Integrated analysis of miRNA and mRNA expression in the blood of patients with Alzheimer's disease

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Abstract. Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is considered the most common type of dementia worldwide. The aim of the present study was to identify key microRNAs (miRNAs/miRs) and mRNAs affecting the pathogenesis of AD, which may be developed as promising biomarkers for the early diagnosis or targeted therapy of patients with AD. Integrative analysis was performed on 12 representative miRNA datasets and three mRNA datasets of the blood from patients with AD, in order to identify differentially expressed (DE)miRNAs and DEmRNAs. Subsequently, the miRWalk database was used to identify the potential miRNA-mRNA interactions among DEmiRNAs and DEmRNAs, and an AD-specific miRNA-mRNA network was constructed using Cytoscape software. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were performed to assess the target mRNAs of DEmiRNAs. A total of 37 DEmiRNAs and 2,011 DEmRNAs were identified between AD and normal control samples. In addition, 853 high confidence miRNA-mRNA interactions were identified and subsequently used to construct the AD specific miRNA-mRNA network. A total of five miRNAs, including hsa-miR-93, hsa-miR-26b, hsa-miR-34a, hsa-miR-98-5p and hsa-miR-15b-5p were identified as the key nodes in the miRNA-mRNA network by topological analysis. Functional enrichment analysis demonstrated that the target mRNAs of DEmiRNAs were enriched in AD-associated pathways, such as the 'neurotrophin signaling pathway' and 'insulin signaling pathway'. Taken together, the results of the present study provide novel insights into the molecular mechanisms underlying AD and contribute to the identification of biomarkers and novel strategies for drug design for AD treatment.

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Introduction

Alzheimer's disease (AD) is the most common form of senile dementia characterized by neuronal death, loss of synaptic function and atrophy in areas of the brain that affect cognitive functions and memory (1,2). The prevalence of AD is estimated to triple by 2050, increasing significant economic and social burden on patients and society (3). Currently, cognitive testing and neuroimaging remain the gold standard for the diagnosis of AD (4); however, these clinical techniques are complicated and expensive (5). Thus, simple and convenient biomarkers are critically required to improve diagnosis of early stage AD (6). Screening for biomarkers in patients with AD is predominantly reported for cerebrospinal fluid, blood and other biological samples, such as urine, breath and saliva (7,8). Increasing evidence has demonstrated that detection of biomarkers in peripheral blood is minimally invasive, low-cost and easily applied for mass screening (9,10).

MicroRNAs (miRNAs/miRs) are 22-23 nucleotide long small non-coding RNAs, which suppress gene expression through binding to the 3'-untranslated region of corresponding mRNAs (11). It has been reported that miRNAs are ideal biomarkers due to their stability in body fluids, and can be attributed to specific organs and pathologies in AD (12). For example, Hara *et al* (13) demonstrated that hsa-miR-501-3p may be a serum biomarker that could correspond to pathological events occurring in the brain of patients with AD. Additionally, Jia and Liu (14) reported that downregulated hsa-miR-223 serum may serve as a biomarker in AD, as demonstrated by quantitative PCR analysis of serum samples from 84 probable sporadic patients with AD and 62 healthy individuals in China.

Currently, a number of studies have identified several miRNAs or mRNAs that are significantly differentially expressed (DE) in the blood from patients with AD compared with normal control samples, indicating their key functions in the pathogenesis of AD (13,14). However, the comparability of these studies is particularly challenging due to their small sample size, as well as differences in their quantification methods and protocols. There is a need to combine the study results using a meta-analysis approach to improve the understanding of the molecular mechanisms underlying AD. Chen *et al* (15) analyzed nine representative miRNA datasets of AD samples, which originated from tissues, serum, extracellular or cerebrospinal fluid, and identified 13 key miRNAs associated with AD. Cătană *et al* (16) evaluated the diagnostic

value of miRNAs expressed in different body fluids of patients with AD using two meta-analytical approaches with different statistic indicators. However, a detailed map of specific biomarkers in the blood of patients with AD is still lacking.

