# Differential Expression and Biological Analysis of circRNA in Acute Ischemic Stroke

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**Research Article**

**Keywords:** Acute ischemic stroke, circRNA, miRNA, Target gene, Bioinformatics

**Posted Date:** May 7th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-476358/v1

# Abstract

**Objective:** The study aimed to analyze the function and signal pathway of target genes by bioinformatics software, and then explore the possible role of circRNA molecules in regulating the pathogenesis of AIS so as to provide new potential molecular targets for AIS therapy.

**Methods:** The study searched the circRNA gene array of AIS from the Gene Expression Omnibus and selected the upregulated and downregulated TOP 10 circRNAs to predict the miRNA binding sites of circRNA, which was then intersected with the differentially expressed miRNAs(DEMs) by miRNA gene array. Furthermore, the downstream target genes of miRNA were predicted jointly by three websites and constructed the ceRNA regulatory network, Database for Annotation Visualization and Integrated Discovery function enrichment analysis and protein–protein interaction analysis by the target genes.

**Results:** miR-337-3p and miR-383 were the binding sites of hsa_circ_0004183, and miR-338-3p and miR-140-3p were the binding sites of hsa_circ_0051778 and hsa_circ_0092342, respectively, in addition, the four miRNAs were closely related to AIS. Kyoto Encyclopedia of Genes and Genomes pathway analysis showed that MAPK signaling pathway, PI3K-Akt signaling pathway, P53 signaling pathway, and erbB signaling pathway were upregulated and downregulated pathways. The MCODE plug-in of Cytoscape was used to screen out a set of the most significant core networks, 21 key protein components, 7 of which were closely related to the occurrence and development of AIS.

**Conclusions:** These findings aim to provide reference for the study of the pathogenesis of AIS by circRNA and broaden the idea of treatment.


# Introduction

Stroke is the second highest mortality cerebrovascular disease, with an incidence of about 0.25–4% and a mortality rate of about 30% [1]. The main types of stroke are hemorrhagic stroke and ischemic stroke (IS). Among them, IS is the most common clinically, accounting for about 80% of all stroke patients, and usually caused by atherosclerotic thrombosis or cerebral artery embolism [2]. At present, intravascular thrombolytic therapy is the most commonly used method for the clinical treatment of acute ischemic stroke (AIS). However, the limitation of treatment time window and the problem of nerve cell ischemia reperfusion injury limit the clinical efficacy. MicroRNA (miRNA) is an endogenous RNA containing about 20 nucleotides that negatively regulate gene expression by targeting the 3 'nontranslated regions of mRNA, leading to mRNA degradation and translation inhibition [3]. CircRNA is a long noncoding RNA molecule, which is a special kind of noncoding RNA produced by the reverse splicing of precursor mRNAs and widely exists in eukaryotic cells [4]. CircRNA does not have the 5 'end cap and multiA tail 3' end of linear RNA but a covalent closed ring structure [5], and the covalent bond in the ring has a strong resistance to ribonuclease degradation, which ensures the structural stability of circRNA molecules [6] and the conserved expression. The function of circRNA is sponge adsorption, protein binding, and protein translation. However, its most prominent function is to regulate gene expression by acting as miRNA

sponge, reducing miRNA activity and upregulating the expression of miRNA-related target genes; thus, it plays a highly competitive role of endogenous RNA (ceRNA) [6] to inhibit the occurrence and development of disease.

Increasing evidence shows that circRNA, as the ceRNA of miRNA, plays a key role in the physiological and pathological functions of AIS. Recent studies found that upregulated circUCK2, as a ceRNA of endogenous miR-125b-5p, can significantly reduce IS infarct volume, reduce neuronal damage, and improve neurological impairment[7]. Another study of cerebral ischemia proved that circHECTD1 negatively regulates the expression of miR-133b and attenuates the apoptosis and NF-κB activation caused by miR-133b[8]; the study also found that circRNA HECTD1 can act as a sponge of miR-142 by regulating autophagy and expanding infarct volume, targeting downstream pathways to promote the activation of astrocytes, thereby increasing the severity of AIS[9]. In addition, circTLK1 acts as an miR-335-3p sponge to inhibit the activity of miR-335-3p, and knockout of circTLK1 can significantly reduce infarct volume, thereby decreasing neuronal damage and improving neurological impairment[10]. The above studies showed that circRNA plays an important role in the pathogenesis of AIS, but specific circRNA target genes and their signaling pathways are still rarely reported.

Therefore, the purpose of this study is to analyze the circRNA gene array in the plasma of patients with AIS in the Gene Expression Omnibus (GEO), screen the abnormally expressed circRNA molecules, and predict the miRNA and mRNA interacting with differentially expressed circRNA (DEcircRNA). The ceRNA regulatory network is constructed, and its target gene function and signal pathway are analyzed by bioinformatic software to explore the possible role of circRNA molecules in regulating the pathogenesis of AIS and to provide new potential molecular targets for AIS therapy.

# Materials And Methods

### Sample acquisition

We obtained a set of standard human plasma circRNA gene chips (GSE133768, GPL21825) from the GEO database (https://www.ncbi.nlm.nih.gov/) and downloaded downloaded platform file and series of matrix file, which included three cases from patients with AIS (GSM3927018–GSM3927020) and three matched normal people (GSM3927015–GSM3927017). Then, a set of miRNA gene array with a large sample size (GSE117064, GPL21263) was obtained from the GEO, including 1612 normal people (GSM3268083–GSM3269694) and 173 patients with stroke (GSM3269695–GSM3269867).

### Quality of gene array

GEO2R online analysis tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) was used to draw Boxplot and UMAP plot by circRNA gene array. In addition, GEO2R online analysis tool also was used to draw expression density plot and UMAP plot by miRNA gene array.

### Screening results of DECs and DEMs

### Screening results of DECs

First, the DEcircRNAs of GSE133768 gene array were obtained by GEO2R. Then, the circRNA name was converted into standard circRNA ID using Perl language. Screening criteria : | log2FC | > 1 and adj. P value < 0.05. GraphPad prism 8.0.1 was used to make a volcanic plot, and heat map and Circos plot were plotted by http://www.bioinformatics.com.cn, an online platform for data analysis and visualization.

### Screening results of DEMs

GEO2R was used to screen the DEmiRNAs in the GSE117064 chip. Screening criteria were | log2FC | > 1, and P value < 0.05. GraphPad prism8.0.1 was used to make the volcanic plot, and heat maps were plotted by http://www.bioinformatics.com.cn, an online platform for data analysis and visualization.

### Construction of the circRNA–miRNA–mRNA regulation

Upgrade or downgrade | log2FC | top 10 circRNAs were selected, the circBase database (http://circbase.org/) was used to define the chromosomal location information of DECs, and the circular RNA Interactom (https://circinteractome.nia.nih.gov/) was used to predict the miRNA combined with DEcircRNA and next intersected the binding miRNA with the DECs as the miRNA regulated by circRNA. Using TargetScan (http://www.targetscan.org/vert_72/), miRDB (http://mirdb.org/index.html) and miRbase (http://www.mirbase.org/) were used to predict the downstream target genes of the intersected miRNAs, and the intersection of the predicted results of the three software was used as the coregulated mRNA. Finally, venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) showed the intersection of miRNAs and mRNAs, and the upregulated and downregulated circRNA–miRNA–mRNA was visualized and analyzed by Cytoscape version 3.7.2.

