

## Bioinformatic prediction of depression-related signaling pathways regulated by miR-146a in peripheral blood of patients with post-stroke depression

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### ABSTRACT

It was aimed to explore the differential expression of miR-146a-5p in peripheral blood of patients with post-stroke depression (PSD), and to analyze its mechanism using bioinformatics. Stroke patients were selected as the research objects, and were divided into PSD ones and non-post-stroke depression (N-PSD) ones with the National Institutes of Health stroke scale (NIHSS) and Hamilton Depression Scale-17 terms (HAMD-17) scores. Peripheral blood of patients was collected for serum miR-146a-5p detection. Targetscan7.1, miRDB, DIANA TOOLS, and more databases were used to predict the target genes of miR-146a-5p. String11.0 was applied to construct a protein interaction network, and GO and KEGG pathway enrichment analysis of target genes was performed. Compared with that of N-PSD patients, serum miR-146a-5p levels in PSD patients were significantly increased ( $P < 0.05$ ). The receiver operator characteristic (ROC) curve suggested that the sensitivity and specificity of miR-146a-5p in predicting PSD were 0.703 and 0.811, respectively. The human miR-146a-5p sequence was highly conserved, with a total of 43 target genes. It involved analysis of activity, signaling pathways, and transcriptional regulation, as well as related signaling pathways such as Toll-like receptors (TLR), neurotrophic factors, and nuclear factor kappa-B (NF- $\kappa$ B). In conclusion, the expression level of miR-146a-5p was abnormally increased in PSD patients, and it could be taken as a candidate marker for the diagnosis of PSD. miR-146a-5p could affect PSD through signaling pathways of TLRs, neurotrophic factors, and NF- $\kappa$ B.

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### Introduction

Depression is one of the most common complications after stroke, and its incidence varies from 20% to 70% due to differences in individual factors, evaluation criteria, and disease time (1). The main clinical symptoms of post-stroke depression (PSD) are low mood, taciturnity, and large mood swings. If treatment is delayed, it will affect the recovery effect on the patients' neurological function. In severe cases, the patients will refuse treatment or commit suicide, which affects the quality of life seriously of PSD patients (2,3). There are evidences that the incidence of PSD is significantly and positively correlated with the risk of stroke recurrence and death (4). PSD patients have not only cognitive dysfunction but also a very long recovery process after stroke. Therefore, early detection and intervention of PSD are of great significance to improve the treatment effect and prognosis of the patients.

The pathogenesis of PSD is still unclear nowadays, but studies have confirmed that PSD is related to neurotrophic and receptor signaling, activation of inflammatory immune responses, secondary degenerative changes, and more factors (5,6). Micro ribonucleic acid (miRNA) is a very important class of regulatory molecules that have

drawn attention in recent years. miRNAs can target specific messenger ribonucleic acid (mRNA), causing their degradation or protein translation arrest, and regulating biological processes of cell proliferation, apoptosis, differentiation, and so on (7). miRNA gets about 19-25 amino acids in length and is stably present in plasma. A large number of researches have confirmed that miRNA, as a new and potential blood biomarker, has been involved in the prediction, diagnosis, and treatment of various psychiatric diseases (8,9). miR-146a-5p was first discovered and characterized in mice, and it was proved to be involved in various malignant tumors (10,11). Studies have also demonstrated that miR-146a-5p plays an important role in certain neuronal diseases, such as stroke, Parkinson's disease, and epilepsy. Pan et al. (2017) (12) showed that miR-146a-5p was involved in the proliferation, migration, and apoptosis of human cerebral vascular smooth muscle cells. Zhang et al. (2021) (13) also illustrated that miR-146a-5p could regulate the neuroinflammatory response mediated by microglia through the IRAK1/TRAF6 pathway, and then participate in the process of ischemic stroke. However, there are relatively few reports on whether miR-146a-5p participates in the occurrence and development of PSD.

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To this end, the expression level of miR-146a-5p in peripheral blood of PSD patients was detected, and its correlation with PSD was analyzed. Then, the potential target genes of hsa-miR-146a-5p were analyzed by bioinformatic methods. The gene ontology (GO) functional annotation of the target genes and the Kyoto Encyclopedia of Genes and Genome (KEGG) pathway enrichment analysis were also conducted. This research aimed to provide ideas for the identification of miR-146a-5p target genes and their roles in the process of PSD.

## Materials and Methods

### Research objects

Patients with acute cerebral infarction, who visited the Department of Neurology of Second Affiliated Hospital of Harbin Medical University from June 2020 to January 2022, were selected as the research objects. Inclusion criteria were as follows. (i) Stroke in patients met the diagnostic criteria of *Key Points for Diagnosis of Various Cerebrovascular Diseases*, and the patients were diagnosed with stroke by brain imaging examination. (ii) The onset time of the patients was within 7 days. (iii) The patients were 18 years old at least. The exclusion criteria below were also followed. (i) The patients were diagnosed with dementia, cognitive dysfunction, or disturbance of consciousness during the treatment process, so they were unable to cooperate with the examination or follow-up. (ii) Those were diagnosed with depression or depressive state after stroke and were treated with antidepressant drugs. (iii) They had other mental illnesses. (iv) They suffered from severe underlying diseases such as malignant tumors and physical disorders. (v) They got severe organic or systemic diseases. (vi) They went with severe hearing or a visual impairment, unable to complete the neuropsychological test. (vii) They were unable to complete the research, withdrew, or died. This research had been approved by the Ethics Committee of Second Affiliated Hospital of Harbin Medical University, and the included patients and their families signed the informed consent.

24 hours after admission, the National Institutes of Health Stroke Scale (NIHSS) of patients were scored by a professional neurologist. The patients were followed up 3 months after stroke, at which the Hamilton Depression Scale-17 terms (HAMD-17) were completed by them and then scored. When the HAMD-17 score was greater than or equal to 7 points, the patient was considered to have depression. According to the HAMD-17 score, the patients were divided into the PSD group and the non-post-stroke depression (N-PSD) group.

