, the understanding of the potential physiological and pathological mechanisms of depression is urgently needed for the development of antidepressant drugs.

Recently, most studies have focused on exploring the pathogenesis of depression, and the results demonstrated that it may be highly related to genetics, immune system effects, oxidative stress, and neuroinflammation in the brain[5]. A previous study found that patients with MDD often cluster within families, which indicates that genetics plays a vital role in MDD[6]. Oxidative stress often leads to emotional stress, depression, and excessive aggression[7]. Moreover, biomarkers of oxidative stress are increased in patients with alcohol-induced aggression, impulsivity, and suicidal behavior[8]. Specific cytokines in the periphery can influence neurons, astrocytes, and microglia in the central nervous system, which leads to mood changes[9]. In addition, patients with cancer or hepatitis virus infection often experience symptoms of depression when they receive cytokine therapy[10]. However, the identification of promising biomarkers for the pathogenesis of MDD still needs further study.

In the present study, datasets related to MDD were downloaded from the Gene Expression Omnibus (GEO) and differentially expressed genes (DEGs) were identified by R software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses and protein-protein interaction (PPI) network and weighted gene coexpression network (WGCNA) analyses were conducted to investigate the corresponding pathways and hub modules, respectively. Finally, the expression of hub genes was validated in the hippocampus of depression-like rats.

2. Materials and methods

2.1. Data collection

Three datasets, namely, GSE98793, GSE52790, and GSE39653, were downloaded from the GEO database. There were 128 patients with MDD (64 patients with anxiety disorders and 64 patients with nonanxiety disorders) and 64 healthy patients in the GSE98793 dataset. There were 10 patients with MDD and 12 coupled healthy patients in the GSE52790 dataset. There were 21 patients with MDD, 8 patients with bipolar disorder, and 24 healthy patients in the GSE39653 dataset. The analyzed samples were whole blood samples in both the GSE98793 and GSE52790 datasets, whereas the collected samples were peripheral blood mononuclear cells in the GSE39653 dataset.

2.2. Data preprocessing and DEG screening

All the data were preprocessed by the Limma package in R

software. The threshold criteria for DEG identification were $P < 0.05$ and $\log_2FC > 0$ between the MDD and control samples.

2.3. GO and KEGG pathway analysis

We identified the commonly upregulated and downregulated genes in the GSE98793, GSE52790, and GSE39653 datasets. The commonly upregulated and downregulated genes were subsequently subjected to GO and KEGG analyses *via* the clusterProfiler package in R software.

2.4. WGCNA and KEGG analysis

Considering that GSE98793 included the largest number of samples among of these three datasets, which makes our results more accurate, GSE98793 was chosen for further analysis. The commonly upregulated and downregulated genes in GSE98793 were used to conduct WGCNA *via* the WGCNA package in R software. In this study, the soft thresholding power was screened out by analysis of the network topology when the degree of scale independence was set as $\beta = 8$. Then, a specific WGCNA procedure was carried out as previously described[11]. Finally, the genes within modules highly related to MDD development were subjected to KEGG pathway analysis.

2.5. PPI analysis and identification of hub genes

The genes within modules highly related to MDD development were subjected to PPI analysis. The potential hub genes were identified *via* the cytoHubba package in Cytoscape using the parameters of betweenness, closeness, degree, radiality, and stress. Finally, we obtained 5 types of potential hub genes, and the interacting genes were identified as the final hub genes.

2.6. Analysis of the diagnostic capabilities of the hub genes

We analyzed the correlations among these genes and the relative levels of the hub genes in the GSE39653, GSE52790, and GSE98793 datasets. Next, we explored the correlation between the hub genes and other genes in the GSE98793 dataset, and a single gene-related Gene Set Enrichment Analysis (GSEA) was conducted on these genes. Finally, we investigated the diagnostic efficiency of these hub genes using receiver operating characteristic (ROC) curves in the GSE39653, GSE52790, and GSE98793 datasets.

2.7. Animal experiments

2.7.1. Main reagents

All chemical reagents were of analytical grade and sourced as follows: Fluoxetine hydrochloride capsules (Lilly Suzhou Pharmaceutical Co., Ltd., China); sucrose (Changde Bikeman

Biotechnology Co., Ltd., China); 75% ethanol solution (Shandong Lierkang Disinfection Technology Co., Ltd., China); Goat anti-Rabbit IgG (H+L) AWS0002 Secondary Antibody, HRP (China Abiowell); MAPK14 14064-1-AP, TLR2 17236-1-AP, PTK2B 17592-1-AP, GRB2 10254-2-AP, GAPDH 10494-1-AP (United States Proteintech); SOCS1 ab65989 (Britain Abcam); Tris-HCl buffer, RIPA lysis buffer, Phosphatase inhibitors (China Abiowell).

2.7.2. Animals and grouping

A total of 14 male Sprague-Dawley rats, weighing 160 g to 180 g, were obtained from the Animal Experiments Centre of Central South University [license number: SCXK (Xiang) 2019-0004] (certificate number: 430727211101906315). All rats were raised under a normal light/dark cycle at a proper temperature $[(22\pm 2)^{\circ}\text{C}]$ and suitable humidity $[(60\pm 10)\%]$ with free access to food and water. The animals were acclimated to the environment for 7 d before the experiments.

These rats were randomly divided into two groups ($n=7$): normal control [normal feeding, no chronic unpredictable mild stress (CUMS) procedure] and CUMS (solitary feeding + CUMS procedure). Each rat was weighed on days 7, 35, and 65 of the experiment.

2.7.3. Ethical statement

The experimental operations on the animals were approved by the Animals Experimental Ethics Committee of Central South University (Approval number: 2021sydw0196) and followed the NIH Guide for the Care and Use of Laboratory Animals.

2.7.4. Sucrose preference test

The rats in the control group were kept in solitary cages before testing. On the first day, 2 bottles of 1% sugar water (w/v) per cage were given for 24 h. On the second day, 1 bottle of 1% sugar water and 1 bottle of distilled water were given for 24 h. On the third day, the rats were deprived of food and water for 24 h. On the fourth day, the baseline sucrose preference test was performed for 4 h (1 bottle of 200 mL of 1% sugar water + 1 bottle of 200 mL of distilled water). The volume of distilled water and 1% sugar water consumed by each rat was recorded after 4 h, and the sucrose preference rate was calculated as follows: sucrose preference (%) = sucrose solution (mL)/[sucrose solution (mL) + water (mL)] $\times 100\%$.