### Gene function analysis

The target genes corresponding to the upregulation or downregulation of circRNAs were analyzed by gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis through Database for Annotation Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/), and data were visualized analyzed by R software package.

### Protein–protein interaction network construction and MCODE key gene screening

Protein–protein interaction (PPI) analysis was performed on the predicted target genes using the online tool String (https://string-db.org/), and TSV data format was imported into Cytoscape. Then, the key genes were screened through MCODE. Standard Settings for MCODE: Degree Cutoff: 2, Node' Score Cutoff: 0.2, K-Core: 5, and Max. Depth: 100.

### This study strictly follows the above process, and the technical route is shown in

# Results

## Screening of DEcircRNAs and DEmiRNAs

The two gene arrays were normalized for further analysis(Fig. 2A–D). A total of 34 upregulated circRNAs and downregulated circRNAs were screened from the circRNA gene array. The differential genes were clustered according to their expression levels to identify the expression patterns of different genes and display the expression level of differential circRNA visually(Fig. 3A,B). The position and expression of DEcircRNA in chromosomes are demonstrated in Figure 3C. Next, 10 upregulated or downregulated circRNA with the most significant expression changes were selected (Table 1) for follow-up analysis. A total of 823 miRNAs had differential expression in miRNA gene array, among which 755 were upregulated and 68 were downregulated( Fig. 4A,B).

Table 1 Basic information of TOP10 circRNA in acute stroke group and normal control group

| circRNA | Expression changes | Chromosomal localization | Gene location | LogFC | adj.p |
|---|---|---|---|---|---|
| hsa_circ_0001649 | up | chr6:146209155-146216113 | SHPRH | 1.6792345 | 0.04364 |
| hsa_circ_0064794 | up | chr3:33853563-33863574 | PDCD6IP | 1.6088067 | 0.04056 |
| hsa_circ_0006987 | up | chr19:34921480-34929671 | UBA2 | 1.5337347 | 0.04838 |
| hsa_circ_0006097 | up | chr1:247039338-247040604 | AHCTF1 | 1.5049683 | 0.02929 |
| hsa_circ_0000832 | up | chr18:13037235-13040955 | CEP192 | 1.4324632 | 0.0459 |
| hsa_circ_0047376 | up | chr18:29691716-29691882 | RNF138 | 1.4010334 | 0.03369 |
| hsa_circ_0050525 | up | chr19:34925772-34929671 | UBA2 | 1.3774908 | 0.04415 |
| hsa_circ_0001630 | up | chr6:99860426-99864304 | PNISR | 1.308806 | 0.01987 |
| hsa_circ_0005227 | up | chr2:162036124-162061304 | TANK | 1.305516 | 0.01268 |
| hsa_circ_0038673 | up | chr16:24788253-24788679 | TNRC6A | 1.2900568 | 0.04744 |
| hsa_circ_0082319 | down | chr7:129662158-129679387 | ZC3HC1 | -3.5193172 | 0.0024 |
| hsa_circ_0004099 | down | chr11:9225206-9229179 | DENND5A | -3.4978114 | 0.01027 |
| hsa_circ_0052372 | down | chr19:59058742-59058878 | TRIM28 | -3.4315232 | 0.00194 |
| hsa_circ_0087631 | down | chr9:99220660-99233376 | HABP4 | -3.4136852 | 0.00186 |
| hsa_circ_0045932 | down | chr17:76823326-76825089 | USP36 | -3.2293832 | 0.00176 |
| hsa_circ_0010027 | down | chr1:13933667-13937011 | PDPN | -3.1615983 | 0.00186 |
| hsa_circ_0092342 | down | chr11:8706439-8707219 | RPL27A | -3.1003026 | 0.00122 |
| hsa_circ_0004183 | down | chr10:13701322-13717044 | FRMD4A | -2.9843806 | 0.02081 |
| hsa_circ_0051778 | down | chr19:49298318- | BCAT2 | -2.9783938 | 0.00176 |

| | | | | | |
|---|---|---|---|---|---|
| | | 49303095 | | | |
| hsa_circ_0007850 | down | chr9:2804256-2812362 | KIAA0020 | -2.8943631 | 0.00176 |

## ceRNA regulatory network

The miRNA prediction of circRNAs previously selected for upregulation and downregulation of TOP10 was performed by using the Circular RNA Interactom database. The results showed the following: The upregulated circRNAs were bound to 100 miRNAs, and the downregulated circRNAs were bound to 281 miRNAs. The upregulated and downregulated miRNAs intersected with the DEmiRNA. Finally, seven upregulated circRNAs corresponded to 14 miRNAs, and seven downregulated circRNAs corresponded to 22 miRNAs(Fig. 5A, B). TargetScan, miRDB, and miRbase were used to predict the target genes of the screened miRNAs. The results showed that 267 genes were upregulated, and 471 genes were downregulated( Fig. 5C,D). Cytoscape was used to construct the upregulated and downregulated circRNA–miRNA–mRNA interaction network( Fig. 6A, B). The results are as follows: In the upregulated ceRNA network, hsa_circ_0006097 and hsa_circ_0005227 had more than five miRNA binding sites, and hsa-miR-581, hsa-miR-485-3p, hsa-miR-767-3p, and hsa-miR-1265 each had more than two circRNA binding sites. In the downregulated ceRNA network, hsa_circ_0087631, hsa_circ_0007850, and hsa_circ_0004183 all had binding sites with hsa-miR-330-5p, and hsa_circ_0007850, hsa_circ_0092342, and hsa_circ_0004183 all had more than five miRNA binding sites, suggesting that the mutual regulation between the above circRNAs and miRNAs plays an important role in the ceRNA network.

## GO and KEGG analysis results

GO and KEGG enrichment analysis were performed on target genes regulated by the upregulated and downregulated circRNAs in the regulatory network diagram using the online database of DAVID. The upregulated GO analysis results in Figure 7A show the following: In terms of biological processes, positive regulation of transcription, DNA-templated, positive regulation of transcription from RNA polymerase II promoter, and negative regulation of transcription from RNA polymerase II promoter were significantly enriched. In terms of cell component, the nucleoplasm, nucleus, cytoplasm, and nuclear matrix were significantly enriched. Molecular functions were mainly concentrated in the binding fields of transcription factors related to gene transcription regulation and sequence-specific DNA binding. These items were closely related to the occurrence and development of AIS. The downregulated GO analysis results in Figure 7B show the following: The upregulated entries were similar to the upregulated entries in terms of biological processes. In terms of cell components, the nucleoplasmic, cytoplasmic, nuclear cyclin-dependent protein kinase holoenzyme complex, and PCG protein complex were significantly enriched. Molecular functions were mainly enriched in protein-bound transcription coactivator activity, transcription factor activity, sequence-specific DNA binding, nucleic acid binding, and DNA binding, which

were closely related to the occurrence and development of AIS. The KEGG enrichment results show the following: Among the top 10 enrichment pathways, MAPK signaling pathway, PI3K-Akt signaling pathway, P53 signaling pathway, and erbB signaling pathway were upregulated and downregulated pathways, among which MAPK signaling, P53 signaling pathway, and erbB signaling pathway interacted with one another in the PI3K-Akt signaling pathway, which could synergically affect the occurrence and development of AIS( Fig. 8A–C).

### PPI network

The 529 genes obtained from the intersection of Targetscan, miRDB, and miRTarbase were input into the online STRING database, and then the data were imported into Cytoscape software to obtain the PPI network diagram with 529 nodes and 1970 pairs of related relationships. The most significant core network was screened out by MCODE plug-in, which was composed of 21 key genes, including 21 nodes and 146 pairs of related relationships(Fig. 9A, B).