The present study systematically analyzed 15 representative miRNA and mRNA datasets of the blood from patients with AD using a series of bioinformatics methods. The present study identified several key miRNAs, mRNAs and pathways affecting the pathogenesis of AD, providing novel insights into the molecular mechanisms underlying AD.

Materials and methods

miRNA and mRNA expression datasets. The miRNA and mRNA expression profiles of patients with AD were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo), which is a public repository for high-throughput gene expression datasets (17). The present study collected 12 representative miRNA and three mRNA expression profiles of blood samples from patients with AD and normal control samples. All datasets contained at least three AD samples and had age-matched normal control samples in each group. Among the 12 miRNA expression datasets, seven focused on serum (9,10,13,14,18-20), three on plasma (21-23), one on both serum and plasma (24), and one focused on whole blood (25). The three mRNA expression datasets all focused on whole blood (5). Detailed information of these datasets are presented in Table I.

Data processing and differential expression analysis. The DEmiRNA information was manually extracted from the publications of 12 miRNA datasets. Only the miRNAs validated by previous reverse transcription-quantitative PCR analysis were retained and categorized into upregulated and downregulated miRNAs in patients with AD compared with normal control samples (Table I). The miRNAs identified in at least one dataset were integrated as high-confidence DEmiRNAs.

The raw data of three mRNA expression profiles (GSE63060, GSE63061 and GSE18309) were downloaded from the GEO database and preprocessed with background correction. Subsequently, the Limma package in R language (version 3.40.6; https://bioconductor.org/packages/limma) was used to normalize the datasets and identify the significantly DEmRNAs with the following cut-off criteria: Adjusted $P<0.05$ and $|\log \text{fold change (logFC)}|>0.5$. In the case where multiple probes corresponded to the same gene, the probe with the maximal value was selected as the expression of that particular gene. The DEmRNAs were clustered using hierarchical clustering and implemented by pheatmap package in R language (version 1.0.12) (26). Euclidean distance was selected as a measure of distance between the samples.

Prediction of miRNA-mRNA interactions. The putative target mRNAs of high-confidence DEmiRNAs were predicted using six bioinformatic algorithms [DIANA-microT (27), miRanda (28), miRDB (29), miRWalk (30), PICTAR (31) and TargetScan (32)]; the default parameters were used for all software programs, and target mRNAs identified by at least four algorithms were retained. Subsequently, target mRNAs identified in the miRWalk database (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk) were selected, which collects data on experiment supported miRNA-mRNA interactions (30). Considering miRNAs suppress expression of their target mRNAs, the DEmRNAs whose expression were inversely associated with the miRNAs were regarded as the miRNA target.

Construction of miRNA-mRNA network and identification of hub nodes. The high-confidence DEmiRNA-mRNA interactions were used to construct the miRNA-mRNA network using Cytoscape software (version 3.5.0; http://www.cytoscape.org). The hub nodes in the network were nodes with high scores of network topology property indictors, which were analyzed using CytoNCA (version 2.1.6) within Cytoscape, including degree centrality, betweenness centrality and closeness centrality. In general, a high score of network topology property indictors indicates important roles in the network.

Functional annotation. Gene Ontology (GO) analysis, which organizes genes into hierarchical categories and determines the gene regulatory network on the basis of biological process (BP), molecular function (MF) and cellular component (CC), was applied to analyze the functions of genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was used to determine which signaling pathways the genes were enriched in. The Database for Annotation, Visualization and Integrated Discovery (DAVID; https:/david.ncifcrf.gov) was used for both GO and KEGG enrichment analyses where a false discovery rate (FDR) <0.05 was considered to indicate a statistically significant difference (33).

Results

Differential analysis of miRNAs and mRNAs in patients with AD. The workflow of the present study is presented in Fig. 1. The present study downloaded 12 separate miRNA expression profiling datasets of blood samples from patients with AD and normal control samples. Detailed information on sample size, blood component and experimentally supported DEmiRNAs were manually extracted from the references of the datasets (Table I). miRNAs identified in at least one dataset were integrated as high-confidence DEmiRNAs. A total of 37 miRNAs were identified to be significant DEmiRNAs, among which seven miRNAs were upregulated and 30 miRNAs were downregulated in patients with AD compared with normal control samples.

