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MicroRNA-146a regulates the expression of the $A\beta_{1-42}$ protein in Alzheimer's disease

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Abstract. – OBJECTIVE: To investigate the expression and clinical significance of microRNA-146a, $A\beta_{1-42}$, and tau protein in the peripheral blood of patients with Alzheimer's disease (AD).

PATIENTS AND METHODS: A total of 98 AD patients admitted to our hospital were selected as the experimental group and 50 healthy individuals were selected as the control group. The correlations between microRNA-146a, $A\beta_{1-42}$, and tau protein were analyzed using receiver operating curves to evaluate the value of microRNA-146a in predicting AD. Bioinformatics analysis was performed to preliminarily explore the possible mechanisms of microRNA-146a in the pathogenesis of AD.

RESULTS: MicroRNA-146a, $A\beta_{1-42}$ and tau protein were differentially expressed between AD patients and healthy individuals ($p<0.05$). microRNA-146a was negatively correlated with $A\beta_{1-42}$ ($r=-0.882$, $p<0.05$) but was not correlated with tau protein ($p>0.05$). The ROC curve showed that the area under the curve for microRNA-146a could be used to predict AD with an accuracy of 0.879 (95% CI 0.812-0.947). Bioinformatics analysis showed that the differentially expressed genes (DEGs) of microRNA-146a may be involved in the pathogenesis of AD through the regulation of multiple signaling pathways, including the Toll-like receptor signaling pathway, the neurotransmitter regulatory signaling pathways, and other pathways that affect the inflammatory response, synapse formation, and other biological processes.

CONCLUSIONS: MicroRNA-146a, $A\beta_{1-42}$ and tau protein are differentially expressed in the peripheral blood of AD patients. These proteins may be involved in the pathogenesis of AD by regulating the activity of multiple signaling pathways and the expression of the $A\beta_{1-42}$ protein.

Key Words:
Alzheimer's disease, MicroRNA-146a, $A\beta_{1-42}$ protein, Tau protein.

Introduction

Alzheimer's disease (AD) is a chronic degenerative disease of the central nervous system that occurs in the old or pre-senile periods. The main clinical manifestations of AD include progressive cognitive dysfunction, memory decline, a decreased ability to cope with daily living and abnormal mental behaviors[1]. In China, it has been estimated that the incidence of AD is between 6 to 8 million and treatment options for these patients remain very limited. The pathogenesis of AD still needs to be fully understood but current theories are based on the roles of the β-amyloid and tau proteins and biological processes, including oxidative stress, inflammatory factors, and apoptosis[2]. The typical neuropathological changes associated with AD include the deposition of β-amyloid (amyloid β, Aβ) protein, hyperphosphorylation of the microtubule-associated protein tau, and neuronal damage[3].

With the discovery of familial AD, a genetic theory was proposed in the pathogenesis of AD. MicroRNAs are a class of non-coding small molecules with lengths of 17-23 nucleotides. MicroRNAs are widely involved in the regulation of body growth, development and the development of the disease. microRNA-106 can regulate the gene expression level of the lipid transporter ABCA1 that is involved in the production of Aβ[4]. Constitutive alpha-secretase ADAM10 is a key gene involved in the pathogenesis of AD that can regulate the proteolytic processing of APP during the formation of Aβ plaques. MicroRNA-1306, microRNA-103, and microRNA-107 can affect the progression of AD by reducing the expression of ADAM10[5]. MicroRNA-146a is a hot-spot microRNA that has multiple functions (Figure 1) in regulating inflammation and oxidative stress. Studies have shown that microRNA-146a is differential-