2.7.5. Forced swimming test

The rats were placed individually in a round transparent glass container (60 cm high, 20 cm diameter, Shenzhen Ruiward Life Technology Co., Ltd., China) filled with water (35 cm depth, 24–26 $^{\circ}\text{C}$) for 6 min. The camera recorded the entire swimming process. The first two minutes were used for adaptation, and the immobility time during the last 4 minutes was recorded by two trained observers independently. The immobility time was defined as the time when

the animal floated in the water without struggling or moving slightly to keep its head above the water's surface.

2.7.6. Reverse transcription–polymerase chain reaction (RT–qPCR)

Total RNA was extracted from the hippocampus of the rats *via* the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The RT-qPCR procedure was carried out as previously described[12]. The following cycling program was used for the SYBR green assay: initial denaturation at 95 $^{\circ}\text{C}$ for 10 min; 40 cycles of 95 $^{\circ}\text{C}$ for 15 s and 60 $^{\circ}\text{C}$ for 60 s; followed by a melt curve step. All primer sequences are shown in Supplementary Table 1.

2.7.7. Western blotting

Tissues were lysed with RIPA buffer (Beyotime, Shanghai, China) containing 1 mM phenylmethanesulfonyl fluoride (Beyotime). Western blotting was conducted as previously described[13], and the levels of MAPK14 (Cat#14064-1-AP), SOCS1 (Cat#ab65989), TLR2 (Cat#17236-1-AP), PTK2B (Cat#17592-1-AP), GRB2 (Cat#10254-2-AP), and GAPDH (Cat#10494-1-AP) in the hippocampi of depression-like rats were measured.

2.8. Statistical analysis

The data analyses were conducted by GraphPad Prism software (CA, USA) and presented as mean $\pm$ SD. An unpaired *t*-test was performed for two groups, and ANOVA was performed for multiple groups to evaluate the expression of central gene difference. $P < 0.05$ was defined as statistically significant.

3. Results

3.1. Identification of DEGs

All three GEO datasets were correctly normalized by R software (Supplementary Figure 1). In the GSE52790 dataset, we identified 5 036 DEGs between patients with MDD and coupled healthy patients (Figure 1A and B), including 2 432 upregulated genes and 2 604 downregulated genes. In the GSE39653 dataset, we identified 2 111 DEGs between patients with MDD and healthy patients (Figure 1C and D), among which 1 091 DEGs were upregulated and 1 020 DEGs were downregulated. In the GSE98793 dataset, we identified 2 941 DEGs between patients with MDD and healthy patients (Figure 1E and F), of which 1 499 DEGs were upregulated and 1 442 DEGs were downregulated. Furthermore, we identified the interacting DEGs among these three datasets, and the results revealed 1 upregulated interacting gene (Figure 1G) and 10 downregulated interacting genes (Figure 1H).

3.2. GO and KEGG analyses

DEGs that were simultaneously dysregulated in two of the three datasets (a total of 658 DEGs) were subjected to GO and KEGG

analyses. The results showed that the DEGs were involved in ribonucleoprotein complex biogenesis, ribosome biogenesis, and positive regulation of the DNA biosynthetic process according to the GO enrichment analysis (Figure 2A). According to the KEGG

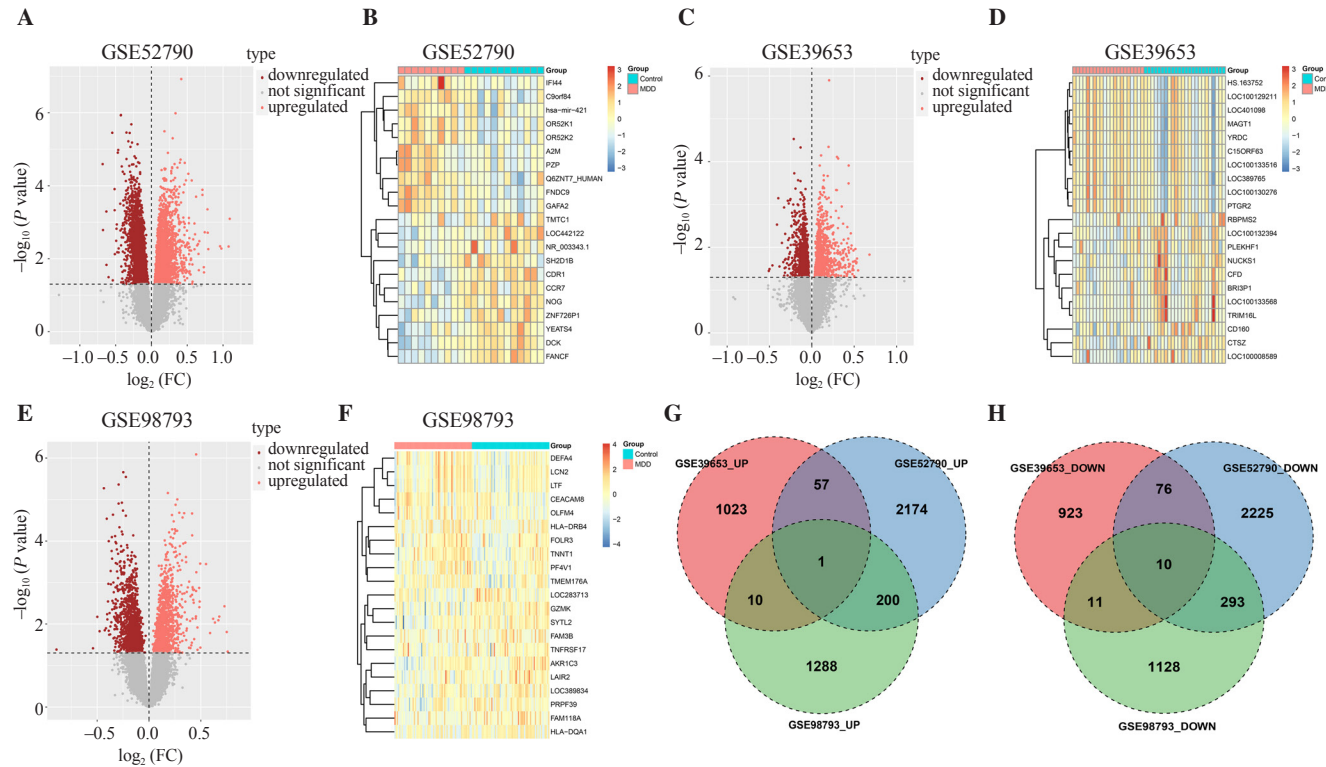


Figure 1. Identification of differentially expressed genes (DEGs) between patients with depression and control patients in GSE52790, GSE39653, and GSE98793 datasets. A, C, and E: Volcano plot of DEGs between patients with depression and control patients in GSE52790 (A), GSE39653 (C), and GSE98793 (E) datasets. B, D, and F: heat map of top 10 upregulated and downregulated DEGs between patients with depression and control patients in GSE52790 (B), GSE39653 (D), and GSE98793 (F). G and H: The commonly upregulated (G) and downregulated (H) DEGs in GSE52790, GSE39653, and GSE98793 datasets. MDD: Major depressive disorder.