## Discussion

In recent years, RNA biomarkers have attracted wide attention in the diagnosis of IS[11]. As a kind of noncoding RNA, circRNA is rich in expression and tissue-specific in the nervous system, which can regulate complex brain functions and various neural activities [4]. Studies by scholars showed that the expression of circRNA in brain tissues is closely related to AIS, and circRNA expressed in brain tissues can be repeatedly detected in human peripheral blood samples [12]. Therefore, in this study, the bioinformatic prediction method was used to analyze the circRNA expression chip in AIS plasma in the GEO database. After the miRNAs predicted by circRNA and DEmiRNAs were intersected, the downstream target genes were predicted and analyzed. In this paper, through the construction of ceRNA regulatory network, functional enrichment analysis, and PPI analysis, to provide a certain value for the diagnosis, treatment, and prognosis of AIS.

According to the ceRNA network of circRNA–miRNA–mRNA, hsa_circ_0004183, hsa_circ_0092342, and hsa_circ_0051778 had 4–6 miRNA binding sites, and miR-337-3p and miR-383 were the binding sites of hsa_circ_0004183. Studies found that after cerebral ischemia, the level of miR-337-3p gradually increased, and LNA antimelanin-337-3p could cross the blood–brain barrier, enter the nervous system, and downregulate the expression of endogenous miR-337-3p, which could reduce ischemic brain injury and mediate neuroprotection in the cerebral ischemia environment[13]. In addition, researchers found that the downregulation of miR-383 could upregulate the expression of PPARγin the early stage of cerebral ischemia to play an anti-inflammatory, neuroprotective function, suggesting that miR-383 might be a potential therapeutic target for stroke[14]. As the binding site of hsa_circ_0051778, Wei et al. found that the expression of miR-338-3p is downregulated after cerebral ischemia, which could increase the survival rate and decrease the apoptosis rate of neurons by negatively regulating the expression of CpKCγ, reduce

the injury of ischemic neurons, and play a protective role[15]. As the binding site of hsa_circ_0092342, miR-140-3p is also one of the prognostic biomarkers of cardiovascular disease identified[16], which is involved in the regulation of oxidative stress and apoptosis after myocardial infarction, and this disease has many common features with IS[17]. In addition, a study found that lncRNA KCNQ1OT1 aggravates cerebral ischemia/reperfusion injury by targeting and binding to miR-140-3p, and the overexpression of miR-140-3p could significantly reduce inflammation, oxidative stress, and apoptosis of oxygen–glucose deprivation/reglucose cells; thus, miR-140-3p provides a new therapeutic target for IS[18]. Future studies are needed to verify the mechanism of action of these circRNAs and bound miRNAs in AIS.

KEGG enrichment analysis found that whether upregulated or downregulated circRNA, PI3K-Akt signal pathway and MAPK signal pathway are two pathways to enrich numerous genes in TOP10, which are involved in the occurrence and development of AIS. Li et al.'s studies found that the PI3K/Akt pathway is involved in several biological processes and closely related to the progress of IS[19]. A study reported that GSK-3 inhibition/β-cateninactivation through PI3K/Akt signaling mediates the neuroprotective and neurogenesis-promoting effects of the ethyl acetate crude extract of Antrodia camphorata and its active ingredientEK100 after an AIS injury in mice[20]. Similarly, miR-18b prevents cerebral ischemic injury by inhibiting ANXA3 and activating the PI3K/AKT pathway[21]. In addition, Wang et al. found that electroacupuncture inhibits autophagy and apoptosis of neurons after IS through PI3K/AKT pathway, which plays a neuroprotective effect after stroke[22]. More evidence showed that the MAPK signal pathway is involved in the pathogenesis of IS. The phosphorylation of the MAPK family (ERK, p38, and JNK) blocks the activation of NF-kappa B and inhibits the expression of many chemokines, which provides substantial therapeutic potential for the treatment of IS[23]. The overexpression of miR-339 inhibits cell viability and induces apoptosis through the MAPK pathway[24]. In addition, miR-128 has neurotoxic effects on ischemia-reperfusion injury by promoting the phosphorylation of ERK and PTEN, and increasing the expression of ERK, PTEN, and cyclin A2, which could aggravate the neuronal damage in patients with AIS. Qingnao dripping pills could reduce neuroinflammation by inhibiting the MAPK signal pathway, which could significantly improve the neurological deficit scores and reduced infarct to play a neuroprotective effect on AIS rats[25]. In addition, a study found that DL-3n-Butylphthalide might protect the integrity of AIS cerebral blood–brain barrier and attenuate brain damage by reducing cerebral edema and infarct volume, and improving the neurological severity score through the MAPK signal pathway[26]. In the future, experiments can be conducted to verify the prognostic effect of the interaction between differentially expressed circRNAs, PI3K/Akt pathway, and MAPK signal pathway on AIS.

The most significant PPI network was screened by MCODE plug-in, which was composed of 21 key proteins, among which seven proteins, namely, STAT3, GSK3B, TP53, NOTCH1, IGF1R, CXCR4, and CDK6, are closely related to the occurrence and development of IS. Liu et al.'s study found that melatonin enhances the expression of phosphorylated STAT3 in microglia induced by oxyglycogen deprivation neurons, promotes the production of anti-inflammatory markers, and inhibits the expression of proinflammatory markers, which is conducive to the improvement of the neurological function of IS[27].

GSK3B is highly expressed in the mammalian brain and deeply involved in nerve cell death caused by cerebral ischemia injury[28]. TP53 is a tumor suppressor gene located on chromosome 17, which is upregulated after ischemic brain injury, and its knockout expression could prevent ischemic injury in transgenic mice[29]. Lentivirus-mediated Notch1 gene knockout could reduce the area around cerebral infarction and induce angiogenesis in ischemic sites[30], In addition, XLOC_035088 silencing reduces the expression of Notch1, affecting cell viability, proliferation, and apoptosis, which promotes the improvement of neural function and inhibits IS[31]. IGF1R is a transmembrane receptor tyrosine kinase. Zhang et al.'s studies found that lncRNA FGD5-AS1 protects IS neurons from injury through miR-223-mediated IGF1R expression[32]. In addition, after lncRNA H19 is inhibited, the expression of IGF1R IS increases, which simultaneously activates the mTOR pathway and promotes axon germination and functional recovery after IS[33]. CXCR4 is a G protein coupled receptor, which could enhance the therapeutic effect of EPC on IS by increasing the expression of CXCR4 and HIF-1a[34]. Moreover, CXCR4 antagonists could destroy the migration of stem cells and prevent newborn neurons from migrating to the ischemic area[35]. miR-99a could inhibit the expression of cyclin D1 and CDK6 to prevent apoptosis, thus reducing neuronal injury after cerebral ischemia reperfusion[36]. In addition, Liu et al. demonstrated that minmonoside could inhibit the overexpression of cyclin D1 and CDK6 caused by cerebral ischemia, thus preventing the abnormal cell cycle of nerve cells, controlling nerve cell apoptosis, and playing a neuroprotective role against IS[37]. The above studies showed that these seven proteins are closely related to AIS, whereas other proteins need more studies to confirm the mechanism of action with AIS in the future.