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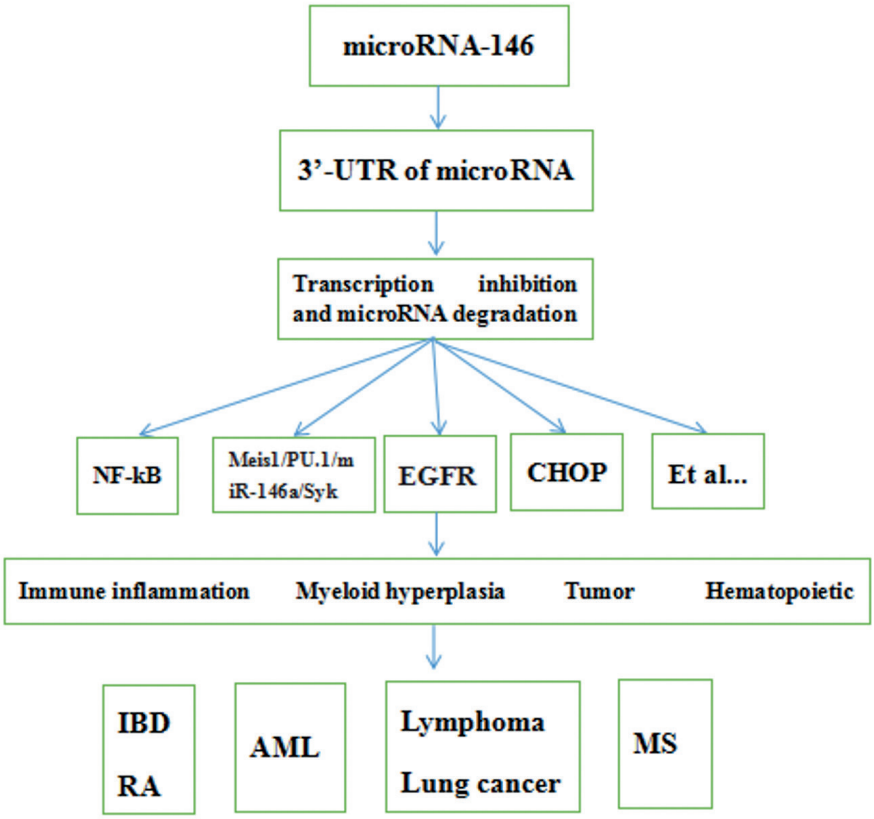

Figure 1. The mechanism of miR-146a in AD.

ly expressed in postoperative cognitive dysfunction, autism, and other diseases[6,7]. However, its role in AD is not fully understood. The current study aimed to understand the role of microRNA-146a in AD patients and identify the mechanism through which it plays a role in the pathogenesis of AD.

Patients and Methods

Study Patients

98 AD patients who attended our hospital from July 2015 to July 2018 were used recruited for the study. Of these patients, 54 were male and 44 were female. All patients were aged between 66-81 years, with an average age of 67.18 (±6.53) years. 50 healthy patients were selected as controls. Of these patients, 26 were male and 24 were females. All patients were aged between 65-80 years, with an average of 68.74 (±6.62) years.

Inclusion Criteria

Patients diagnosed with AD according to the criteria in the Manual of Diagnosis and Statistics of Mental Disorders[8]; 2. Patients older than 60 years of age; 3. Patients with cognitive dysfunction indicated by the mini-mental state examination (MMSE) score in which illiterate patients had MMSE scores ≤17, elementary school pupils have scores ≤20, and middle school students and above have scores ≤24; 4. Patients capable of completing the study; 5. Patients with no cerebral hemorrhage, cerebral infarction or intracranial space and identified by cranial CT, MRI, clinical manifestations, and laboratory examinations.

Exclusion Criteria

Patients with other types of dementia such as vascular dementia or advanced Parkinson's disease; 2. Patients with intracranial infections, schizophrenia, and depression; 3. Patients with severe heart, liver, lung and renal insufficiencies; 4. Patients already prescribed drugs for the treatment of dementia; 5. AD patients with MMSE scores >24.

Inclusion Criteria For The Control Group

Patients in the control group had no central nervous system diseases, no dementia or

non-neurological disease, and no serious diseases of the heart, liver, kidney or lungs. This study was approved by the hospital Ethics Committee, and all patients or legally authorized persons were recruited under written informed consent. No statistically significant difference was observed between general characteristics of the patient groups such as age and gender.

Sample Collection

5 ml of fasting peripheral venous blood was collected in the morning from all participants. Mononuclear cells were separated for the total RNA extraction. RT-PCR was performed to detect microRNA-146a. Serum was collected for detection of $A\beta_{1-42}$ protein and tau protein by ELISA.