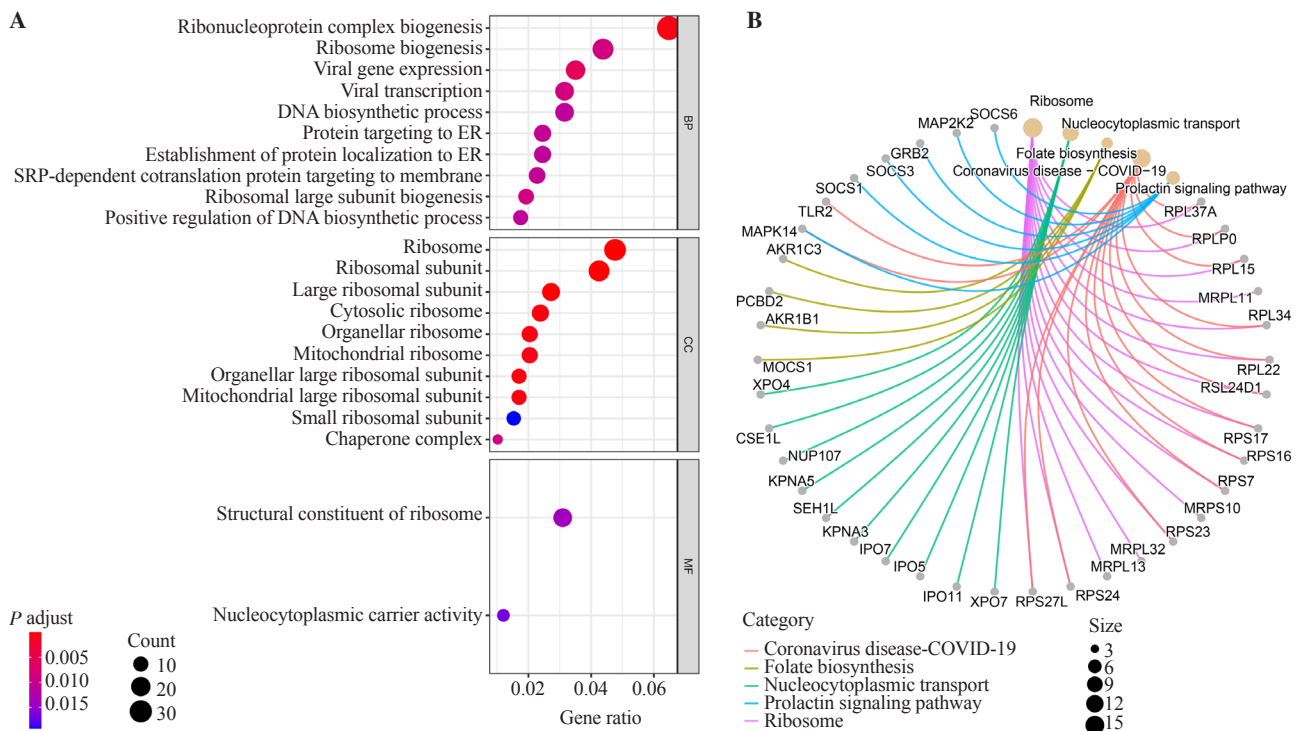


Figure 2. GO (A) and KEGG (B) pathway enrichment analyses for DEGs. ER: endoplasmic reticulum.

pathway enrichment analysis, these genes were highly correlated with ribosome biosynthesis, nucleocytoplasmic transport, and folate biosynthesis (Figure 2B).

3.3. WGCNA

The commonly upregulated and downregulated genes in GSE98793 were subjected to WGCNA. The clustering results with sample characteristics are illustrated in Figure 3A. A soft threshold of $\beta = 8$ was chosen, as illustrated in Figure 3B. As shown in Figure 3C, these modules display and contain different numbers of genes in different colors, with the grey module comprising a set of genes

that could not be merged into other modules. As shown in Figure 3D and E, each module is separated from the others in the network, and there is a correlation between some modules. We found that the MEgreen, MEyellow, and MEgrey modules were highly correlated with MDD (Figure 3D), thus we selected the genes (a total of 244 genes) associated with these modules to conduct further analyses.

3.4. KEGG pathway and PPI analyses of the module genes

These 244 module-related genes were subjected to KEGG analysis, and we found that these genes were related to axon guidance, the Ras signaling pathway, and regulation of the actin cytoskeleton (Figure