# Conclusion

In summary, bioinformatic methods are used in this study to identify DEcircRNAs by AIS and predict DEcircRNAs' bound miRNA, take the intersection with DEmiRNAs, and finally predict and analyze the downstream target genes. The results show that miR-337-3p, miR-383, miR-338-3p, and miR-140-3p are related to AIS from the ceRNA regulatory network. In addition, functional enrichment analysis shows that the MAPK signal path and PI3K-AKT signal path are closely related to AIS. Moreover, MCODE plug-in is used to find the most significant core network of PPI network, consisting of 21 key proteins, among which TAT3, GSK3b, TP53, Notch1, IGF1R, CXCR4, and CDK6 are important protein molecules in the occurrence and development of AIS. The analysis of the above content provides a theoretical basis and reference direction for the mechanism of circRNA in AIS.

# Declarations


## Funding

National Natural Science Foundation of China (81960768)


## Disclosure of interest

The authors declare that they have no conflict of interest.

### Acknowledgements

Thanks to the Second Affiliated Hospital of Guangxi Medical University for its support to the research. We acknowledge GEO database for providing their platforms and contributors for uploading their meaningful datasets.

### Authors' contributions

Zheng and Liu followed up and collected the research data, and wrote the paper after analysis. Lin and He were responsible for analyzing the rationality of the audit data and participating in the writing of the paper. Liao and Yang participated in the writing of the paper. Long, the correspondent author, proposed the overall design of the paper, participated in the writing of the paper, and put forward guiding opinions on the paper, which was finally finalized.

### Disclosure

GEO belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

## References

## Figures

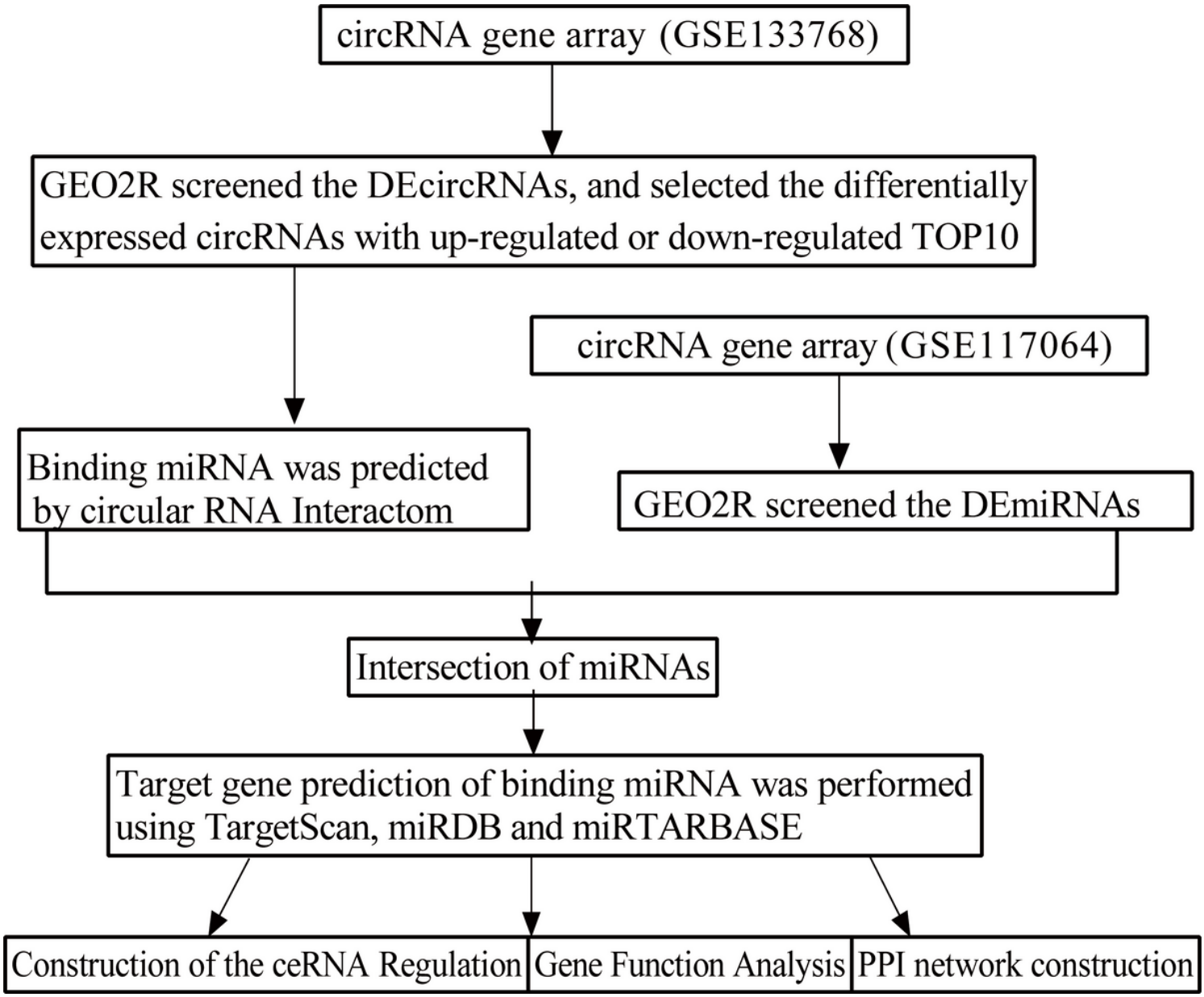
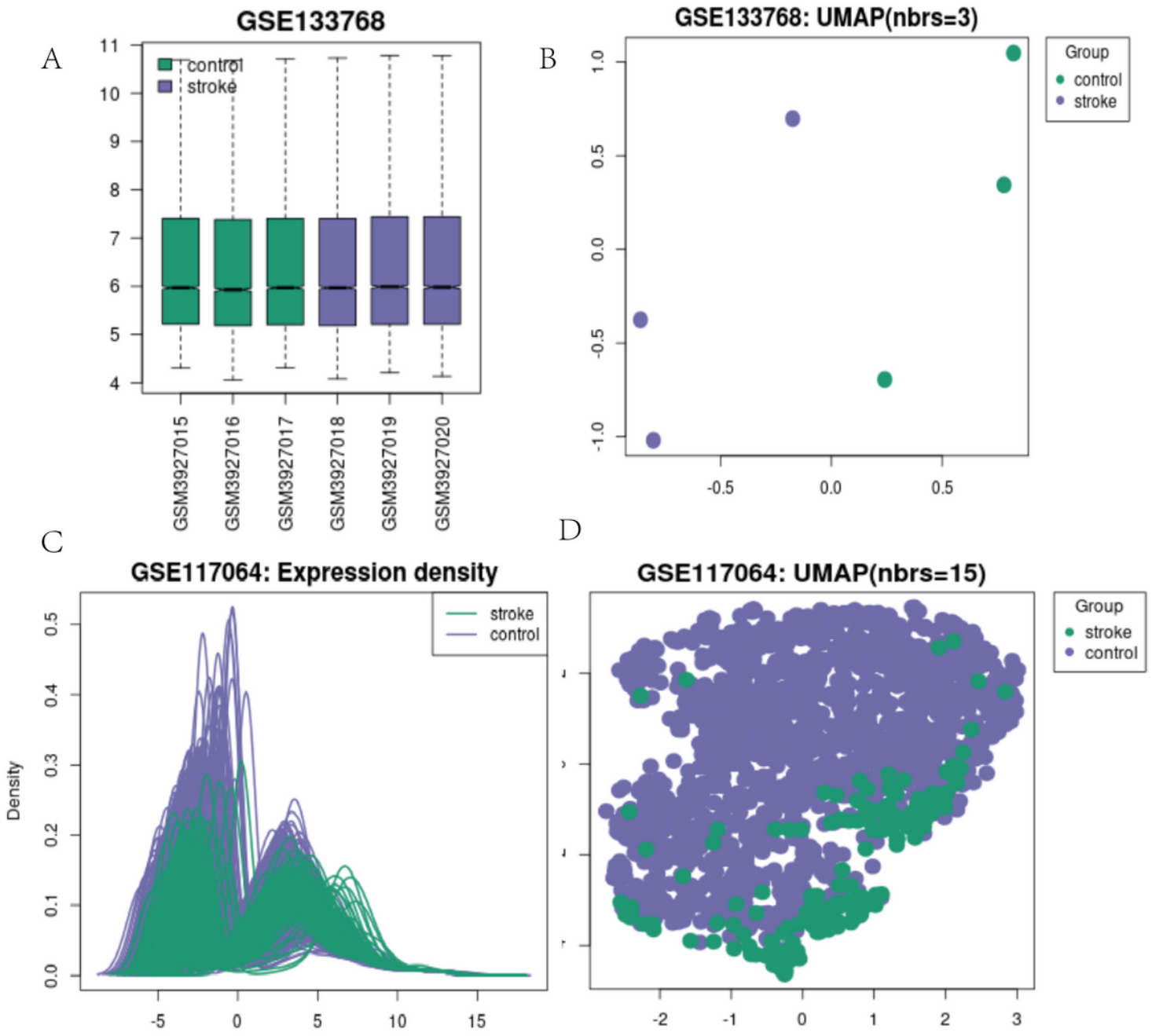