A total of three mRNA expression profiles of whole blood samples from patients with AD and normal control samples were downloaded from the publicly accessible database GEO (Table I). Following background correction and normalization, 2,011 DEmRNAs were identified using the Limma package, under the following cut-off criteria; adjusted $P<0.05$ and $|\text{logFC}|>0.5$. Among these, 911 mRNAs were upregulated and 1,100 were downregulated in patients with AD compared with normal control samples. Subsequently, hierarchical clustering of the DEmRNAs was performed, which is displayed in the heatmap (Fig. 2).

Construction of the miRNA-mRNA network and identification of hub nodes. The DEmiRNAs-mRNA interactions

Table I. Summary of the microRNA and mRNA datasets analyzed.

Author (year)	Study ID	RNA type	Patient count	Control count	Blood component	Upregulated	Downregulated	(Refs.)
Tan *et al* (2014)	1	miRNA	105	150	Serum	hsa-miR-9	hsa-miR-125b, hsa-miR-181c	(9)
Galimberti *et al* (2014)	2	miRNA	7	6	Serum		hsa-miR-125b, hsa-miR-23a, hsa-miR-26b	(10)
Tan *et al* (2014)	3	miRNA	50	50	Serum		hsa-miR-98-5p, hsa-miR-885-5p, hsa-miR-483-3p, hsa-miR-342-3p, hsa-miR-191-5p, hsa-let-7d-5p	(18)
Dong *et al* (2015)	4	miRNA	127	123	Serum		hsa-miR-31, hsa-miR-93, hsa-miR-143, hsa-miR-146a	(19)
Zhu *et al* (2014)	5	miRNA	26	42	Serum		hsa-miR-210	(20)
Jia *et al* (2016)	6	miRNA	84	62	Serum	hsa-miR-519	hsa-miR-29, hsa-miR-125b, hsa-miR-223	(14)
Hara *et al* (2017)	7	miRNA	27	18	Serum		hsa-miR-501-3p, has-miR-26b-5p	(13)
Kumar *et al* (2013)	8	miRNA	11	20	Plasma		hsa-let-7d-5p, hsa-let-7g-5p, hsa-miR-15b-5p, hsa-miR-142-3p, hsa-miR-191-5p, hsa-miR-301a-3p, hsa-miR-545-3p	(21)
Kiko *et al* (2014)	9	miRNA	10	10	Plasma		hsa-miR-34a, hsa-miR-146a	(22)
Wang *et al* (2015)	10	miRNA	97	81	Plasma		hsa-miR-107	(23)
Liu *et al* (2014)	11	miRNA	7	7	Serum, plasma		hsa-miR-384	(24)
Leidinger *et al* (2013)	12	miRNA	48	22	Whole blood	hsa-miR-151a-3p, hsa-miR-161, hsa-let-7d-3p, hsa-miR-112, hsa-miR-5010-3p	hsa-miR-103a-3p, hsa-miR-107, hsa-miR-532-5p, hsa-miR-26b-5p, hsa-let-7f-5p	(25)
GSE63060	13	mRNA	145	104	Whole blood	-	-	(5)
GSE63061	14	mRNA	140	135	Whole blood	-	-	(5)
GSE18309	15	mRNA	3	3	Whole blood	-	-	(5)

miRNA/miR, microRNA.