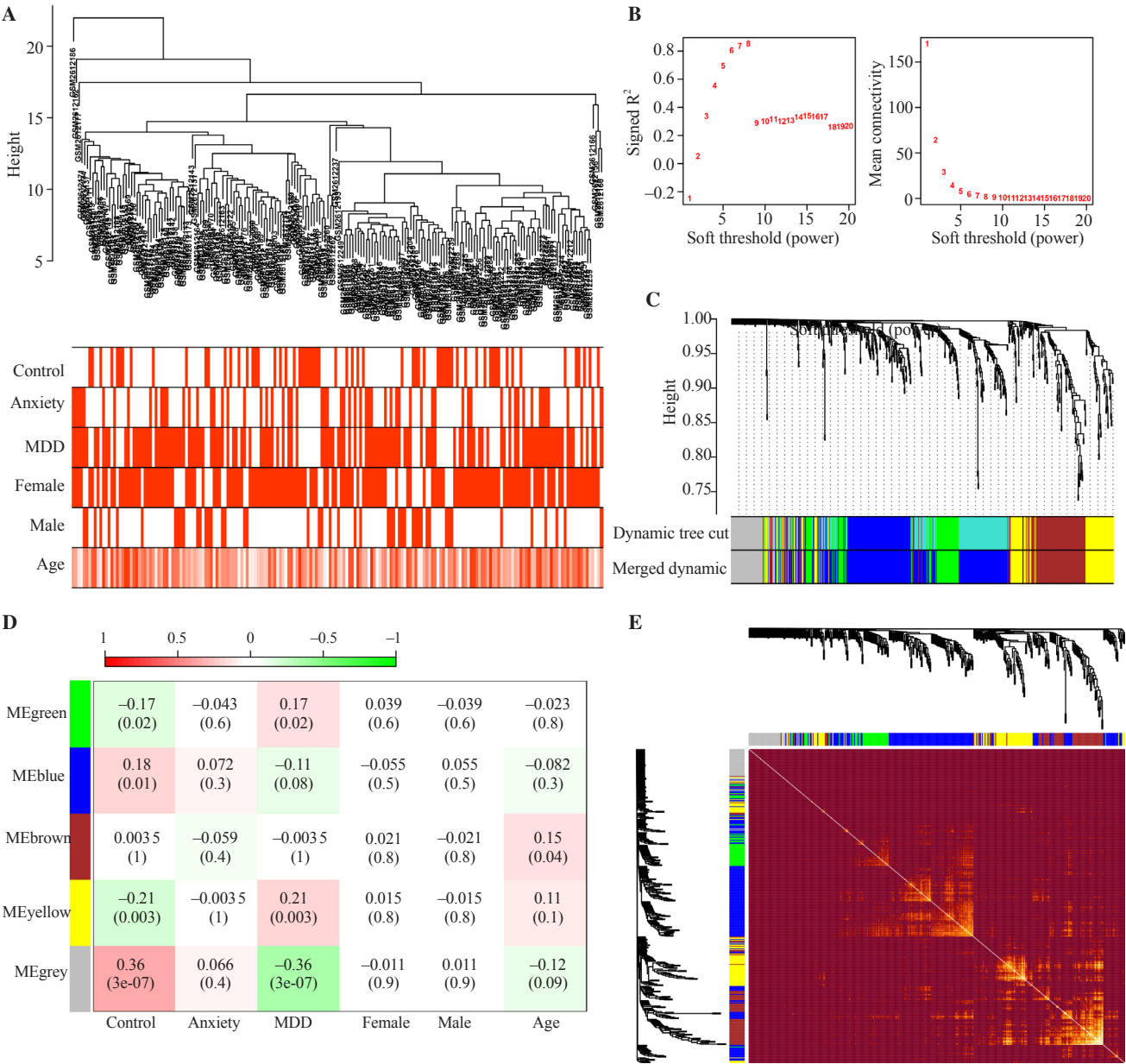


Figure 3. Weighted correlation network by weighted gene coexpression network analysis package. (A) Sample clustering molecular map; (B) Calculation and selection of the optimal soft threshold power; (C) The cluster dendrogram of genes in GSE98793; (D) Relationship between trait and module; (E) Network heatmap plot of the selected genes.

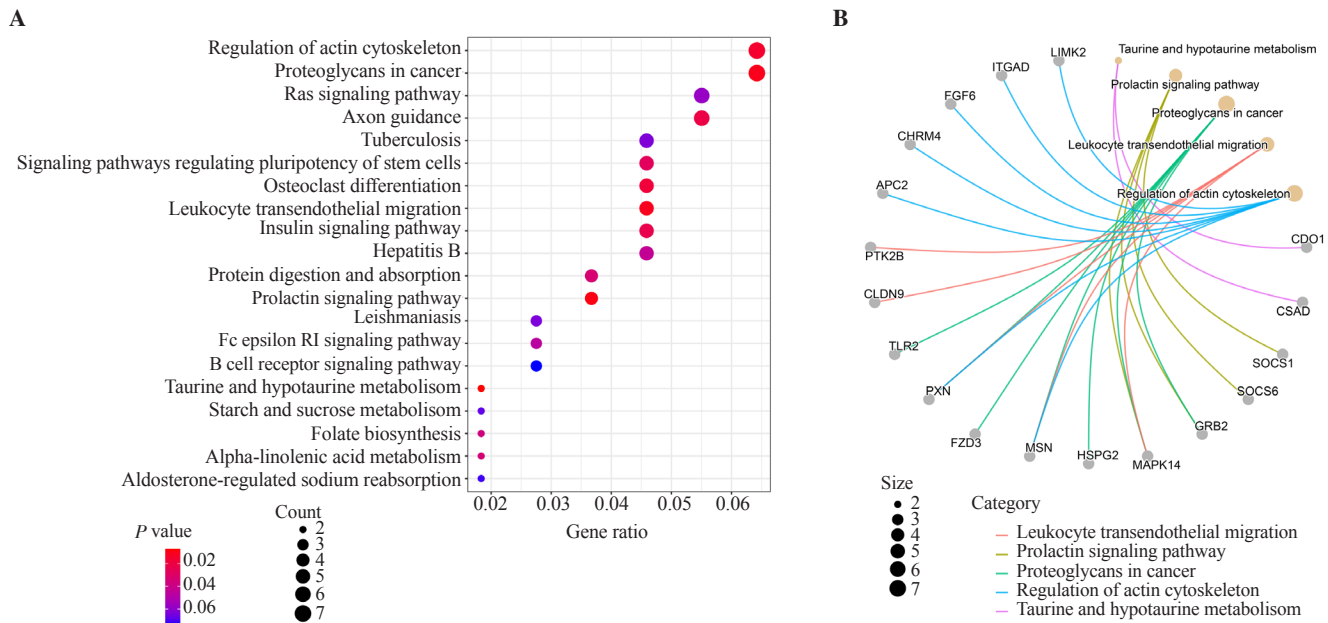


Figure 4. KEGG pathway enrichment analysis of module-related genes. The top 20 (A) and top 5 (B) pathways of module-related genes in KEGG pathway enrichment analysis.

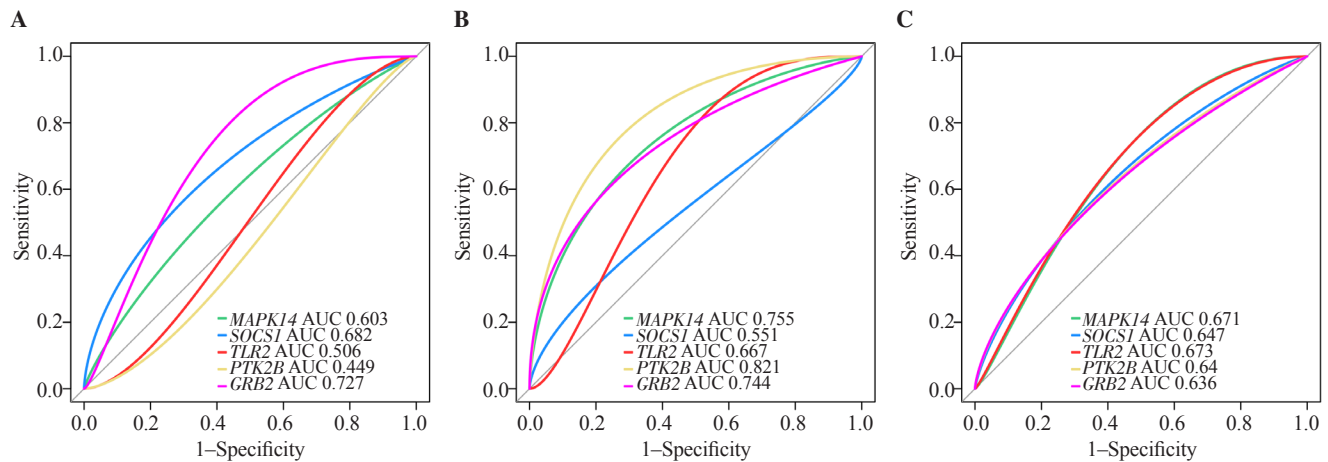


Figure 5. Diagnostic efficiency of hub genes in GSE39653 (A), GSE52790 (B) and GSE98793 (C) datasets.