RT-PCR Detection

According to the manufacturer's instructions, total RNA was collected from the serum samples using a TRIzol kit (Invitrogen™, 15596-026). The O260/O280 values for each sample were measured using a nucleic acid-protein analyzer (Shanghai Qite Co., Ltd., Shanghai, China, WXJ-9388) with an O260/O280 value of 1.8-2.0 considered as a threshold for RNA quality. A M-MLV reverse transcriptase kit was used to synthesize cDNA (Invitrogen™, C-28025). After the synthesis of the first-strand cDNA, the RT-PCR reaction mixture was amplified using a cation instrument (Hangzhou Bori Technology, Hangzhou, China, TC-XP). In a 96-well reaction plate, 2 x PCR mix (5.0 μl) was combined with 1 μl of primer working solution (2.5μM), 1.0 μl of template, 2.8 μl of ddH2O and 0.2 μl of Rox in a total volume of 10 μL. Each of the samples was plated in 3 duplicate wells. The amplification cycle conditions were as follows; 95°C for 1 min; 95°C for 15 s; 58°C for 20 s; 72°C for 45 s, for a total of 40 cycles. The primer sequence of the microRNA-146a was: Forward: 5'-CCTGAGAAGTGAATTCCATGGG-3'; reverse: 5'-CTCAACTGGTGTCGTGGAGTC-3'. The primer sequence of the internal reference gene U6 was: Forward: 5'-CTCGCTTCGGCAGCACAT-3'; reverse: 5'-AACGCTTCACGAATTTGCGT-3'. As the Ct value in RT-PCR cannot be used as raw data for statistical analysis, this study used the $2^{-\Delta Ct}$ value to represent the relative expression of microRNA. $^{\Delta}Ct$ = (target microRNA *Ct* value-U6 *Ct* value).

Detection of $A\beta_{1-42}$ Tau Protein Concentrations

A double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of $A\beta_{1-42}$ and tau proteins in the serum of the patient groups. The kit was provided by Wuhan Bayer Biotechnology Co., Ltd. (Wuhan, China), and a microplate reader was provided by Beijing Pulang New Technology. Ltd. (model DNM-9602, Beijing, China). ELISA was performed according to the manufacturer's instructions. A standard curve was established and used to calculate the concentrations of $A\beta_{1-42}$ and tau proteins based on the absorbance values of each well.

Bioinformatics Analysis

The UCSC genome online tool (http://genomeasia.ucsc.edu) was used to analyze the position and conservation of microRNA-146a in the human genome. The Targetscan (http://www.targetscan.org/vert_72/) and CoMeTa (https://www.co-meta-systems.com/) databases were used to predict the differentially expressed genes (DEGs) of microRNA-146a. The DAVID database was used to perform GO and KEGG enrichment analysis on the potential target genes of microRNA-146a.

Statistical Analysis

SPSS17.0 software (SPSS Statistics for Windows, Chicago, IL, USA) was used to analyze and process the data. The measured data were normally distributed and expressed as $x\pm s$. An independent sample *t*-test was used for comparison between the groups. Pearson correlation analysis was used for correlation analysis with a *p*-value <0.05 set as the threshold for statistical significance.

Results

Baseline Data Comparison

All participants from the AD and control groups that completed the study were included in the analysis. The demographic characteristics, comorbid diseases, and laboratory tests are summarized in Table I. All variables were not significantly different between the two groups.

Comparison of MicroRNA-146a, $A\beta_{1-42}$, Tau Protein, and MMSE Scores in the Two Groups

The relative expression of microRNA-146a in the peripheral blood of the AD group patients was significantly higher than the control group ($p<0.05$). The concentration of $A\beta_{1-42}$ in the peripheral blood of the AD group was significantly