### Clinical sample collection and reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

15 mL of peripheral venous blood samples were collected from patients, and anticoagulated with sodium citrate anticoagulant. Then, the samples were centrifuged at 3000 rpm for 5 min, and the supernatant was collected. Serum total RNA was extracted by the Trizol method; the concentration, purity, and integrity of the extracted RNA were detected by 1% agarose gel electrophoresis and a multifunctional microplate reader. According to the instructions of the cDNA reverse transcription kit [Takara Biotechnology (Dalian) Co., Ltd.], the cDNA reverse transcription of the total RNA was extracted. The concentration and purity of

cDNA were also detected with a multi-function microplate reader. The reaction system and reaction procedure of RT-qPCR were set up in accordance with the instructions of the TB Green fluorescence quantitative kit [Takara Biotechnology (Dalian) Co., Ltd.]. U6 quantification primer sequences were 5'-AAAGCAAATCATCGGACGACC-3' (upstream) and 5'-GTACAACACATTGTTTCCTCGGA-3' (downstream). The quantification primer sequences of miR-146a-5p were, 5'-ACTACTCCAGCTGGGAA-TG-3' (upstream) and 5'-TGGTCTGGTGGTGATGAG-GTTG-3' (downstream). As U6 was taken as the internal reference gene, the relative expression level of the target genes was detected by the  $2^{-\Delta\Delta CT}$  method.

### Conservation analysis of miR-146a-5p sequence

Online databases such as UCSC (<http://genome.ucsc.edu>) were utilized to find the gene sequence, chromosomal location, and species conservation of human miR-146a-5p. Clustal Omega software was used for the analysis of sequence conservation of miR-146a-5p in different species, including Homo sapiens, Pan troglodytes (chimpanzees), Mus musculus (mice), and so on.

### Tissue differential expression of miR-146a-5p

miRgator v3.0 database (<http://mirgator.kobic.re.kr/>) recorded the deep sequencing datasets of human miRNAs distributed by institutions such as Gene Expression Omnibus (GEO), Sequence Read Archive (SRA), and The Cancer Genome Atlas (TCGA). The sequencing datasets were composed of 4.1 billion short sequences and 2.5 billion matched sequences. The expression of human miR-146a-5p in different tissues and organs were searched using miRgator v3.0 database, in which the predictive analysis was also made.

### miR-146a-5p target gene prediction

miRGen v4.0 (<http://diana.cslab.ece.ntua.gr/mirgen/>), TargetScan v8.0 (<https://www.targetscan.org/>), DIANA-microT-CDS v5.0 ([https://dianalab.e-ce.uth.gr/html/dianauniverse/index.php?r=microT\\_CDS](https://dianalab.e-ce.uth.gr/html/dianauniverse/index.php?r=microT_CDS)), and miR DIP (<http://ophid.utoronto.ca/mirDIP/>) were utilized for the prediction of human miR-146a-5p target genes. A Venn diagram was drawn using the online tool Venny v2.1 (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>), and the intersections were included in the target gene dataset.

### GO and KEGG enrichment analysis of miR-146a-5p target genes

GO analysis of human miR-146a-5p target genes was made using the FUNRICH online tool (<http://www.funrich.org/>), in the directions of cellular component, molecular function, and biological process. The enrichment analysis of KEGG signaling pathway of human miR-146a-5p target genes was performed using DAVID database (<http://David.abcc.ncif-crf.gov/>) and KOBAS database (<http://kobas.cbi.pku.edu.cn/>). The Fisher's exact test came with the DAVID database used for the calculation of the  $P$  value, and  $P < 0.05$  was thought as a significant threshold.

### miR-146a-5p target gene protein interaction analysis

The protein interaction predictive parsing was operated on human miR-146a-5p target genes through the String v11.0 database (<http://www.string-db.org/>). Combined with Cytoscape v3.6 software, the target gene-encoded

protein interaction network was drawn.

**Statistical analysis**

Statistical analysis of experimental data was made using SPSS 19.0. Continuous variables were expressed as mean ± standard deviation, and differences between the two groups were compared using an independent samples t-test. Dichotomous variables were expressed by the number of cases (percentage), in which the differences between the two groups were compared using the  $\chi^2$  test. The differential analysis of GO molecules and KEGG pathway enrichment was performed with the one-way analysis of variance. It was considered that a difference was significant statistically when  $P < 0.05$ .

**Results**

**General data analysis of the two groups of patients**

The clinical data of patients were compared between the PSD and N-PSD groups, as the results were shown in Table 1. No significant difference was discovered in gender ratio, average age, history of high blood pressure, history of diabetes, history of coronary heart disease, course of a stroke, and NIHSS score between the two groups ( $P > 0.05$ ). The HAMD-17 score in the PSD group was greatly higher than that in the N-PSD group, showing a significant difference statistically ( $P < 0.05$ ).

**miR-146a-5p expression in serum and correlation analysis in PSD patients**

The miR-146a-5p expression levels in serum were compared between the PSD and N-PSD groups, shown in Figure 1A. It could be observed that the expression level of miR-146a-5p in serum of PSD patients was higher than that of N-PSD patients significantly, with a difference of statistical significance ( $P < 0.05$ ).

As shown in Figure 1B, the receiver operator characteristic (ROC) curve was drawn for analyzing the sensitivity and specificity of miR-146a-5p in predicting PSD. The area under the curve (AUC) was 0.812 of miR-146a-5p for the prediction of PSD (95% confidence interval (CI): 0.733-0.982,  $P < 0.05$ ). The cut-off value was 1.439, and the sensitivity, as well as specificity, were 0.703 and 0.811, respectively.