4A and B). The PPI analysis network is shown in Supplementary Figure 2. The potential hub genes were screened *via* the cytoHubba package in Cytoscape according to the parameters of betweenness (Supplementary Figure 3A), closeness (Supplementary Figure 3B), degree (Supplementary Figure 3C), radiality (Supplementary Figure 3D), and stress (Supplementary Figure 3E). Finally, we identified the genes that interact under these five conditions, including mitogen-activated protein kinase 14 (*MAPK14*), *SOCS1*, *TLR2*, *PTK2B*, and *GRB2* (Supplementary Figure 3F).

3.5. Diagnostic efficiency of the hub genes

We found that these five hub genes were highly correlated with each other (Supplementary Figure 4). In the GSE39653 dataset, *GRB2* was significantly downregulated and *SOCS1* was upregulated in patients with MDD compared with those in the control group

(Table 1). In the GSE52790 dataset, *GRB2* was significantly downregulated and *TLR2*, *PTK2B*, and *MAPK14* were upregulated in patients with MDD compared with those in the control group (Table 1). In the GSE98793 dataset, we found that *GRB2*, *TLR2*, *SOCS1*, *PTK2B*, and *MAPK14* were upregulated in patients with MDD compared with those in the control group (Table 1). *GRB2*, *TLR2*, *SOCS1*, *PTK2B*, and *MAPK14* all showed better diagnostic efficiency in the GSE39653 (Figure 5A), GSE52790 (Figure 5B) and GSE98793 (Figure 5C) datasets.

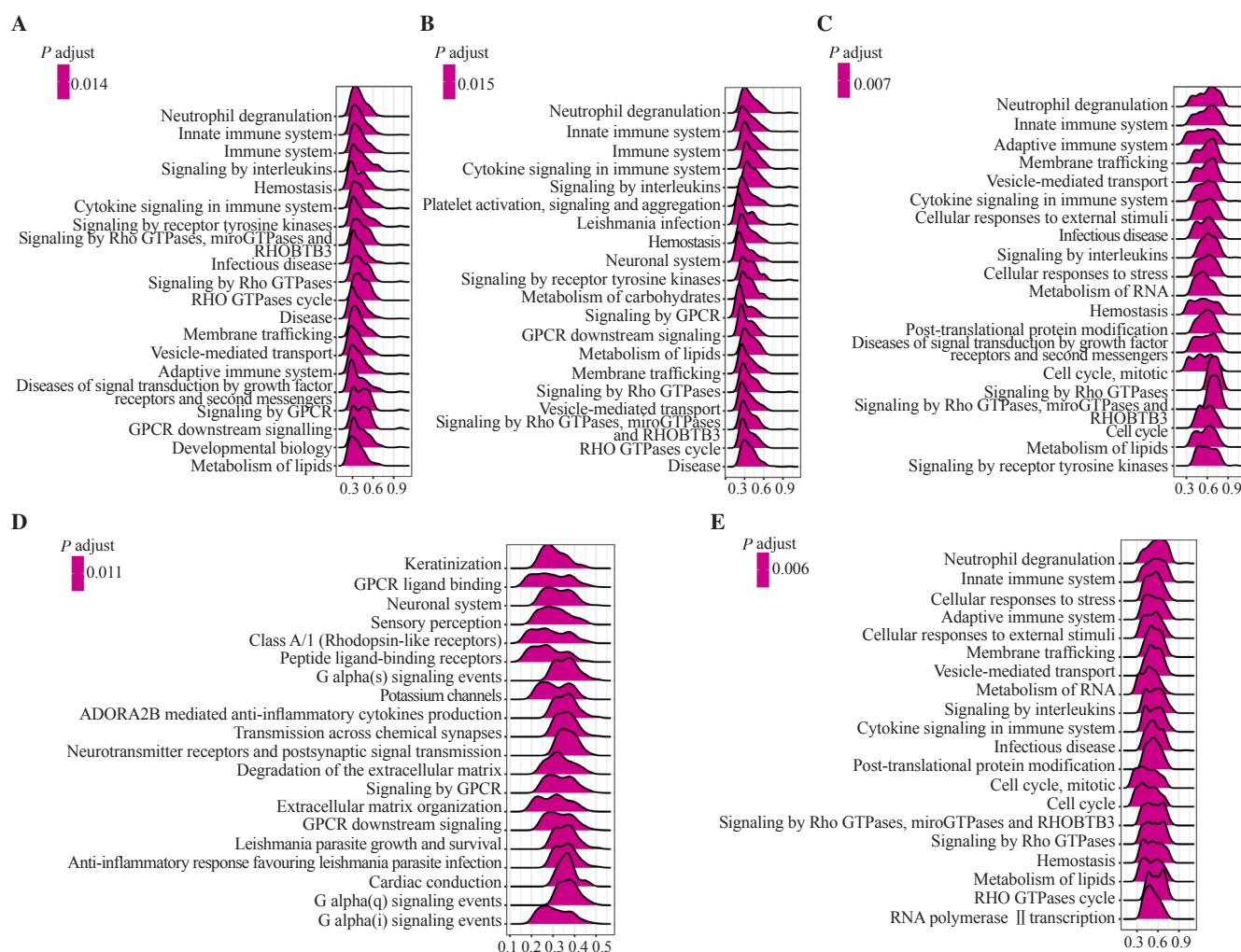
3.6. Correlation analysis and single-gene GSEA of the hub genes

Supplementary Figure 5 shows the top 50 genes positively correlated with *GRB2*, *TLR2*, *SOCS1*, *PTK2B*, and *MAPK14* in the GSE98793 dataset. Single-gene GSEA revealed that *GRB2* was

Table 1. Expression of five hub genes.

Groups	<i>GRB2</i>	<i>TLR2</i>	<i>SOCs1</i>	<i>PTK2B</i>	<i>MAPK14</i>
GSE39653 dataset					
Model	9.10±0.18*	7.06±0.10	7.56±0.23*	7.75±0.10	7.25±0.09
Control	9.29±0.27	7.07±0.15	7.42±0.19	7.72±0.13	7.28±0.08
GSE52790 dataset					
Model	7.74±0.22*	7.62±0.15*	8.38±0.14	7.52±0.09*	8.52±0.20*
Control	7.94±0.17	7.52±0.20	8.38±0.12	7.40±0.07	8.34±0.17
GSE98793 dataset					
Model	10.11±0.17*	10.19±0.39**	5.67±0.26*	8.14±0.25*	8.94±0.32**
Control	10.03±0.20	9.95±0.50	5.59±0.26	8.05±0.25	8.76±0.28

Compared with the control group, * $P < 0.05$, ** $P < 0.001$.