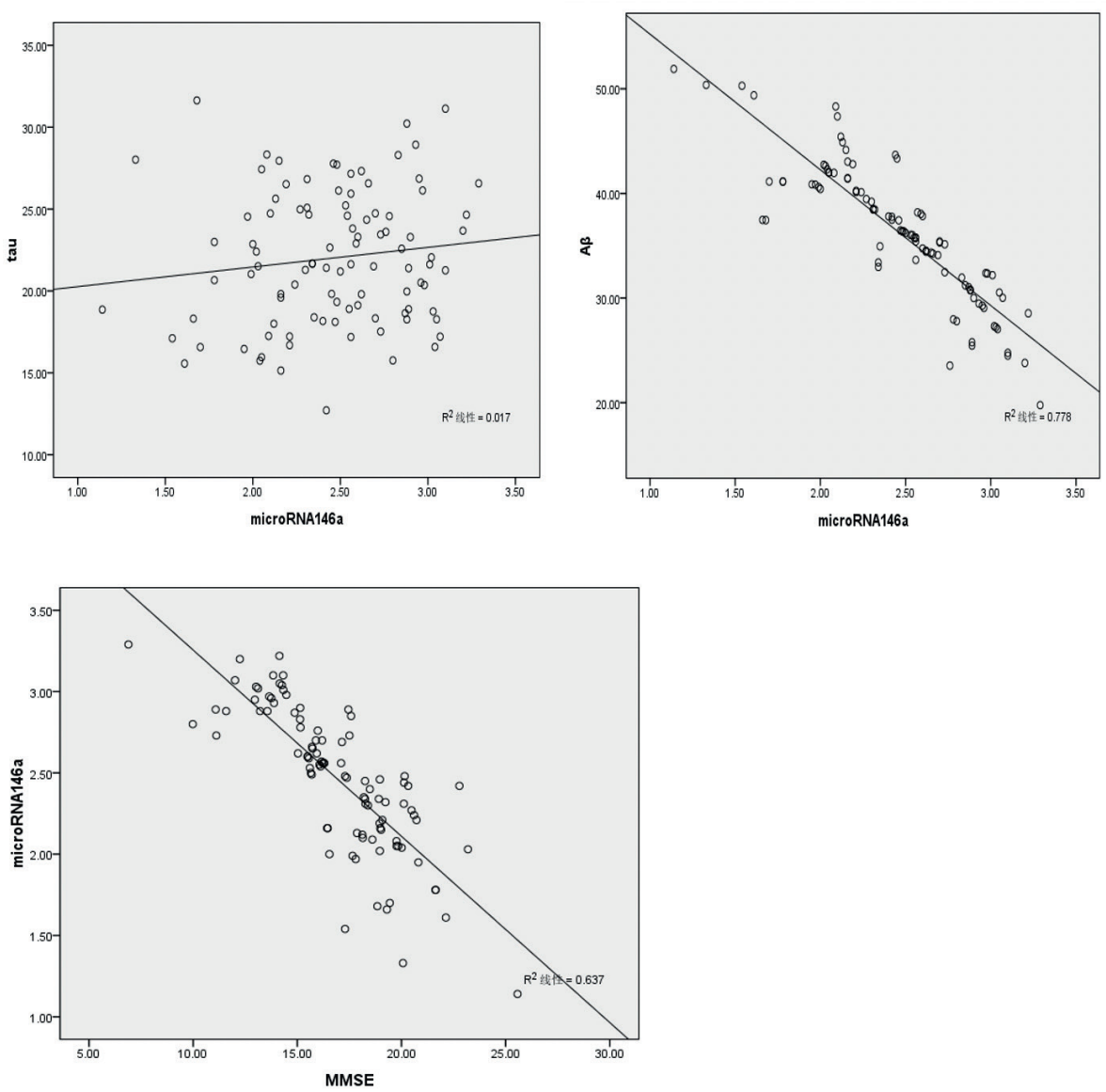

Figure 2. Correlation analysis between microNRA-146a and $A\beta_{1-42}$, Tau protein, MMSE scores.

lower than the control group. The tau protein concentration was significantly higher in AD patients compared to the control group ($p<0.05$). Data are summarized in Table II.

Correlation of MicroRNA-146a with $A\beta_{1-42}$ Tau Protein, and MMSE Score

The expression of microRNA-146a in the AD group was positively correlated with the $A\beta_{1-42}$ protein and negatively correlated with tau protein and MMSE score levels. The differences were statistically significant ($p<0.05$) as summarized in Table III and Figure 2.

The Value of MicroRNA-146a in Predicting AD

The ROC curve showed that microRNA-146a had a strong predictive value for AD with an AUC of 0.879 (95% CI 0.812-0.947). The optimum cut-off value was shown to be 1.45 with a sensitivity of 91.8% and a specificity of 88.0%. Data are summarized in Table IV and Figure 3.

Analysis of the Position and Conservation of MicroRNA-146a in the Human Genome

MicroRNA-146a is located at positions 160485325-160485450 on the 5q33.3 human chromosome. The

Table I. Comparison of baseline data between the two groups.