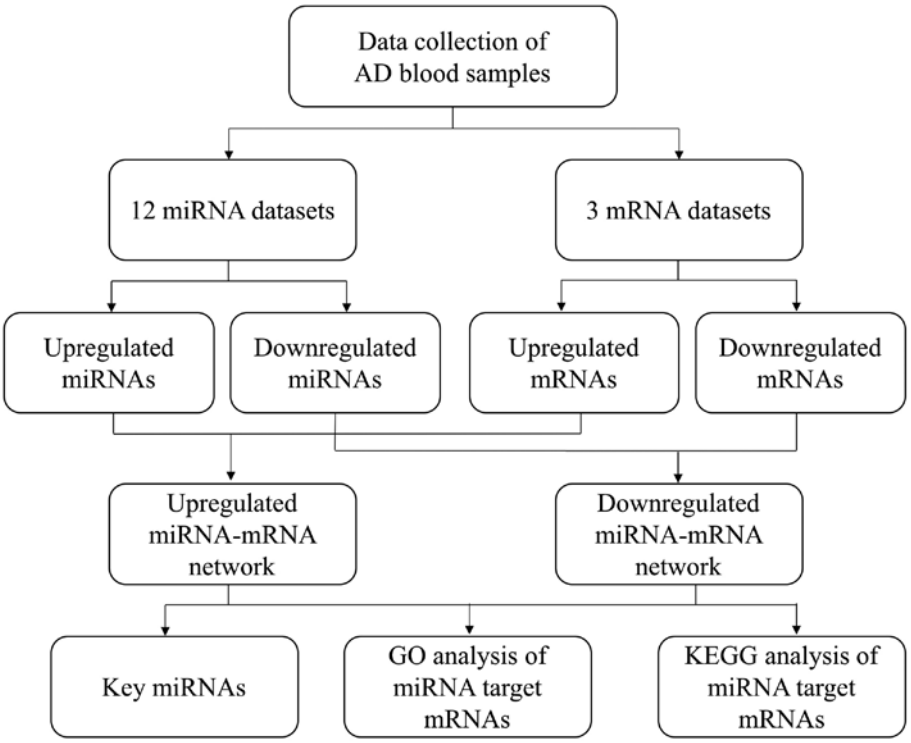

Figure 1. Schematic diagram of the study workflow to determine the association between microRNA and AD. AD, Alzheimer's disease; miRNA, microRNA; GO, Gene Ontology; KEGG, Kyoto of Encyclopedia of Genes and Genomes.

were identified using four algorithms and validated by subsequent experimentation in the miRWalk database. Target mRNAs of the DEmiRNAs were inversely associated with the expression of corresponding miRNAs between patients with AD and normal control samples. In total, 853 high-confidence DEmiRNA-mRNA interactions were identified, of which 17 were upregulated miRNAs-mRNA and 836 were downregulated miRNAs-mRNA interactions. The upregulated miRNAs-mRNA network and downregulated miRNAs-mRNA network were constructed based on these miRNA-mRNA interactions using Cytoscape software. The upregulated miRNAs-mRNA network consisted of 20 nodes and 17 edges (Fig. 3A). Of these, hsa-miR-9 had the highest connectivity and was demonstrated to negatively interact with 14 target mRNAs. A high score of network topology property indictors suggests a notable role in the network. According to the rankings of network topology property indictors, including degree centrality, betweenness centrality and closeness centrality, the top five nodes of the downregulated miRNAs-mRNA network, which consisted of 413 nodes and 836 edges, are listed in Table II. A total of five miRNAs, including hsa-miR-93, hsa-miR-26b, hsa-miR-34a, hsa-miR-98-5p and hsa-miR-15b-5p were among the top nodes for all topology property indictors, suggesting their critical roles in the pathogenesis of AD. The interactions of these five key miRNAs and their target mRNAs are presented in Fig. 3B.

GO terms annotation of the target mRNAs of DEmiRNAs. The online tool DAVID was used to identify significantly enriched GO terms for the target mRNAs of DEmiRNAs between patients with AD and normal control samples (Table III). The results indicated that the target mRNAs were predominantly enriched in BP terms, including 'regulation of transcription' and 'apoptotic process'. Regarding CC, the target mRNAs were enriched in the 'nucleus' and 'intracellular'. In addition, MF analysis displayed that the target mRNAs were significantly enriched in 'metal ion binding' and 'DNA binding'.

KEGG pathway enrichment of the target mRNAs of DEmiRNAs. The significantly enriched pathways of the target mRNAs of DEmiRNAs between patients with AD and normal control samples are presented in Table IV. The results demonstrated that the target mRNAs were enriched in the 'neurotrophin signaling pathway', 'insulin signaling pathway', 'MAPK signaling pathway', 'lysosome', 'Alzheimer's disease' and 'Huntington's disease' (Fig. 4).