**Figure 6.** The top 20 Reactome pathways in single-gene GSEA analysis of *GRB2* (A), *MAPK14* (B), *PTK2B* (C), *SOCs1* (D) and *TLR2* (E).

correlated with cytokine and Rho GTPase signaling in the immune system (Figure 6A). *MAPK14* was correlated with neutrophil degranulation, cytokine signaling in the immune system, signaling by receptor tyrosine kinases, signaling by Rho GTPases, vesicle-mediated transport, and signaling by interleukins (Figure 6B). *PTK2B* was correlated with post-translational protein modification and cytokine signaling in both the adaptive immune system and the innate immune system (Figure 6C). *SOCs1* was correlated with the neuronal system, postsynaptic signal transmission, and GPCR ligand

binding (Figure 6D). *TLR2* was correlated with RNA polymerase II transcription, cytokine signaling in the adaptive and innate immune system, and signaling by Rho GTPases (Figure 6E).

3.7. In vivo experiments

We constructed a model of depressive-like rats *via* the CUMS procedure and assessed the accuracy of the model *via* various behavioral tests. Our results indicated that there was no significant

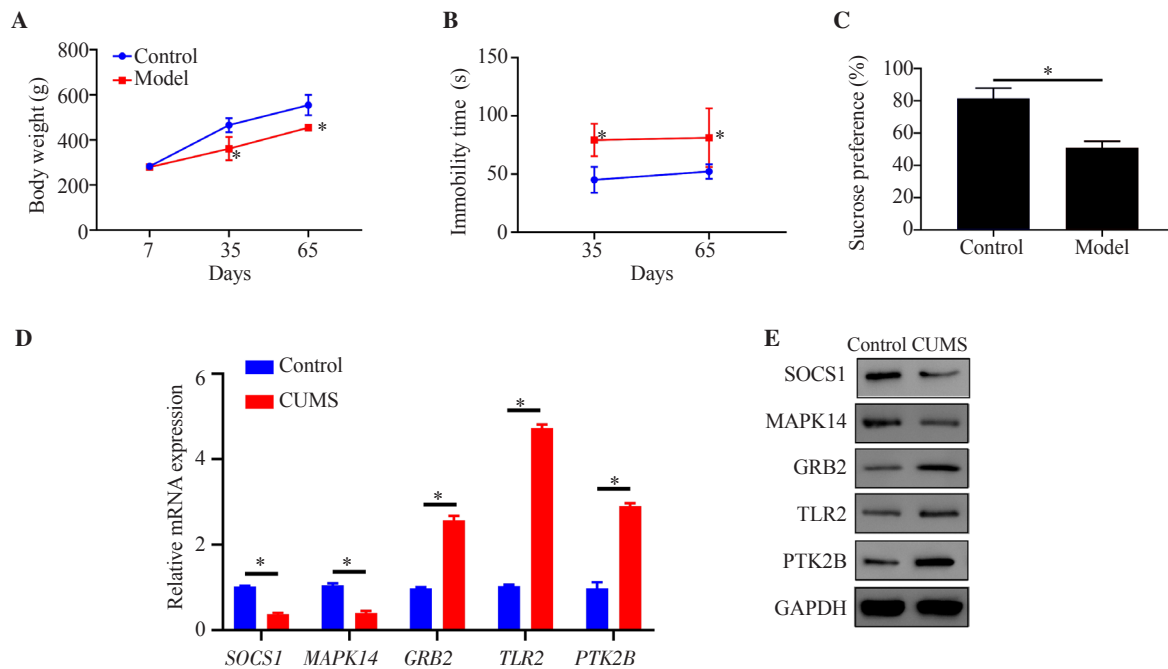


Figure 7. Assessment of chronic unpredictable mild stress (CUMS) model and validation of expression of hub genes in the hippocampus of rats. (A) The weight of rats on days 7, 35 and 65 of the experiments. (B) Immobility time of rats in a forced swimming test on days 35 and 65. (C) Sucrose preference percentage on day 64. (D) The mRNA expression of *SOCS1*, *MAPK14*, *GRB2*, *TLR2*, and *PTK2B* using the RT-qPCR methods. (E) The protein expression of *SOCS1*, *MAPK14*, *GRB2*, *TLR2*, and *PTK2B* in the hippocampus of depression-like rats using the Western blotting methods. Compared with the control group, * $P < 0.001$.

difference in the weight of the rats between the control and model groups after 7 days of adaptive feeding (Figure 7A). Compared with those in the control group, the rats in the model group presented decreased weight and longer immobility time in the forced swimming test on days 35 and 65 of the experiment (Figure 7A and B). Compared with those in the control group, the rats in the model group presented a lower sucrose preference rate in the sucrose preference test on day 64 of the experiment (Figure 7C). RT-qPCR and Western blotting results revealed that the *SOCS1* and *MAPK14* expression levels were significantly decreased; however, the *GRB2*, *TLR2*, and *PTK2B* expression levels were significantly increased in the hippocampal tissues of depression-like rats compared with those of control rats (Figure 7D&E).

4. Discussion

Although progress has been made in understanding the neurobiology of major depression, further research is needed to identify the unique pathophysiology of major depression compared with many other brain disorders[14]. Therefore, the identification of promising biomarkers associated with depression is urgently needed. In our research, we obtained three GEO datasets related to depression, namely, the GSE98793, GSE52790, and GSE39653 datasets, and identified the DEGs between control and depressed

patients *via* cut-offs of $P < 0.05$ and $\log_2FC > 0$. GO and KEGG analyses were performed on the DEGs that were simultaneously dysregulated in two of the three datasets (a total of 658 DEGs). These genes were highly correlated with ribosome biosynthesis, nucleocytoplasmic transport, and folate biosynthesis according to the KEGG analysis.

Subsequently, we conducted WGCNA and found that the MEgreen, MEyellow, and MEgrey modules, which included a total of 244 genes, were highly correlated with MDD. KEGG analysis revealed that these 244 module-related genes were enriched in axon guidance, the Ras signaling pathway, and the regulation of the actin cytoskeleton. Dephosphorylation of presynaptic actin and tubulin and consequent changes in the cytoskeleton may regulate low-frequency depression[15]. Additionally, the hub proteins in the actin cytoskeleton may regulate synaptic plasticity by interacting with various proteins and play a vital role in depression[16]. Li *et al.* reported that inhibiting the Ras signaling pathway can ameliorate depression-like behaviors in rats[17]. Another study revealed that the granulocyte colony-stimulating factor-Ras signaling pathway improved depression-like behaviors[18]. Axonal guidance is involved mainly in the formation of the corpus callosum and neural circuits; the corpus callosum is a bundle of nerve fibers connecting the left and right cerebral hemispheres, and it plays an important role in the coordinated activities of the two hemispheres[19].