Item	Exp group (n=98)	Control group (n=50)	t/X^2	p
Sex (Male/Female)	54/44	26/24	0.12	0.72
Age (year)	67.18±6.53	68.74±6.62	1.36	0.17
BMI (kg/m^2)	22.87±3.23	23.65±3.41	1.34	0.18
Hypertension (n,%)	33 (33.67%)	14 (28.00%)	0.49	0.48
Diabetes mellitus (n,%)	18 (18.36%)	6 (12.00%)	0.98	0.32
TG (mmol/l)	1.42±0.21	1.46±0.22	0.59	0.55
TC (mmol/l)	4.17±0.85	4.10±0.92	0.52	0.60
HDL-C (mmol/l)	0.93±0.35	0.84±0.32	1.55	0.12
LDL-C (mmol/l	2.71±0.85	2.92±0.91	1.49	0.14
Education duration (year)	8.75±2.31	8.92±2.12	0.53	0.59

Table II. Comparison of microRNA-146a, $A\beta_{1-42}$ and tau protein between the two groups.

Item	Exp group	Control group	t	p
microRNA-146a	2.45±0.43	0.98±0.12	31.52	0.00
$A\beta_{1-42}$ (pg/ml)	37.43±6.75	51.26±9.24	9.38	0.00
tau (pg/ml)	22.87±4.51	13.64±2.91	15.03	0.00
MMSE score	16.92±3.07	28.59±0.72	34.50	0.00

sequence of microRNA-146a is 99 bp long and is highly conserved amongst six species, including humans, rhesus monkeys, mice, dogs, elephants, and chickens.

Screening Results of MicroRNA-146a DEGs

The Targetscan and Cometa databases were used to predict the target genes of microRNA-146 and identified 251 and 939 DEGs, respectively. A total of 88 DEGs in the two databases were identified using a Venn diagram (Table V). These target genes were mainly related to gene transcription, the process of immunoglobulin, inflammatory response, calmodulin production, and synaptic differentiation.

Enrichment Analysis of MicroRNA-146a DEGs GO and KEGG

GO analysis showed that DEGs were mainly enriched in biological processes such as cell proliferation regulation, neuronal differentiation, phosphorus metabolism, immune inflammation,

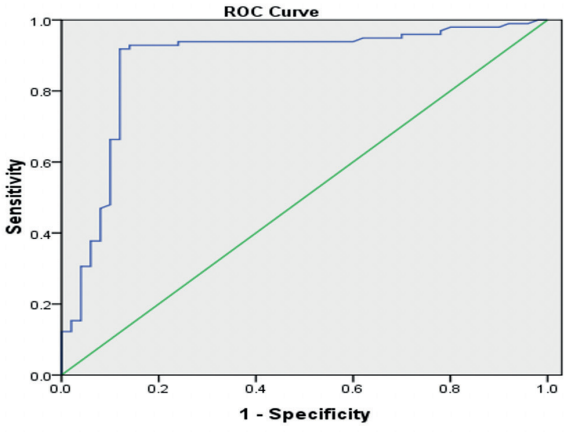

Figure 3. AUC curve.

Table III. Correlation analysis between microRNA-146a, $A\beta_{1-42}$ and Tau protein.

	microRNA-146a	
	r	p
$A\beta_{1-42}$	-0.882	<0.01
Tau	0.129	0.205
MMSE	-0.789	<0.01

Table IV. AUC curve.

AUC	Standard error	p	95%CI
0.879	0.035	<0.01	0.812-0.947

Table V. MicroRNA-146a differentially expressed genes.

ERBB4 IRAK1 WWC2 TAF9B USP47 ABL2 BMPR1A CDC14A CLCN6 RNF32 ZNF148 MYO6 MARK1 SORT1 IGSF1 MMP16 SLC2A3 SEC23IP CNTF NRP2 QKI BAG1 ARMC8 RARB TDRKH NRAS FRYL FBXW2 GRSF1 C10orf76 PHOX2B MYT1 CAMSAP1 FLOT2 CCDC6 STC1 SEMA3G TCF21 TANC2 LTB EDNRB ROBO1 EIF5A2 RABGAP1 RUNX1T1 LRRC15 SMAD4 VAT1 POU3F2 LFNG BNC1 MYBL1 SYT1 SIAH2 TRAF6 DGKG KLF7 CD80 CDON BCORL1 CARD10 DNPEP SLI1 TRK3 RNASEL C16orf72 LRP2 PRX KCMF1 NUMB KIF24 ZNF532 PPP1R11 RIMS2 NOVA1 DLGAP2 SH3GL2 EIF4G2 MFHAS1 FBXO28 KPNA6 NF2 STRN CASK PBX2 SLC10A3 USP3 SCN3B PGK1

Table VI. PGO analysis of microRNA-146a differentially expressed genes.