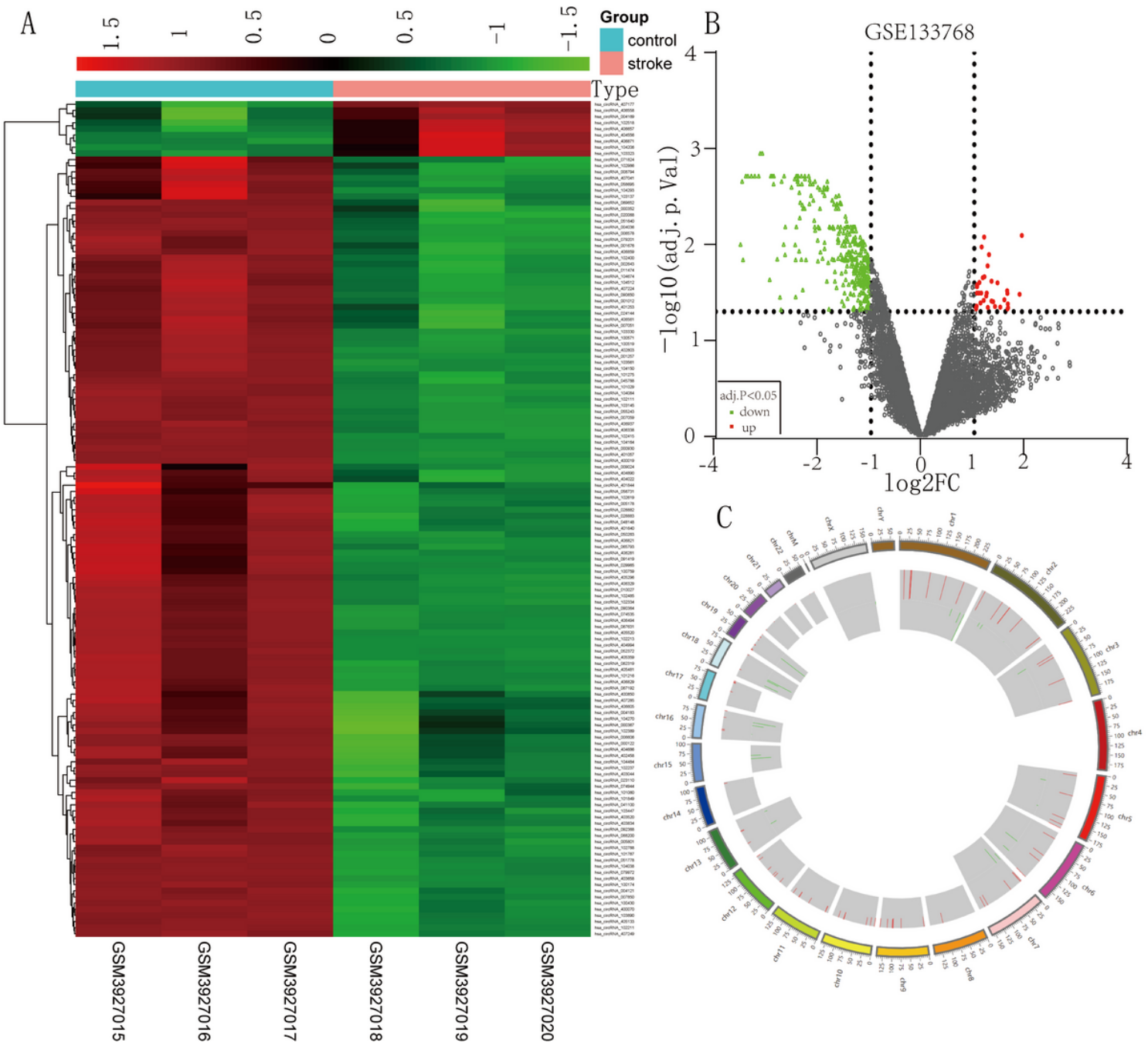

**Figure 1**

Flow chart of data acquisition and analysis




## Figure 2

Quality of gene array (A) BoxplotA, (B) UMAP plot, (C) Expression density plot, and (D) UMAP plot. A and B test the quality of the circRNA microarray. C and D test the quality of the miRNA microarray.




## Figure 3

DEcircRNAs in patients with AIS and normal people. (A) Heat map: Red is upregulated gene expression, and green indicates downregulated gene expression. (B) Volcanic plot: Red means upregulated, and green means downregulated. (C) Circos plot: In the position of circRNA on the chromosome, the short red line in the inner ring indicates upregulation, and the green line indicates downregulation.