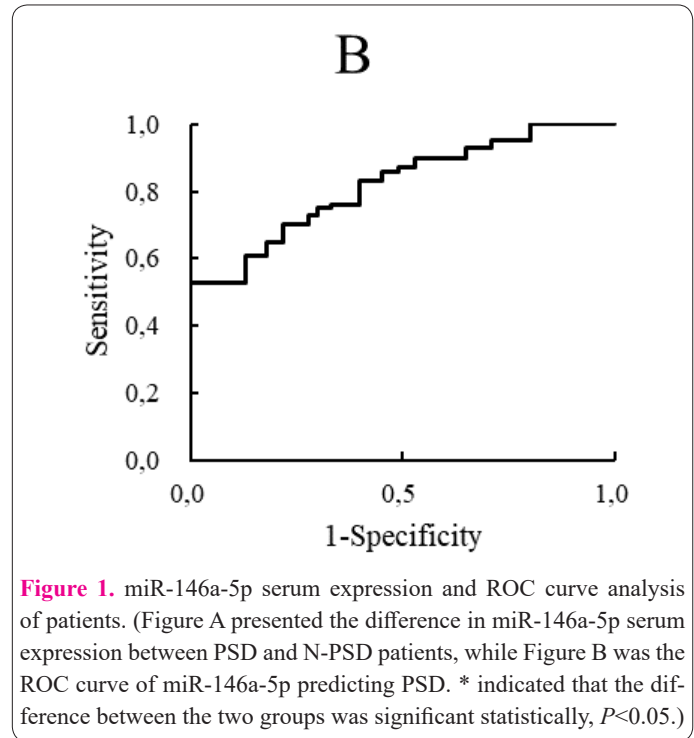
**Sequence conservation analysis of hsa-miR-146a-5p**

miR-146a-5p was located in the chr5q33.3 chromosome of the human genome and between chr5:160485373-

1460485393. The mature sequences of miR-146a-5p were retrieved and compared among different species, as the results were represented in Figure 2. The miR-146a-5p sequence was highly conserved in multiple species, and the mature sequence was UGAGAACUGAAUCCAUGGGUU.

**Differential analysis of tissue expression of hsa-miR-146a-5p**

The expression of miR-146a-5p was analyzed in different human tissues and organs, which were summarized in Figure 3. It could be found that the expression abun-



Species	Name	Sequence
<i>Homo sapiens</i>	hsa-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Mus musculus</i>	mmu-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Rattus norvegicus</i>	rno-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Musca domestica</i>	mdm-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Gallus domesticus</i>	gga-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Sus scrofa</i>	ssc-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Trypaena chinensis</i>	tch-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Cavia porcellus</i>	cpo-miR-146a-5p	UGAGAACUGAAUCCAUGGGUUG
<i>Oryzotilus cincticollis</i>	ocu-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Anolis carolinensis</i>	aca-miR-146a-5p	UGAGAACUGAAUCCAUGGCG
<i>Alligator mississippiensis</i>	ami-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
<i>Salmo salar</i>	ssa-miR-146a-5p	UGAGAACUGAAUCCAUGGAUGG
<i>Ornithorhynchus anatinus</i>	oan-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU

**Figure 2.** Mature sequence comparison of miR-146a-5p among multiple species.

**Table 1.** Comparative analysis of patients' clinical data in PSD and N-PSD groups.

Clinical data	PSDgroup (n=12)	N-PSD group (n=31)	P-value
Gender [n (%)]			0.906
Male	5 (41.7)	15 (48.4)	
Female	7 (58.3)	16 (51.6)	
Age (years old)	61.3±5.8	60.5±4.4	0.552
History of high blood pressure [n (%)]	3 (25.0)	8 (25.8)	0.911
History of diabetes [n (%)]	5 (41.7)	15 (48.4)	0.729
History of coronary heart disease [n (%)]	4 (33.3)	10 (32.3)	0.668
Course of stroke (d)	66.9±7.4	67.3±6.1	0.527
NIHSS score	5.8±1.3	5.9±2.0	0.518
HAMD-17 score	19.6±3.3	6.3±1.7	0.000



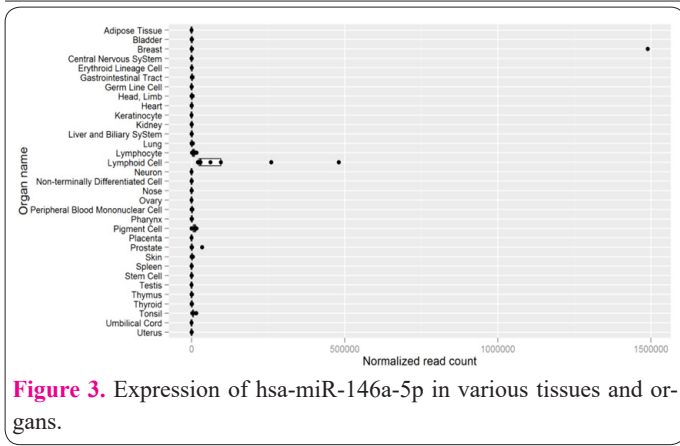


Figure 3. Expression of hsa-miR-146a-5p in various tissues and organs.

dance of miR-146a-5p was relatively high in lymphocytes, while was relatively low in tissues such as the heart, kidney, and gastrointestinal tract.

**Target gene prediction of hsa-miR-146a-5p**

The target genes of hsa-miR-146a-5p were predicted using each database, then the results were displayed in Figure 4. TargetsCan database predicted 283 target genes, miRGen predicted 249 target genes, miR DIP predicted 220 target genes, and miRBD predicted 488 target genes. After the predicted target genes of the databases were intersected, 42 target genes were obtained in total. The specific target genes obtained were listed in Table 2.

**GO analysis of hsa-miR-146a-5p target genes**

GO functional annotation analysis was performed of hsa-miR-146a-5p target genes, as the results were represented in Figure 5. hsa-miR-146a-5p target genes were mainly involved in biological processes, cellular components, and molecular functions. The biological processes included cellular processes, biological regulation, and regulation of RNA metabolic process. Cellular components referred to cells, intracellular components, and organelles.