GO ID	Term	Gens	Count	*p*	Benjamini
30182	neuron differentiation	NRP2, KLF7, SLITRK3, BAG1, et al	10	4.5E-5	3.5E-1
01060	positive regulation of macromolecule metabolic process	TCF21, IRAK1, EDNRB, KLF7, et al.	14	4.5E-4	2.0E-1
10557	positive regulation of macromolecule biosynthetic process	TCF21, IRAK1, KLF7, CD80, et al.	12	5.6E-4	1.6E-1
42127	regulation of cell proliferation	NF2, ERBB4, SMAD4, STRN, et al.	13	7.4E-4	1.6E-1
31328	positive regulation of cellular biosynthetic process	TCF21, IRAK1, KLF7, CD80, et al.	12	8.3E-4	1.5E-1
09891	positive regulation of biosynthetic process	ZNF148, SMAD4, CASK, POU3F2, et al	12	9.3E-3	1.4E-1
48598	embryonic morphogenesis	TCF21, NF2, CDON, SMAD4, et al.	8	1.1E-3	1.4E-1
07250	activation of NF-kappaB-inducing kinase activity	IRAK1, TRAF6, CARD10, et al.	3	2.0E-3	2.2E-1
48729	tissue morphogenesis	TCF21, NF2, SMAD4, GRSF1, et al.	6	2.5E-3	2.4E-1
51130	positive regulation of cellular component organization	NF2, ROBO1, NUMB, SMAD4, et al.	6	2.6E-3	2.2E-1
42476	odontogenesis	ERBB4, NF2, TRAF6, BMPR1A, et al.	4	2.8E-3	2.2E-1
42325	regulation of phosphorylation	DGKG, SMAD4, TRAF6, CARD10, et al.	9	3.0E-3	2.1E-1
19220	regulation of phosphate metabolic process	SMAD4, TRAF6, CARD10, BMPR1A	7	3.1E-3	8.1E-1
51174	regulation of phosphorus metabolic process	NF2, CD80, DGKG, SMAD4, et al.	9	3.8E-3	2.4E-1
07398	ectoderm development	NF2, FLOT2, BNC1, POU3F2, et al	9	3.8E-3	2.4E-1
60284	regulation of cell development	PHOX2B, ROBO1, NUMB, SMAD4, et al.	6	3.9E-3	2.3E-1
08284	positive regulation of cell proliferation	NRAS, ERBB4, CD80, BNC1, et al.	6	4.4E-3	2.5E-1
45597	positive regulation of cell differentiation	PHOX2B, CD80, ROBO1, NUMB, et al.	8	4.9E-3	7.9E-1
10647	positive regulation of cell communication	TRAF6, LTB, BMPR1A,NRAS, et al.	8	5.9E-3	3.0E-3
10769	cell morphogenesis involved in differentiation	ROBO1, NUMB, SMAD4, POU3F2, et al.	6	7.0E-3	3.3E-1
48666	neuron development	NRP2, KLF7, SLITRK3, ROBO1, et al.	7	7.5E-3	3.3E-1
45941	positive regulation of transcription	TCF21, IRAK1, KLF7, ZNF148, et al.	8	7.6E-3	8.5E-1

and cytokine synthesis. KEGG pathway enrichment analysis showed that differentially expressed genes were mainly enriched in signaling pathways such as Toll-like receptor signaling, tumor signaling, neurotransmitter regulatory signaling, viral myocarditis, and EB signaling as shown in Tables VI and VII.

Discussion

The histopathological changes of AD include the formation of neuritic plaques (formed by argyrophilic nerve axon protrusions surrounding Aβ), neurofibrillary tangles (formed by hyperphosphorylated microtubule tau protein in a high-

Table VII. Predictors of clinical remission in CD patients at 2-month follow-up.

Signal pathway	Gene	Count	p	Benja mini
Neurotrophin signaling pathway	IRAK1, NRAS, SORT1, TRAF6	4	2.4E-2	7.8E-1
Viral myocarditis	EIF4G2, CD80, ABL2	3	5.0E-2	8.0E-1
ErbB signaling pathway	NRAS, ERBB4, ABL2	3	7.2E-2	7.9E-1
Pathways in cancer	NRAS, CCDC6, RUNX1T1, SMAD4, TRAF6	5	8.3E-2	7.4E-1
Toll-like receptor signaling pathway	IRAK1, CD80, TRAF6	3	9.3E-2	7.0E-1

ly spiralized neuron), and the loss of neurons and gliosis. In the brain, β-hydrolase and γ-hydrolase hydrolyze amyloid precursor protein to form $A\beta_{1-40}$ and $A\beta_{1-42}$ proteins. Studies have shown that monomeric Aβ protein is not neurotoxic, and soluble Aβ aggregation occurs when monomers are hydrolyzed[9]. Aβ oligomers can recognize and bind to a variety of receptors in the central nervous system causing neuronal cell calcium overload, decreased microglia membrane potential and increased glutamate levels that lead to the development of AD.