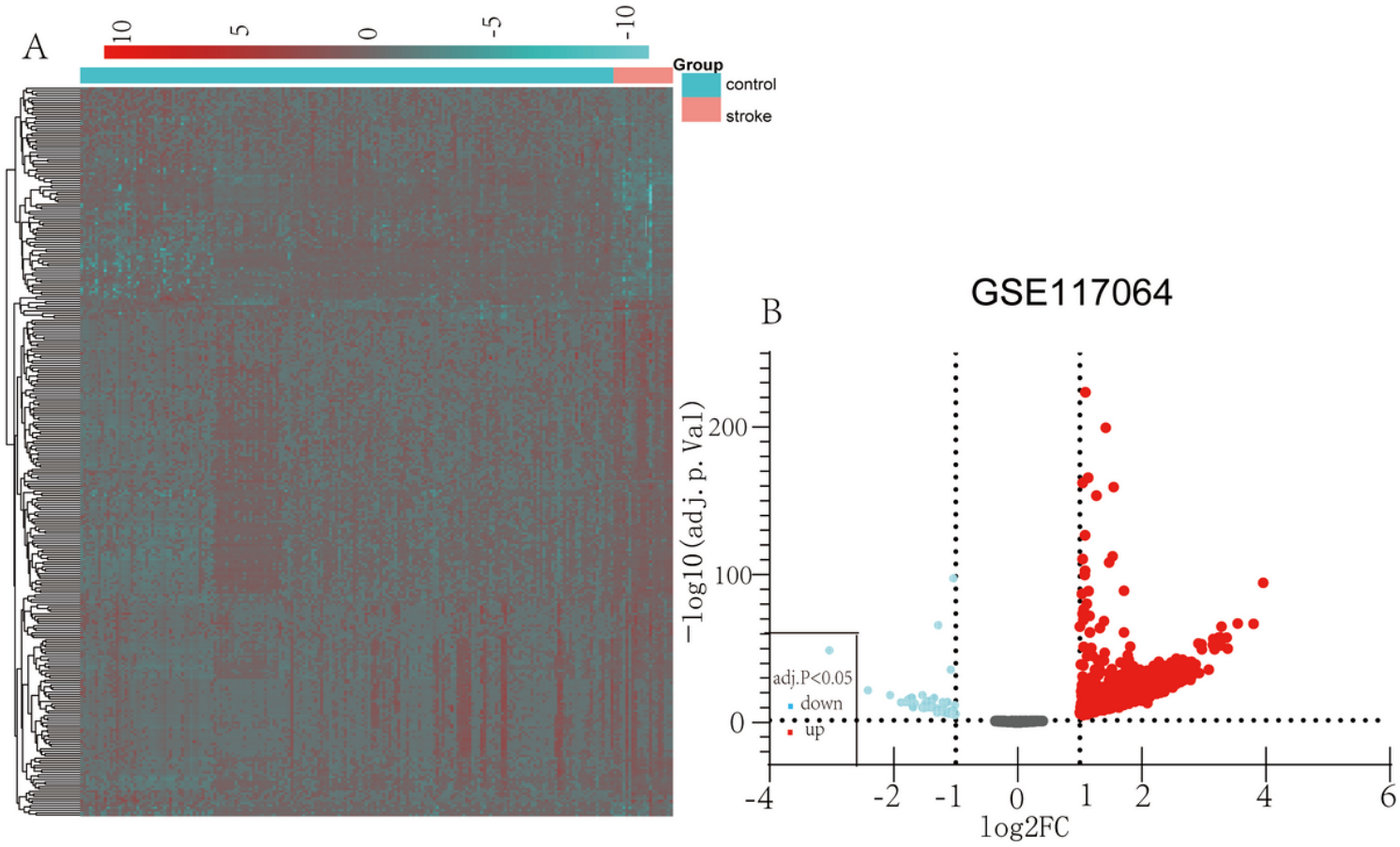
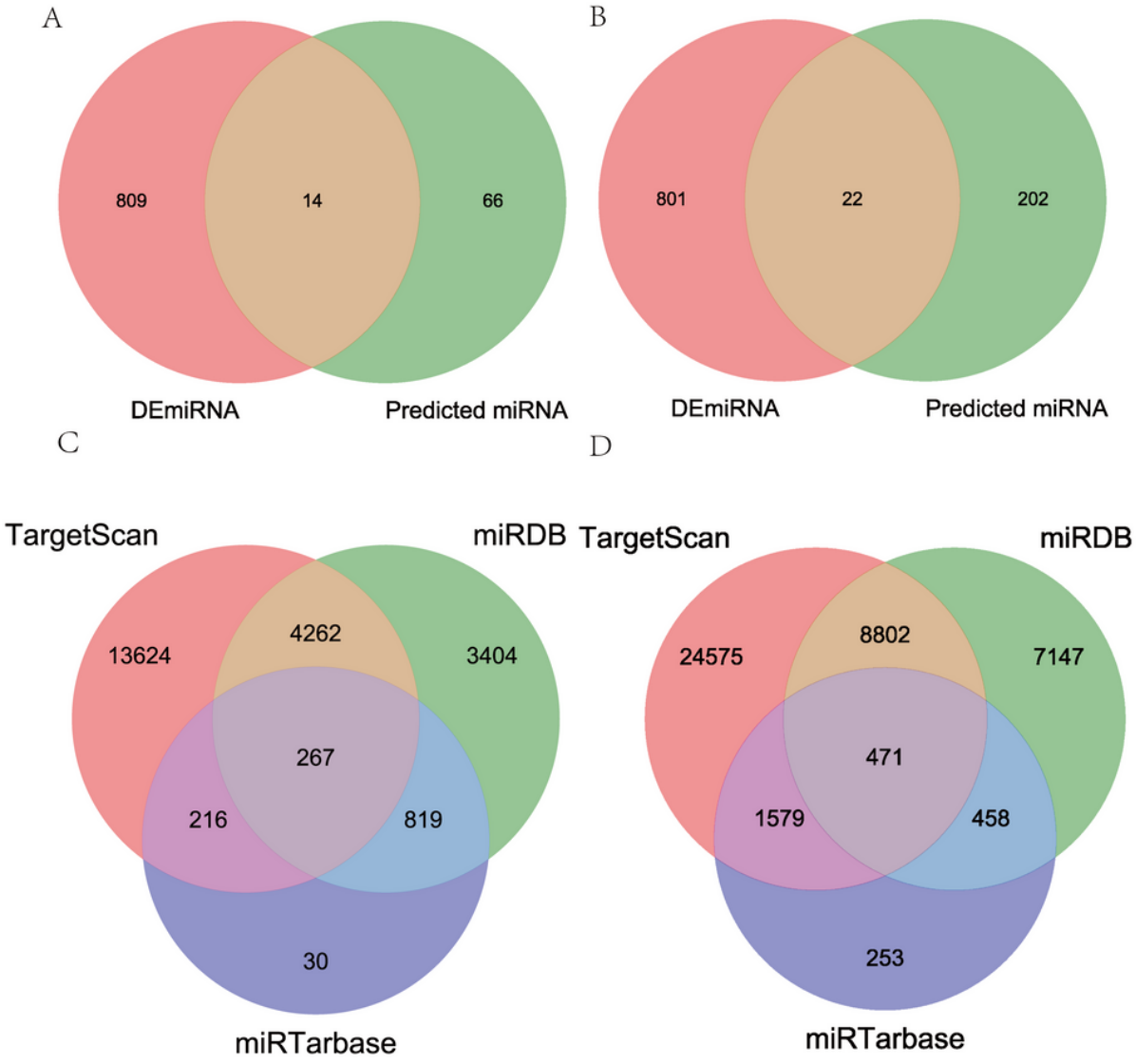