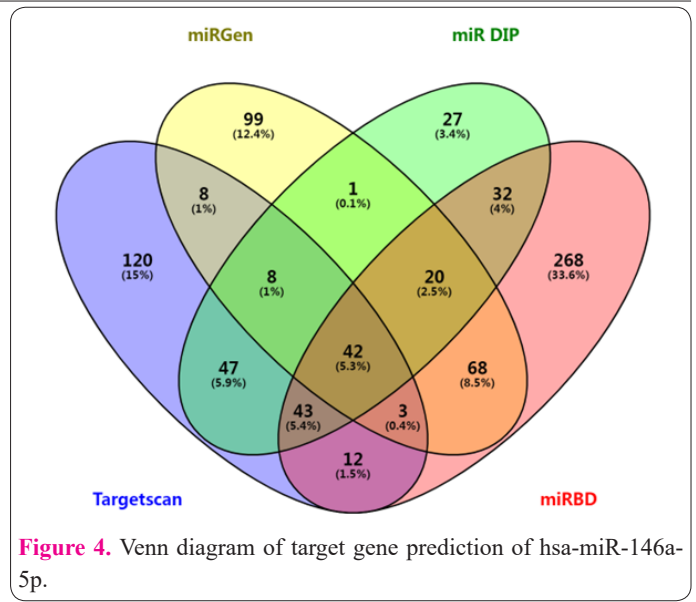


Figure 4. Venn diagram of target gene prediction of hsa-miR-146a-5p.

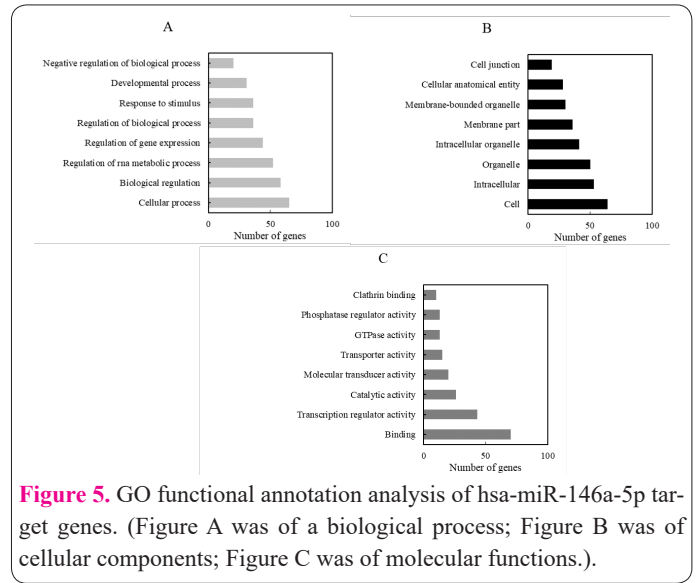


Figure 5. GO functional annotation analysis of hsa-miR-146a-5p target genes. (Figure A was of a biological process; Figure B was of cellular components; Figure C was of molecular functions.)

Table 2. Information about target genes of hsa-miR-146a-5p.

No.	ID of genes	Name of target genes	No.	ID of genes	Name of target genes
1	ENST00000370900.1	<i>IGSF1</i>	22	ENST00000540052.1	<i>BCORL1</i>
2	ENST00000302350.4	<i>CDKN2AIP</i>	23	ENST00000288266.3	<i>APPL1</i>
3	ENST00000325144.4	<i>ZBTB2</i>	24	ENST00000362063.2	<i>ZNF652</i>
4	ENST00000369980.3	<i>IRAK1</i>	25	ENST00000369075.3	<i>SEC23IP</i>
5	ENST00000369649.4	<i>SLC10A3</i>	26	ENST00000327827.7	<i>C16orf72</i>
6	ENST00000313899.7	<i>HNRNPD</i>	27	ENST00000312960.3	<i>SLAH2</i>
7	ENST00000446113.2	<i>ZDHHC13</i>	28	ENST00000371103.3	<i>LCOR</i>
8	ENST00000465357.2	<i>NOVA1</i>	29	ENST00000544604.2	<i>ZNRF3</i>
9	ENST00000526995.1	<i>TRAF6</i>	30	ENST00000297107.6	<i>GALNT10</i>
10	ENST00000449592.2	<i>TMEM120B</i>	31	ENST00000457153.2	<i>SYT1</i>
11	ENST00000437042.2	<i>RARB</i>	32	ENST00000522677.3	<i>MYBL1</i>
12	ENST00000402045.1	<i>LFNG</i>	33	ENST00000375256.4	<i>ZNF367</i>
13	ENST00000264246.3	<i>CD80</i>	34	ENST00000436010.2	<i>ROBO1</i>
14	ENST00000554546.1	<i>NUMB</i>	35	ENST00000376773.1	<i>PPP1R11</i>
15	ENST00000423015.1	<i>KLF7</i>	36	ENST00000347624.3	<i>LRRC15</i>
16	ENST00000526148.1	<i>EIF4G2</i>	37	ENST00000323669.5	<i>DDHD1</i>
17	ENST00000403733.3	<i>WWC2</i>	38	ENST00000369888.1	<i>ZNF512B</i>
18	ENST00000447404.2	<i>STRBP</i>	39	ENST00000342788.4	<i>ERBB4</i>
19	ENST00000336078.4	<i>ZNF532</i>	40	ENST00000502732.1	<i>ABL2</i>
20	ENST00000361264.4	<i>DCAF12</i>	41	ENST00000263816.3	<i>LRP2</i>
21	ENST00000608872.1	<i>FBXW2</i>	42	ENST00000507740.1	<i>RIMS2</i>

The molecular functions here included binding, transcription regulator activity, and catalytic activity.

**KEGG enrichment analysis of hsa-miR-146a-5p target genes**

The enrichment analysis of the KEGG signaling pathway was performed on the hsa-miR-146a-5p target genes. The top 10 results were selected for display in Figure 6. It was shown that hsa-miR-146a-5p target genes were mainly enriched in the Toll-like receptor (TLR) signaling pathway, nuclear factor kappa-B (NF-κB) signaling pathway, neurotrophic signaling, and other processes.