Although the expression level of $A\beta_{1-42}$ is significantly lower than that of $A\beta_{1-40}$, the neurotoxicity of $A\beta_{1-42}$ is much higher than that of $A\beta_{1-40}$, and it can more easily form amyloid leading to the formation of neuritis plaques[10]. The tau protein is distributed in neurons and forms microtubules after binding to tubulin. This complex plays an important role in maintaining the axonal transport of nerve cells and the stability of the cytoskeleton. The tau protein has many phosphorylation sites in its structure that become hyperphosphorylated under pathological conditions. Phosphorylation of tau results in loss of the ability of the protein to bind to microscopic proteins and promotes the mutual polymerization and winding of tau proteins to form neurofibrillary tangles (NFTs) that occur during AD[11].

Previous studies on the $A\beta_{1-42}$ and tau proteins are focused mainly on the detection in the cerebrospinal fluid. However, it is difficult to obtain specimens from the cerebrospinal fluid and peripheral blood is more convenient for routine analysis. The identification of $A\beta_{1-42}$ and tau proteins in the peripheral blood is particularly important in the diagnosis of AD. However, due to the blood-brain barrier and the large peripheral blood volume, the concentrations of related proteins are low and require highly sensitive detection methods. Compared to other detection methods, enzyme-linked immunosorbent assay (ELISA) is highly sensitive and can be used to detect low concentrations of biomarkers in the blood or other fluids. ELISA is based on the use of enzymes as organic catalysts that can induce a large number of catalytic reactions to produce an observable color reaction product. This approach uses an enzyme amplification system that is sufficiently accurate to trace the location of the antigens or antibodies at the cellular or subcellular levels and to quantify the levels of proteins at the microgram or nanogram levels[12,13].

In this study, the ELISA technique was used to quantitatively detect the concentrations of the $A\beta_{1-42}$ and tau proteins in the serum of the AD and control groups of patients. Our results showed that the concentration of $A\beta_{1-42}$ protein in the serum of AD patients was significantly lower than that of the control group suggesting the use of $A\beta_{1-42}$ as a potential biomarker for AD.

The data presented in this study do not comprehensively agree with other published reports in the literature, which may be due to a number of reasons. During the development of AD, a large amount of $A\beta_{1-42}$ protein in the cerebrospinal fluid entangles in the brain to form neuronal inflammatory plaques that leads to a decrease in the secreted levels of $A\beta_{1-42}$ protein in the peripheral blood. The $A\beta_{1-42}$ protein can also be secreted by platelets and skeletal muscle, and its metabolism is affected by the functions of the liver and kidnies[14]. The molecular structure of the $A\beta_{1-42}$ protein determines its hydrophobic activity, that is affected by binding to lipoproteins and albumin that may affect its detection in the blood[15].

We also found that the concentration of tau protein in AD patients was significantly higher than that of the control group. Similar to the conclusions from other studies, it is currently thought that the concentration of tau protein in the cerebrospinal fluid can be used to better detect and identify the stages of AD in patients. The levels of tau protein in the peripheral blood can be used to distinguish AD patients from those free from disease.

MicroRNA is a type of small RNA that can regulate protein expression and transcription. Mi-

croRNAs are widely involved in the regulation and development of diseases that are stable in the peripheral blood and can be used as biomarkers in many diseases[16]. MicroRNAs have diverse functions and are differentially expressed in different tissues, organs, and species[17]. It is known that the levels of some microRNAs are closely related to the pathogenesis of AD and can participate in Aβ deposition. Amyloid precursor protein (APP) is cleaved by β-secretase 1 (BACE1) to produce sAPPa, and the remaining short peptides are cleaved and processed by r-secretase to produce CTFr and Aβ. The deposition of Aβ protein is a central part of the pathological changes in AD. BACE1 is the rate-limiting enzyme for the production of Aβ. Studies have shown that microRNAs-29a, 29b-1, 9, 107, and 328 can indirectly regulate the expression of BACE1 and affect the pathogenesis of AD[18-21]. MicroRNA-520c and microRNA-153 can also directly regulate the expression of APP and affect the pathogenesis of AD[22-23].