PPI network analysis revealed the hub genes involved in

depression, including *MAPK14*, *SOCS1*, *TLR2*, *PTK2B*, and *GRB2*. We investigated the relative expression and diagnostic capabilities of the hub genes. *GRB2*, *TLR2*, *SOCS1*, *PTK2B*, and *MAPK14* all showed better diagnostic efficiency in the GSE39653, GSE52790, and GSE98793 datasets. We detected the levels of these five hub genes in the hippocampal tissues of depression-like and control rats. The results revealed that the mRNA and protein levels of *SOCS1* and *MAPK14* were significantly lower in the hippocampal tissues of depression-like rats than in those of control rats; however, compared with those of control rats, *GRB2*, *TLR2*, and *PTK2B* expression levels were increased in the hippocampi of depression-like rats. He *et al.* reported that *SOCS1* was downregulated in CUMS-induced rats and suppressing the expression of *SOCS1* could ameliorate the depression-induced inflammatory response[20]. Sun *et al.* reported that shanzhiside methylester protects against depression by inhibiting inflammation *via* the miRNA-155-5p/*SOCS1* axis[21]. Another study showed that miR-345-5p/*SOCS1* could activate microglia in hippocampal neurons[22]. Liu *et al.* reported that *MAPK14* was decreased in CUMS-induced rats and that Chaihu-Shugan-San exerts an antidepressive effect by downregulating miR-124 and stopping the inhibition of *MAPK14*[23]. In terms of *TLR2*, Su *et al.* reported that *TLR2*-mediated neuroinflammation triggers depression-like behaviors in mice[24]. Medina-Rodriguez *et al.* reported that *TLR2* impaired the recovery of male mice from a depression-like state[25]. Another study revealed that depression-like rats were characterized by high *TLR2/4* levels in the brain[26]. These results indicate that *GRB2*, *TLR2*, *SOCS1*, *PTK2B*, and *MAPK14* are promising biomarkers for depression.

However, several limitations should be addressed in this study. First, even though several *in vivo* experiments were conducted, most of the results were derived from data analysis, which may have caused deviation to some extent. The sample size of these three datasets is relatively small, and a large sample dataset should be included in a future study. Finally, *in vivo* experiments should be conducted to further improve our analysis.

In conclusion, our bioinformatic analysis focused on identifying the molecular targets and biological pathways associated with depression. Axon guidance, regulation of the actin cytoskeleton, and regulation of the Ras signaling pathway were highly correlated with the development of depression. Furthermore, *GRB2*, *TLR2*, *SOCS1*, *PTK2B*, and *MAPK14* are promising hub genes, and targeting these five genes may be effective treatment strategies for depression.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

YZ performed the experiments and wrote the paper. MZ, LQP, RZ, and KCN contributed to data analysis and interpretation, and provided experimental reagents and materials. WH was responsible for designing the experimental framework and supervising the entire project. All authors reviewed and approved the final manuscript.

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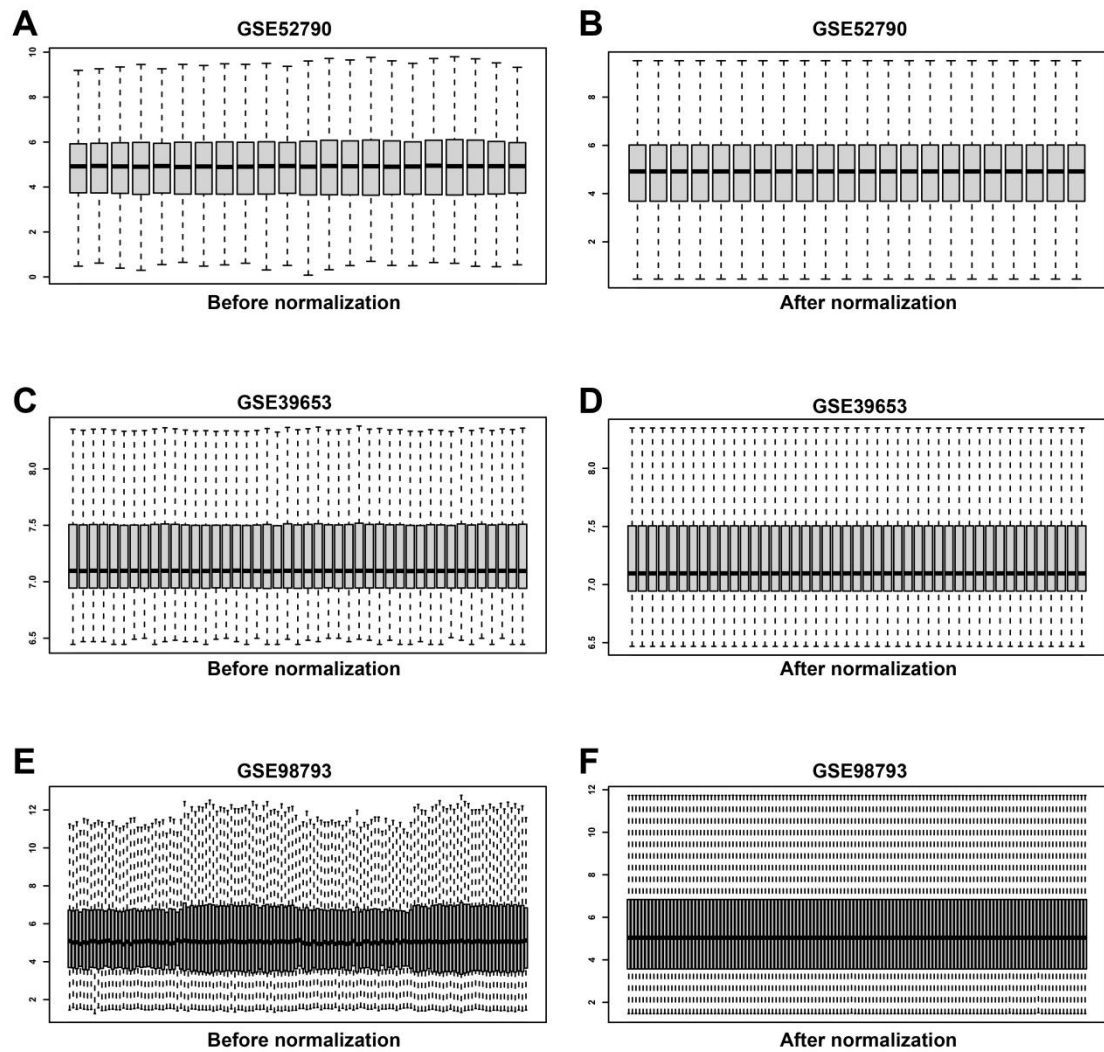
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Supplementary Table 1. Sequences of primers used in RT-qPCR.