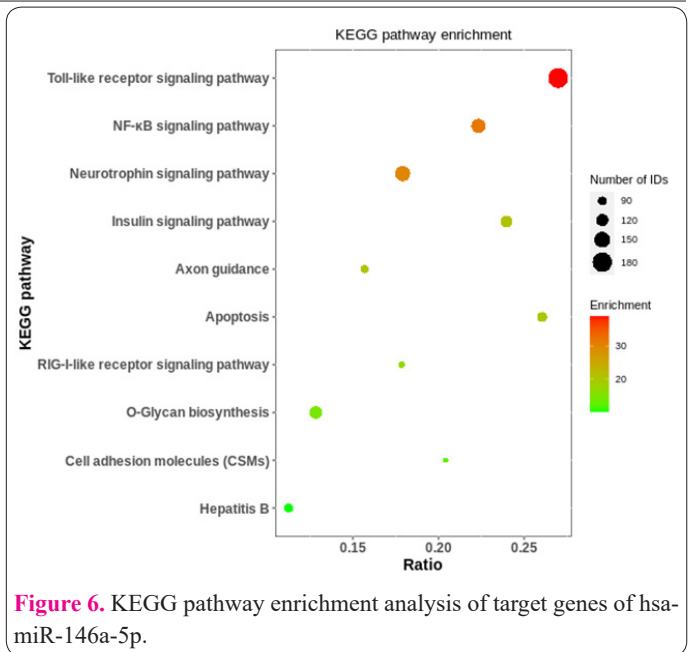
**hsa-miR-146a-5p target gene-encoded protein interaction analysis**

The interaction network diagram of the proteins encoded by the hsa-miR-146a-5p target genes was drawn through String software, which was presented in Figure 7. It could be found that there was an interaction relationship among the proteins encoded by most target genes, and only a few target genes did not interact with other genes. Then, the most strongly associated gene, endothelial ras-related C3 botulinum toxin substrate 1 (Rac1), was selected to map the protein interaction network encoded by the strongly associated target genes; the results were displayed in Figure 8.

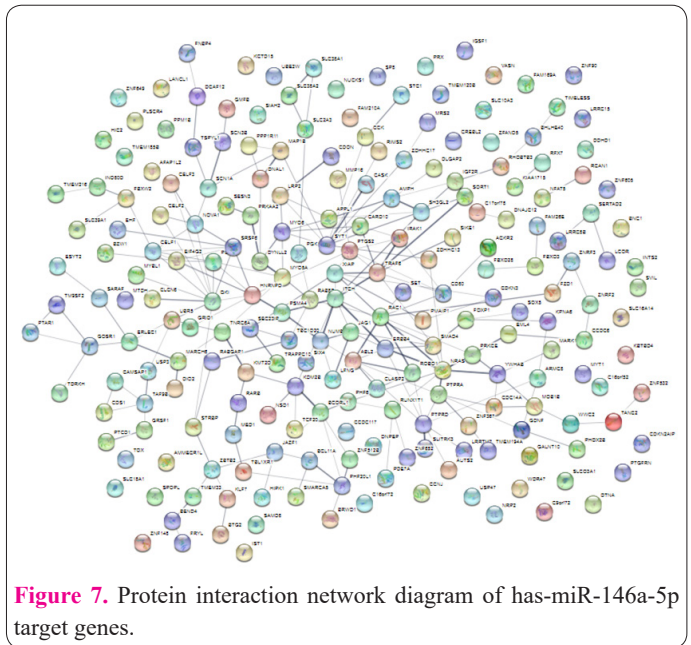
**Discussion**

Brain tissue damage occurs in patients after stroke, which may damage the emotional center (14). The reduction of the biochemical transmitter 5-HT and norepinephrine in brain tissues can lead to depression, and the blockade of nerve conduction pathways can also make patients in low spirits (15). Depression will hinder the recovery of neurological functions in stroke patients; in severe cases, the patient's ability to live and quality of life will be affected, even it will cause death (16). PSD is a secondary depression, closely related to factors such as age, neurological impairment, and lesion location (17,18). However, there is a lack of stable and convenient biochemical indicators for PSD prediction currently.

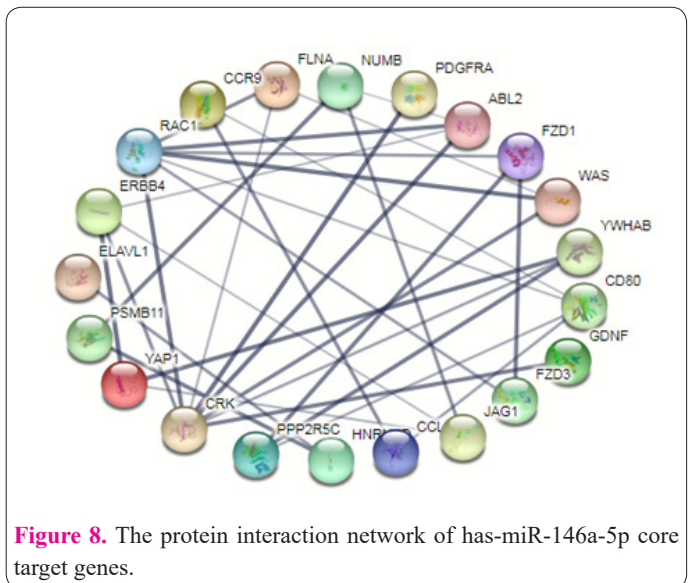
miRNA is a class of micro non-coding RNAs of about 22 nucleotides in length, highly conserved in evolution (19). miRNA can interact with the 3' untranslated region of mRNA and affect its post-transcriptional regulation, thereby participating in important biological processes such as cellular proliferation, apoptosis, and differentiation (20). A large number of studies at present have proved that miRNAs can play the role of tumor-promoting or tumor-suppressing genes in cancers (21,22). miRNAs can also be involved in the inflammatory response, oxidative stress response, neurogenesis, and other processes of the central nervous system, and are in close relation with the process of PSD (23,24). Hu *et al.* (2020) (25) illustrated that miR-30-5p was highly expressed in the peripheral blood of PSD patients, and the sensitivity and specificity for PSD prediction were 0.727 and 0.840, respectively. Panta *et al.* (2019) (26) showed that miR-363-3p treatment can reduce the infarct volume of acute stroke in women effectively and was closely related to PSD. In recent years, it has been found that hsa-miR-146a-5p plays an important role in the inflammatory response and is strongly linked to acute stroke (27). But whether hsa-miR-146a-5p is related



**Figure 6.** KEGG pathway enrichment analysis of target genes of hsa-miR-146a-5p.



**Figure 7.** Protein interaction network diagram of has-miR-146a-5p target genes.



**Figure 8.** The protein interaction network of has-miR-146a-5p core target genes.

to PSD remains to be verified. To this end, the expression of miR-146a-5p was detected in peripheral blood of both PSD and non-PSD patients in this research, and bioinfor-

matic technology was utilized to predict and analyze the target genes of hsa-miR-146a-5p.