Discussion

Increasing evidence has demonstrated that identifying biomarkers using meta-analysis is helpful to the diagnosis and targeted therapy of patients with AD at an early stage (15,16). However, previous studies have used datasets that originate from different samples, for example, Chen *et al* (15) combined datasets of tissues, serum, extracellular and cerebrospinal fluid, and Cătană *et al* (16) evaluated datasets from different body fluids. Conversely, the present study focused on datasets from the blood. In addition, the strategies of these previous studies only considered miRNAs, whereas the present study identified key miRNAs, mRNAs and pathways affecting the pathogenesis of AD by integrating the miRNA and mRNA expression profiling datasets. Although different strategies were used, some of the key miRNAs identified in the present study were in agreement with previous studies, for example, hsa-miR-26b, hsa-miR-15b

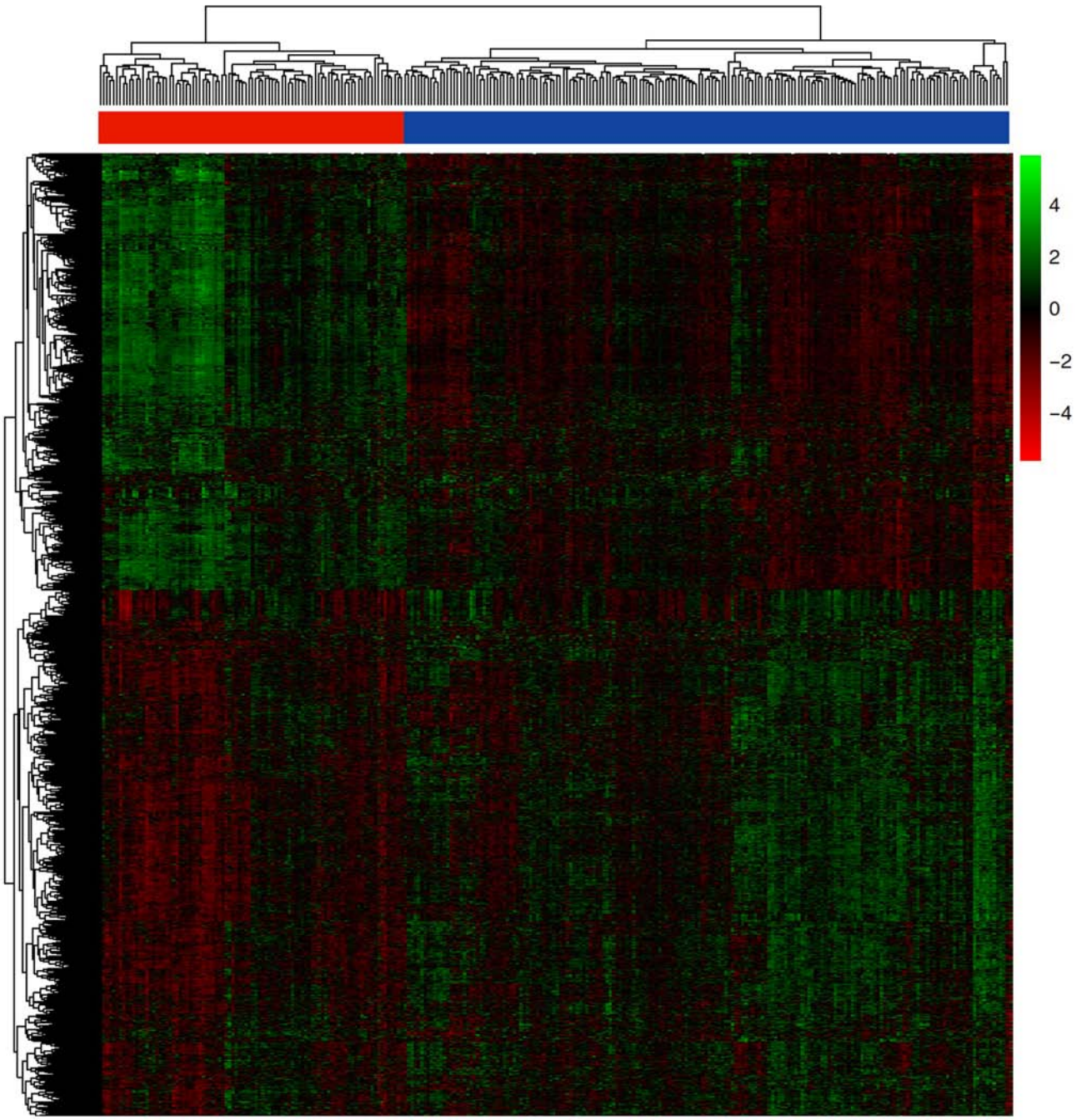

Figure 2. Heatmap of cluster analysis of differentially expressed mRNAs between patients with AD and normal control samples. Rows represent genes and columns represent samples. The values indicate the expression levels of the genes. Red represents normal control blood samples and blue represents blood samples from patients with AD. AD, Alzheimer's disease.