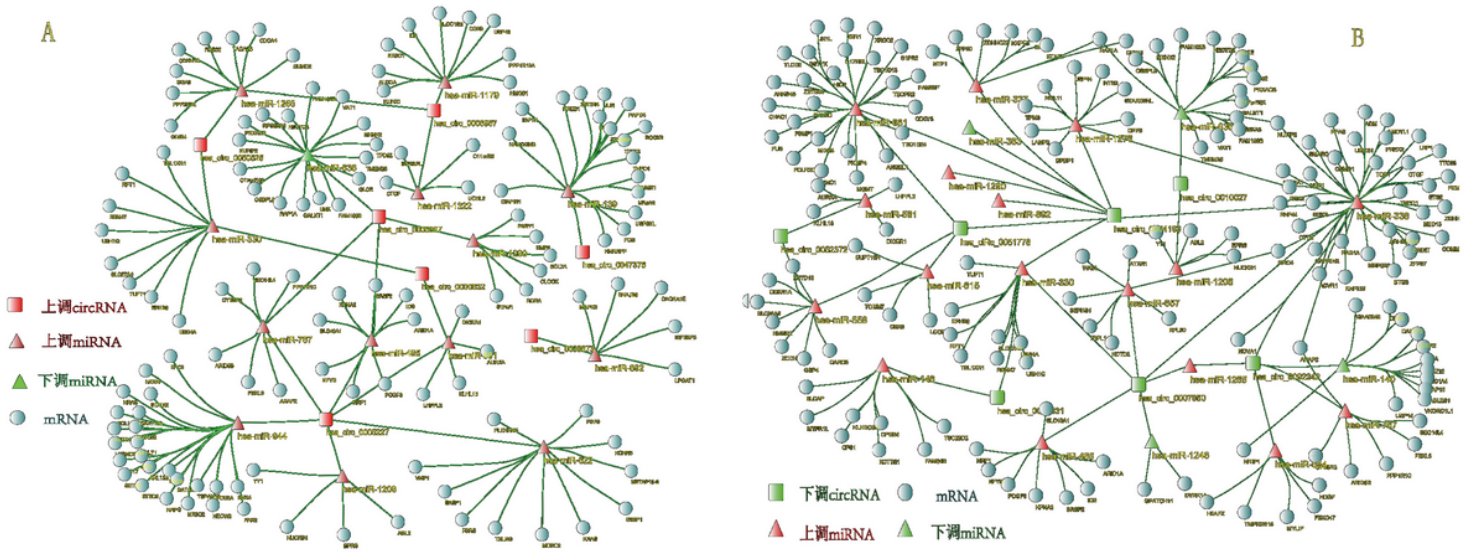

**Figure 4**

DEmircRNAs in patients with AIS and normal people. (A) Heat map: Red is upregulated gene expression, and blue indicates downregulated gene expression. (B) Volcanic plot: Red means upregulated, and blue means downregulated.



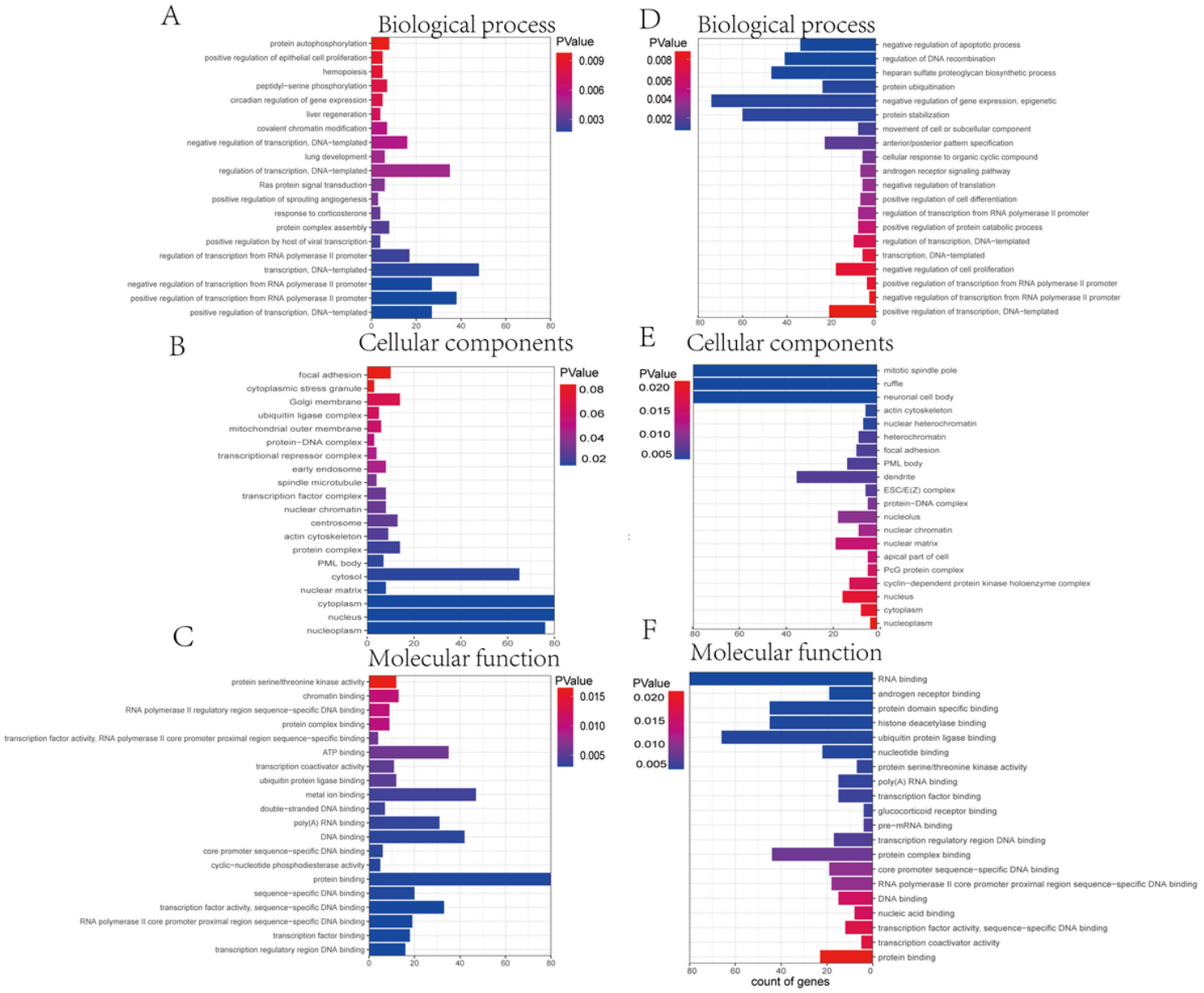

## Figure 5

Venn diagram. (A) is the intersection of upregulated miRNA, (B) is the intersection of downregulated miRNA, (C) is the intersection of upregulated mRNA, and (D) is the intersection of downregulated mRNA.