The results of this work suggested that hsa-miR-146a-5p showed a trend of high expression in clinical peripheral blood samples of PSD patients. Meanwhile, its AUC for PSD prediction was 0.812 (95% CI: 0.733-0.982,  $P < 0.05$ ), the cut-off value was 1.439, and the sensitivity and specificity were 0.703 and 0.811, respectively. Liang et al. (2019) (28) showed that miR-140-5p was an independent risk factor for late-onset PSD prediction, and its sensitivity and specificity were 0.833 and 0.726, respectively for predicting late-onset PSD. It was proved in this work that hsa-miR-146a-5p levels in the serum of stroke patients might be a novel biomarker for PSD prediction. To further understand the possible mechanism of hsa-miR-146a-5p on PSD, the hsa-miR-146a-5p was analyzed with bioinformatic technology. The results suggested that hsa-miR-146a-5p owned highly conserved sequences in multiple species, and 42 target genes were predicted from multiple databases.

The GO function annotation and KEGG pathway enrichment analysis were carried out on the target genes of hsa-miR-146a-5p. The target genes were mainly involved in biological processes like cellular processes, biological regulation, and RNA metabolic process regulation; cell components including cells, intracellular components, and organelles; as well as molecular functions such as binding, transcription regulator activity, and catalytic activity. The target genes of hsa-miR-146a-5p were majorly enriched in the TLR pathway, NF- $\kappa$ B pathway, neurotrophic signal transduction, and more. TLR signaling pathway is an innate immune pattern recognition receptor (29). The research of Chen et al. (2016) (30) demonstrated that miR-146a-5p is a key regulator of the innate immune response and inhibits the secretion of pro-inflammatory cytokines through the TLR4/NF- $\kappa$ B pathway. The continuous activation of the NF- $\kappa$ B signaling pathway involves processes such as cell apoptosis, immune response, inflammatory response, and cell survival (31). Lei et al. (2020) (32) confirmed that miR-146a-5p had such an expression trend in Alzheimer's disease models; NF- $\kappa$ B could induce miR-146a-5p to upregulate the target gene TIGAR, which promoted oxidative stress and cell apoptosis in Alzheimer's disease. Neurotrophic signaling and axonal development are related to the process of PSD closely, and both axonal development and nerve cell regeneration play a vital role in the functional repair of nerve cells after injury (33). Therefore, the target genes of miR-146a-5p may participate in neural remodeling by regulating the inflammatory response, neurotrophic factor signaling, and other processes in vivo, and thereby participate in the occurrence and development of PSD.

Afterward, the protein interaction network of miR-146a-5p target genes was constructed. Activation of Rac1 plays different roles in multiple types of cells after central nervous system injury. A study by Bu et al. (2019) (34) proved that Rac1 in endothelial cells is quite important in post-stroke recovery and angiogenesis. The inhibition of Rac1 expression could promote the activation of regenerative molecules such as CREB and ERK1/2, and promote the generation of brain-derived neurotrophic factors. To the research of Li et al. (2017) (35), Rac1 is involved in the regulation of neuroinflammation and neuro-oxidative stress, which promotes neuronal death and affects cerebral

ischemia/reperfusion injury in turn. Liu et al. (2018) (36) also illustrated that Rac1 played a central role in the regeneration of damaged brain axons. Inhibition of Rac1 could lead to the inactivation of pro-regenerative molecules and reduce the regeneration of neuronal axons, which was of important significance in the recovery of neural functions after stroke. As Rac1 is a potential target gene of miR-146a-5p, miR-146a-5p can inactivate it by target binding to target genes such as Rac1, affecting neuronal axonal remodeling, and then participating in the PSD process.

In this work, RT-qPCR technology was used for the detection of differential expression levels of miR-146a-5p in peripheral blood of both PSD and non-PSD patients. It was indicated that miR-146a-5p might be involved in the process of PSD. Subsequently, the biological characteristics and functions of miR-146a-5p were analyzed using bioinformatic methods, from which miR-146a-5p was found to participate in and affect the process of PSD by targeting mRNAs like Rac1. miR-146a-5p could be applied in clinical practice as a potential therapeutic target for PSD. However, a great number of basic and clinical trials were needed in the future for the verification of this hypothesis.

## References

1. Medeiros GC, Roy D, Kontos N, Beach SR. Post-stroke depression: A 2020 updated review. *Gen Hosp Psychiatry*. 2020 Sep-Oct;66:70-80. doi: 10.1016/j.genhosppsych.2020.06.011. Epub 2020 Jun 27. PMID: 32717644.
2. Das J, G K R. Post stroke depression: The sequelae of cerebral stroke. *Neurosci Biobehav Rev*. 2018 Jul;90:104-114. doi: 10.1016/j.neubiorev.2018.04.005. Epub 2018 Apr 12. PMID: 29656030.
3. Zhang E, Liao P. Brain-derived neurotrophic factor and post-stroke depression. *J Neurosci Res*. 2020 Mar;98(3):537-548. doi: 10.1002/jnr.24510. Epub 2019 Aug 5. PMID: 31385340.
4. Cai W, Mueller C, Li YJ, Shen WD, Stewart R. Post stroke depression and risk of stroke recurrence and mortality: A systematic review and meta-analysis. *Ageing Res Rev*. 2019 Mar;50:102-109. doi: 10.1016/j.arr.2019.01.013. Epub 2019 Jan 31. PMID: 30711712.
5. Starkstein SE, Hayhow BD. Treatment of Post-Stroke Depression. *Curr Treat Options Neurol*. 2019 Jun 25;21(7):31. doi: 10.1007/s11940-019-0570-5. PMID: 31236751.
6. Schöttke H, Giabbiconi CM. Post-stroke depression and post-stroke anxiety: prevalence and predictors. *Int Psychogeriatr*. 2015 Nov;27(11):1805-12. doi: 10.1017/S1041610215000988. Epub 2015 Jul 16. PMID: 26178418.
7. Chen L, Heikkinen L, Wang C, Yang Y, Sun H, Wong G. Trends in the development of miRNA bioinformatics tools. *Brief Bioinform*. 2019 Sep 27;20(5):1836-1852. doi: 10.1093/bib/bby054. PMID: 29982332; PMCID: PMC7414524.
8. Baloun J, Bencurova P, Totkova T, Kubova H, Hermanova M, Hendrych M, Pail M, Pospisilova S, Brazdil M. Epilepsy miRNA Profile Depends on the Age of Onset in Humans and Rats. *Front Neurosci*. 2020 Sep 15;14:924. doi: 10.3389/fnins.2020.00924. PMID: 33041753; PMCID: PMC7522367.
9. Bielefeld P, Mooney C, Henshall DC, Fitzsimons CP. miRNA-Mediated Regulation of Adult Hippocampal Neurogenesis; Implications for Epilepsy. *Brain Plast*. 2017 Nov 9;3(1):43-59. doi: 10.3233/BPL-160036. PMID: 29765859; PMCID: PMC5928558.
10. Iacona JR, Lutz CS. miR-146a-5p: Expression, regulation,