MicroRNAs can also affect the process of Aβ deposition by regulating enzymes and signal pathways. They can also affect the phosphorylation of tau. Highly phosphorylated tau weakens the stability of the protein and its interactions with microtubules, causing nerve fiber degeneration and function loss. These changes then lead to the formation of double-stranded spiral nerve filaments and neurofibrillary tangles that are common features in AD. MicroRNA-26b and microRNA-132-3b can directly affect the phosphorylation of tau. MicroRNA-15a and microRNA-9 can affect the kinases involved in the phosphorylation of tau protein such as glycogen synthesis kinase 3β and Bcl-2 related anti-apoptotic proteins that regulate the non-ubiquitin-dependent proteasomal degradation of tau protein[24-27].

To explore the expression of microRNA-146a in the serum of AD patients, this study first demonstrated that microRNA-146a is highly conserved in humans using bioinformatics analyses. We used these data to design microRNA-146a primer sequences and then performed subsequent *in vitro* experiments. RT-PCR results also showed that the relative expression level of microRNA-146a was higher in the serum of AD patients than in the control group, suggesting that microRNA-146a may be related to the pathogenesis of AD. To investigate the potential mechanisms of microRNA-146a in AD, we used correlation analysis to show that microRNA-146a was negatively correlated with the levels of the $A\beta_{1-42}$ protein and did not correlate with the levels of tau protein. Current studies have shown that the expression of microRNA-146a is up-regulated in AD patients whilst the levels of microRNA-146b are down-regulated. The regulatory mechanism and functional impact behind these differences still needs to be fully clarified[28].

In the early stage of AD disease, interleukin-1 (IL-1) is overexpressed. This change is induced by the nuclear factor-kappa B (NF-KB) transcription pathway, that can also induce the expression of microRNA-146a but not microRNA-146b[29]. MicroRNA performs biological functions by binding to specific target gene mRNA. To clarify the potential target genes of microRNA-146a, we used bioinformatics methods to predict the target genes of microRNA-146a in AD and identified a total of 88 genes. Further enrichment analysis of the potential target genes using GO and KEGG helped to identify and predict the molecular regulatory networks involved in AD. GO analysis showed that the relevant target genes were enriched in many functions, including cell proliferation regulation, neuronal differentiation, phosphorus metabolism response, and immunity. These identified genes were involved in many biological processes, including the synthesis of inflammatory cytokines that is an important mechanism in the pathogenesis of AD. KEGG analysis showed that microRNA-146a was mainly enriched in signaling pathways such as Toll-like receptors and neurotransmitter regulation. The Toll-like receptor signaling pathway is an important inflammatory signaling pathway. Inflammation of the central nervous system is also one of the causes of AD. An important mechanism is that the inflammatory factors, induced by microglia in the brain of AD patients, can reduce the levels of key enzymes known to degrade the Aβ protein-insulin degrading enzyme resulting in decreased Aβ clearance and deposition and the formation of inflammatory plaques in the brain[30,31].

MicroRNA-146a can participate in the inflammatory response in a variety of ways. It can negatively regulate important inflammatory signaling pathways such as the TLR pathway, suggesting that the increased expression of microRNA-146a in AD patients may negatively regulate the inflammatory response. Researches have also found that inflammation can affect the transcriptional and post-transcriptional modification of tau protein and the spread of oligomeric tau protein during the pathogenesis of AD. However, in this study, we did not find a correlation between microRNA-146a and the tau protein. It has been

hypothesized that inflammation is involved in the pathogenesis of AD. MicroRNA-146a does not regulate the inflammatory response or affect the metabolism of tau protein in AD patients, yet the specific mechanism needs to be further investigated. Our data require further validation as our study had a small sample size, was a single-center study and only a correlation analysis. Also, we did not investigate the underlying mechanism of microRNA-146a in AD that requires further study.

Conclusions

This is the first report that shows that microRNA-146a is differentially expressed in AD and demonstrates that it may be involved in the pathogenesis of the disease through the Aβ1-42 protein instead of the Tau protein. Our data suggest that microRNA-146a may be a new target for the treatment of AD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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