Primers	Forward sequence (5'→3')	Reverse sequence (5'→3')
<i>SOCS1</i>	CGAGAACCTGGCACGCATCCCT	TCCACACCCACTCCCTCCGACCT
<i>MAPK14</i>	AGCTTACCGATGACCACGTT	CACGTAGCCGGTCATTTCGTC
<i>GRB2</i>	TTACGGAATCTCGCCGCTAC	TCCGGCTCCTCTAGTCCATC
<i>TLR2</i>	GGAGGTCTCCAGGTCAAATCT	AGTCACCATGGCCAATGTAGG
<i>PTK2B</i>	GCCCGAGGGACTGCAA	TCTTCCCTGGGTTGAAGCTG
<i>GAPDH</i>	ACAGCAACAGGGTGGTGGAC	TTTGAGGGTGCAGCGAACTT

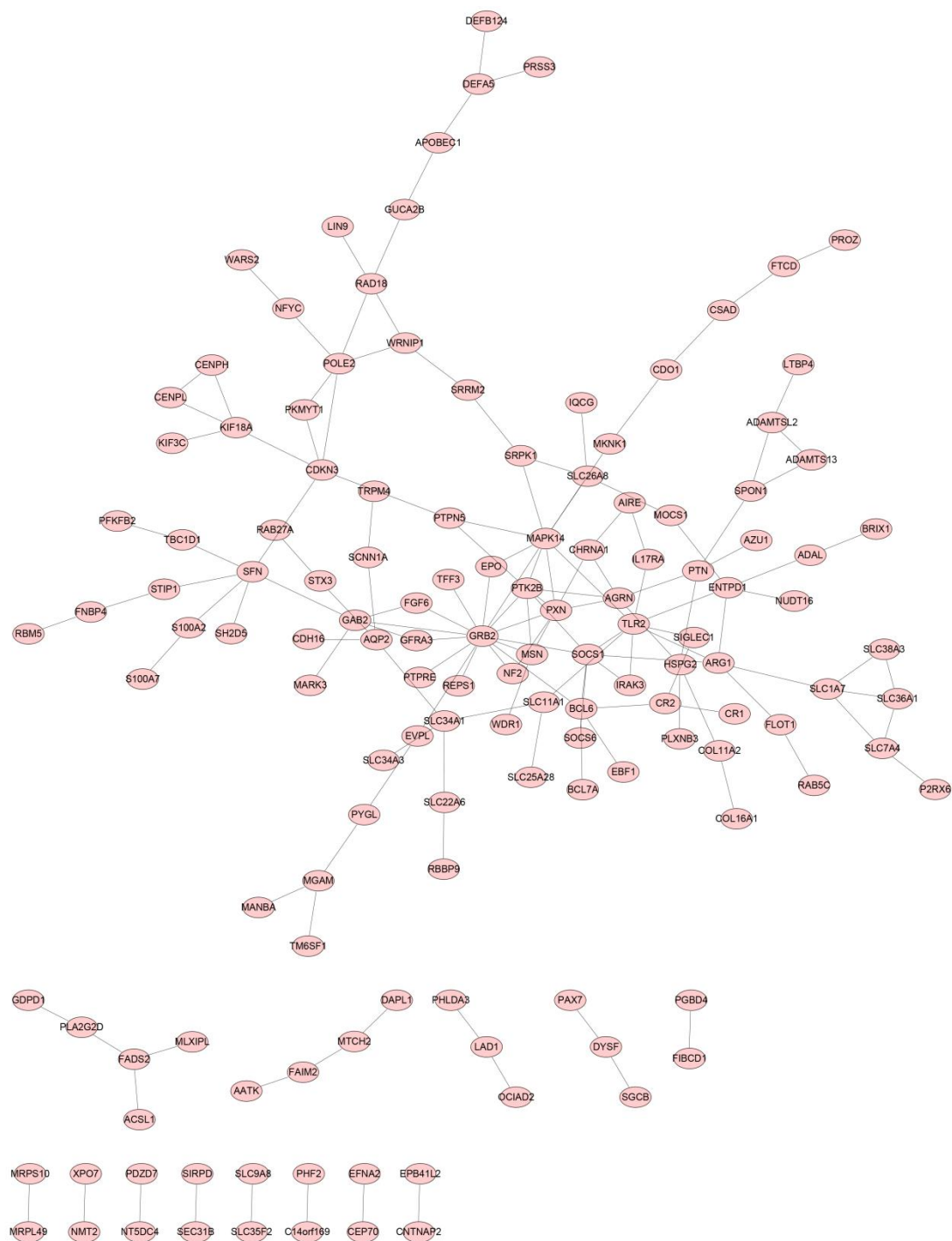


Supplementary Figure 1. Normalization by R software in GSE52790, GSE39653 and GSE98793 datasets.

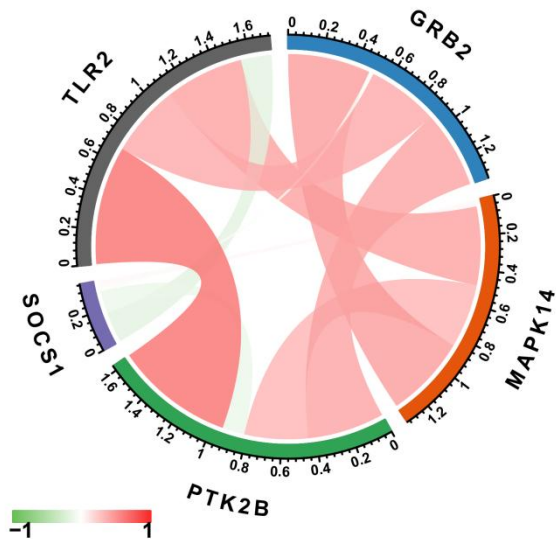
(A and B) Before (A) and after (B) normalization by R software in GSE52790 dataset.

(C and D) Before (C) and after (D) normalization by R software in GSE39653 dataset.

(E and F) Before (E) and after (F) normalization by R software in GSE98793 datasets.



Supplementary Figure 2. PPI interaction analyses network of the 244 module-related genes.



Supplementary Figure 4. The correlation analysis of these five hub genes.