- and functions in cancer. *Wiley Interdiscip Rev RNA*. 2019 Jul;10(4):e1533. doi: 10.1002/wrna.1533. Epub 2019 Mar 20. PMID: 30895717.
11. Meng Q, Liang C, Hua J, Zhang B, Liu J, Zhang Y, Wei M, Yu X, Xu J, Shi S. A miR-146a-5p/TRAF6/NF- $\kappa$ B p65 axis regulates pancreatic cancer chemoresistance: functional validation and clinical significance. *Theranostics*. 2020 Mar 4;10(9):3967-3979. doi: 10.7150/thno.40566. PMID: 32226532; PMCID: PMC7086345.
  12. Pan Q, Liu H, Zheng C, Zhao Y, Liao X, Wang Y, Chen Y, Zhao B, Lazartigues E, Yang Y, Ma X. Microvesicles Derived from Inflammation-Challenged Endothelial Cells Modulate Vascular Smooth Muscle Cell Functions. *Front Physiol*. 2017 Jan 12;7:692. doi: 10.3389/fphys.2016.00692. PMID: 28127288; PMCID: PMC5226944.
  13. Zhang Z, Zou X, Zhang R, Xie Y, Feng Z, Li F, Han J, Sun H, Ouyang Q, Hua S, Lv B, Hua T, Liu Z, Cai Y, Zou Y, Tang Y, Jiang X. Human umbilical cord mesenchymal stem cell-derived exosomal miR-146a-5p reduces microglial-mediated neuroinflammation via suppression of the IRAK1/TRAF6 signaling pathway after ischemic stroke. *Aging (Albany NY)*. 2021 Jan 21;13(2):3060-3079. doi: 10.18632/aging.202466. Epub 2021 Jan 21. PMID: 33479185; PMCID: PMC7880318.
  14. Sebestova M, Lackner I, Inayat M, Ademaj A, Mikutta C. Post Stroke Depression [Post stroke depression]. *Ther Umsch*. 2021 Aug;78(6):299-304. German. doi: 10.1024/0040-5930/a001274. PMID: 34291664.
  15. Zhao A, Ma B, Xu L, Yao M, Zhang Y, Xue B, Ren J, Chang D, Liu J. Jiedu Tongluo Granules Ameliorates Post-stroke Depression Rat Model via Regulating NMDAR/BDNF Signaling Pathway. *Front Pharmacol*. 2021 May 20;12:662003. doi: 10.3389/fphar.2021.662003. PMID: 34093193; PMCID: PMC8173625.
  16. Villa RF, Ferrari F, Moretti A. Post-stroke depression: Mechanisms and pharmacological treatment. *Pharmacol Ther*. 2018 Apr;184:131-144. doi: 10.1016/j.pharmthera.2017.11.005. Epub 2017 Nov 9. PMID: 29128343.
  17. Shi Y, Yang D, Zeng Y, Wu W. Risk Factors for Post-stroke Depression: A Meta-analysis. *Front Aging Neurosci*. 2017 Jul 11;9:218. doi: 10.3389/fnagi.2017.00218. PMID: 28744213; PMCID: PMC5504146.
  18. Mitchell AJ, Sheth B, Gill J, Yadegarfar M, Stubbs B, Yadegarfar M, Meader N. Prevalence and predictors of post-stroke mood disorders: A meta-analysis and meta-regression of depression, anxiety and adjustment disorder. *Gen Hosp Psychiatry*. 2017 Jul;47:48-60. doi: 10.1016/j.genhosppsych.2017.04.001. Epub 2017 Apr 3. PMID: 28807138.
  19. Zhang Y, Cheng L, Chen Y, Yang GY, Liu J, Zeng L. Clinical predictor and circulating microRNA profile expression in patients with early onset post-stroke depression. *J Affect Disord*. 2016 Mar 15;193:51-8. doi: 10.1016/j.jad.2015.12.061. Epub 2016 Jan 1. PMID: 26766035.
  20. Qinlin F, Qi X, Qiong C, Lexing X, Peixia S, Linlin H, Yupu D, Lijun Y, Qingwu Y. Differential expression analysis of microRNAs and mRNAs in the mouse hippocampus of post-stroke depression (PSD) based on transcriptome sequencing. *Bioengineered*. 2022 Feb;13(2):3582-3596. doi: 10.1080/21655979.2022.2027061. PMID: 35100085.
  21. Mishra S, Yadav T, Rani V. Exploring miRNA based approaches in cancer diagnostics and therapeutics. *Crit Rev Oncol Hematol*. 2016 Feb;98:12-23. doi: 10.1016/j.critrevonc.2015.10.003. Epub 2015 Oct 8. PMID: 26481951.
  22. Wu M, Wang G, Tian W, Deng Y, Xu Y. MiRNA-based Therapeutics for Lung Cancer. *Curr Pharm Des*. 2018 Feb 12;23(39):5989-5996. doi: 10.2174/1381612823666170714151715. PMID: 28714413.
  23. Zhang Y, Cheng L, Chen Y, Yang GY, Liu J, Zeng L. Clinical predictor and circulating microRNA profile expression in patients with early onset post-stroke depression. *J Affect Disord*. 2016 Mar 15;193:51-8. doi: 10.1016/j.jad.2015.12.061. Epub 2016 Jan 1. PMID: 26766035.
  24. Cai L, Li WT, Zhang LL, Lu XQ, Chen M, Liu Y. Long noncoding RNA GAS5 enhanced by curcumin relieves poststroke depression by targeting miR-10b/BDNF in rats. *J Biol Regul Homeost Agents*. 2020 May-Jun;34(3):815-823. doi: 10.23812/20-113-A-25. PMID: 32627518.
  25. Hu J, Zhou Z, Yang Q, Yang K. [Differential expression of miR-30a-5p in post stroke depression and bioinformatics analysis of the possible mechanism]. *Nan Fang Yi Ke Da Xue Xue Bao*. 2020 Jul 30;40(7):922-929. Chinese. doi: 10.12122/j.issn.1673-4254.2020.07.02. PMID: 32895153; PMCID: PMC7386218.
  26. Panta A, Pandey S, Duncan IN, Duhamel S, Sohrabji F. Mir363-3p attenuates post-stroke depressive-like behaviors in middle-aged female rats. *Brain Behav Immun*. 2019 May;78:31-40. doi: 10.1016/j.bbi.2019.01.003. Epub 2019 Jan 10. PMID: 30639697; PMCID: PMC6488367.
  27. Duan S, Wang F, Cao J, Wang C. Exosomes Derived from MicroRNA-146a-5p-Enriched Bone Marrow Mesenchymal Stem Cells Alleviate Intracerebral Hemorrhage by Inhibiting Neuronal Apoptosis and Microglial M1 Polarization. *Drug Des Devel Ther*. 2020 Aug 5;14:3143-3158. doi: 10.2147/DDDT.S255828. PMID: 32821084; PMCID: PMC7425091.
  28. Liang HB, He JR, Tu XQ, Ding KQ, Yang GY, Zhang Y, Zeng LL. MicroRNA-140-5p: A novel circulating biomarker for early warning of late-onset post-stroke depression. *J Psychiatr Res*. 2019 Aug;115:129-141. doi: 10.1016/j.jpsychires.2019.05.018. Epub 2019 May 17. PMID: 31129437.
  29. Wu Y, Wang L, Hu K, Yu C, Zhu Y, Zhang S, Shao A. Mechanisms and Therapeutic Targets of Depression After Intracerebral Hemorrhage. *Front Psychiatry*. 2018 Dec 17;9:682. doi: 10.3389/fpsy.2018.00682. PMID: 30618863; PMCID: PMC6304443.
  30. Chen Y, Zeng Z, Shen X, Wu Z, Dong Y, Cheng JC. MicroRNA-146a-5p Negatively Regulates Pro-Inflammatory Cytokine Secretion and Cell Activation in Lipopolysaccharide Stimulated Human Hepatic Stellate Cells through Inhibition of Toll-Like Receptor 4 Signaling Pathways. *Int J Mol Sci*. 2016 Jul 7;17(7):1076. doi: 10.3390/ijms17071076. PMID: 27399683; PMCID: PMC4964452.
  31. Li C, Xu X, Wang Z, Wang Y, Luo L, Cheng J, Chen SF, Liu H, Wan Q, Wang Q. Exercise ameliorates post-stroke depression by inhibiting PTEN elevation-mediated upregulation of TLR4/NF- $\kappa$ B/NLRP3 signaling in mice. *Brain Res*. 2020 Jun 1;1736:146777. doi: 10.1016/j.brainres.2020.146777. Epub 2020 Mar 22. PMID: 32171705.
  32. Lei B, Liu J, Yao Z, Xiao Y, Zhang X, Zhang Y, Xu J. NF- $\kappa$ B-Induced Upregulation of miR-146a-5p Promoted Hippocampal Neuronal Oxidative Stress and Pyroptosis via TIGAR in a Model of Alzheimer's Disease. *Front Cell Neurosci*. 2021 Apr 16;15:653881. doi: 10.3389/fncel.2021.653881. PMID: 33935653; PMCID: PMC8085266.
  33. Syafrita Y, Amir D, Susanti R, Fadhilah I. Relationship of brain-derived neurotrophic factor, malondialdehyde, and 8-Hydroxy 2-Deoxyguanosine with post-ischemic stroke depression. *Dement Neuropsychol*. 2020 Jan-Mar;14(1):41-46. doi: 10.1590/1980-57642020dn14-010007. PMID: 32206197; PMCID: PMC7077855.
  34. Bu F, Min JW, Munshi Y, Lai YJ, Qi L, Urayama A, McCullough LD, Li J. Activation of endothelial ras-related C3 botulinum toxin substrate 1 (Rac1) improves post-stroke recovery