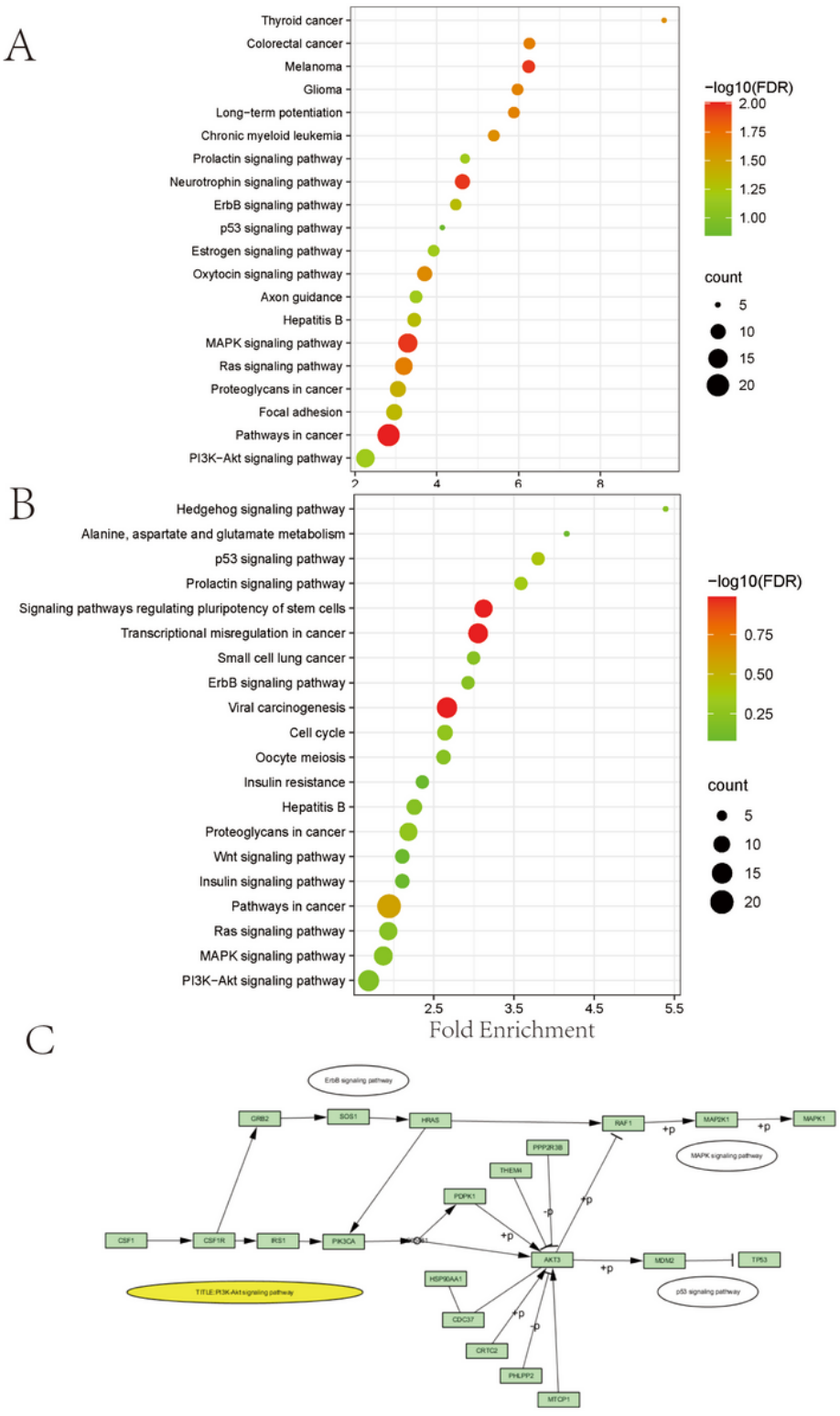

## Figure 6

(A) Upregulated CERNA regulatory network. (B) Downregulated ceRNA regulatory network. The red square represents upregulated circRNA, the green square represents downregulated circRNA, the red triangle represents upregulated miRNA, the green triangle represents downregulated miRNA, and the circle represents mRNA.



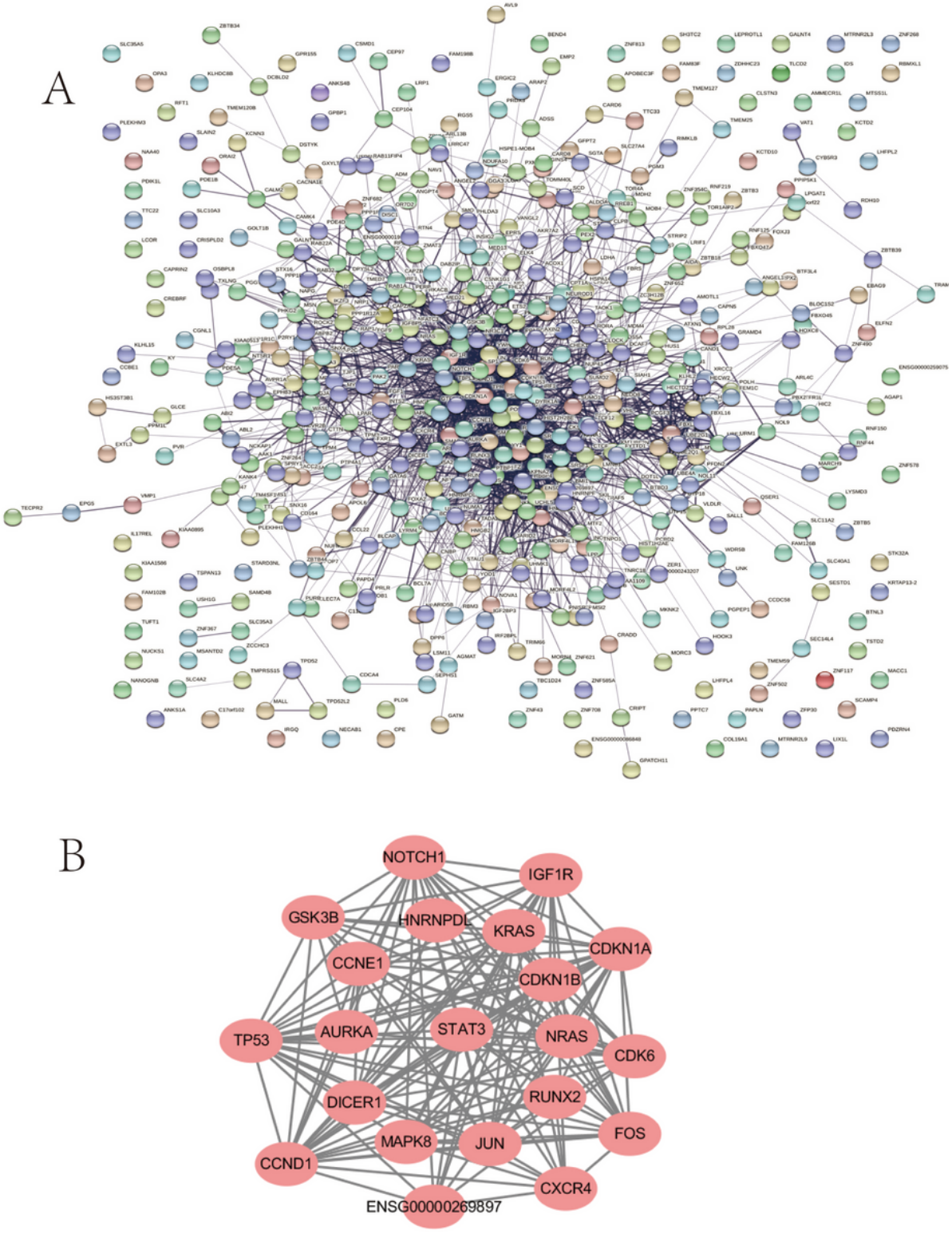

## Figure 7

GO enrichment analysis, including biological processes, cell components, and molecular functions. (A–C) are upregulated GO analysis, and (D–F) are downregulated GO analysis. The vertical axis is the enriched items, and the abscissa is the number of enriched genes. The redder the color is, the more significant the enriched items are.




## Figure 8

KEGG enrichment analysis, (A) is upregulated KEGG bubble plot, and (B) is downregulated KEGG bubble plot. The vertical axis represents the enrichment pathway, and the horizontal axis represents the enrichment multiple. The smaller the FDR value is, the redder the round color is, the more significant the difference is, the more the number of enrichment genes is, and the larger the circle is. (C) is part of the Akt-PI3K pathway.




## Figure 9

Interaction network of target gene proteins. (A) is the total protein interaction network, and (B) is the most critical group of proteins screened by the ECODE plugin.