- and angiogenesis via activating Pak1 in mice. *Exp Neurol.* 2019 Dec;322:113059. doi: 10.1016/j.expneurol.2019.113059. Epub 2019 Sep 6. PMID: 31499064; PMCID: PMC6864282.
35. Li T, Qin JJ, Yang X, Ji YX, Guo F, Cheng WL, Wu X, Gong FH, Hong Y, Zhu XY, Gong J, Wang Z, Huang Z, She ZG, Li H. The Ubiquitin E3 Ligase TRAF6 Exacerbates Ischemic Stroke by Ubiquitinating and Activating Rac1. *J Neurosci.* 2017 Dec 13;37(50):12123-12140. doi: 10.1523/JNEUROSCI.1751-17.2017. Epub 2017 Nov 7. PMID: 29114077; PMCID: PMC6596816.
36. Liu L, Yuan H, Yi Y, Koellhoffer EC, Munshi Y, Bu F, Zhang Y, Zhang Z, McCullough LD, Li J. Ras-Related C3 Botulinum Toxin Substrate 1 Promotes Axonal Regeneration after Stroke in Mice. *Transl Stroke Res.* 2018 Oct;9(5):506-514. doi: 10.1007/s12975-018-0611-5. Epub 2018 Feb 24. PMID: 29476448; PMCID: PMC6598679.