and hsa-miR-93 were also identified by Chen *et al* (15). In the present study, the DEmiRNA-mRNA crosstalk was assessed between the blood from patients with AD and normal control samples, by integrating the largest count of miRNA and mRNA datasets. Topological analysis of the AD-specific miRNA-mRNA network identified five miRNAs, including hsa-miR-93, hsa-miR-26b, hsa-miR-34a, hsa-miR-98-5p and hsa-miR-15b-5p as hub nodes, suggesting their critical roles in the pathogenesis of AD. Functional enrichment analysis demonstrated that the target mRNAs of DEmiRNAs were enriched in AD-associated pathways, such as the 'neurotrophin signaling pathway' and 'insulin signaling pathway'.

hsa-miR-93 was the hub node with the highest topology property indictors, which was demonstrated to negatively regulate 87 DEmRNAs in the AD-specific downregulated miRNA-mRNA network, including GADPH, ATP synthase F1 subunit β (ATP5B) and MAPK1. These protein-coding genes were significantly upregulated in patients with AD and enriched in the AD-associated pathway in the present study. Dong *et al* (19) reported that hsa-miR-93 was markedly decreased in the serum of patients with AD compared with controls, while screening the expression profile of serum miRNAs by Solexa sequencing. In addition, it was demonstrated that the panel of hsa-miR-93, along with hsa-miR-31

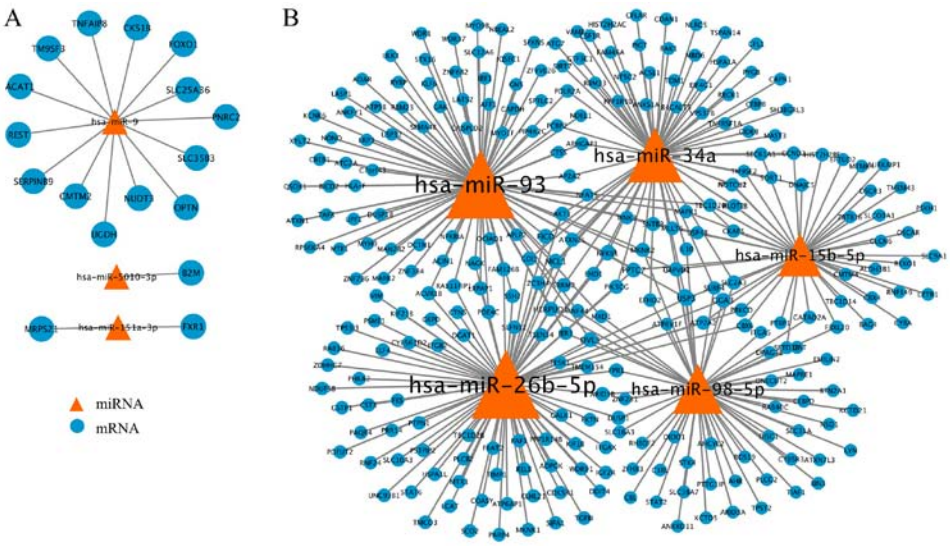

Figure 3. DEmiRNAs-mRNA regulatory network in Alzheimer's disease. (A) Upregulated and (B) downregulated miRNA-mRNA network of the key five miRNAs. Orange triangles represent miRNAs and blue circles represent target mRNAs. The solid lines represent high-confidence DEmiRNA-mRNA interactions. DE, differentially expressed; miRNA, microRNA.

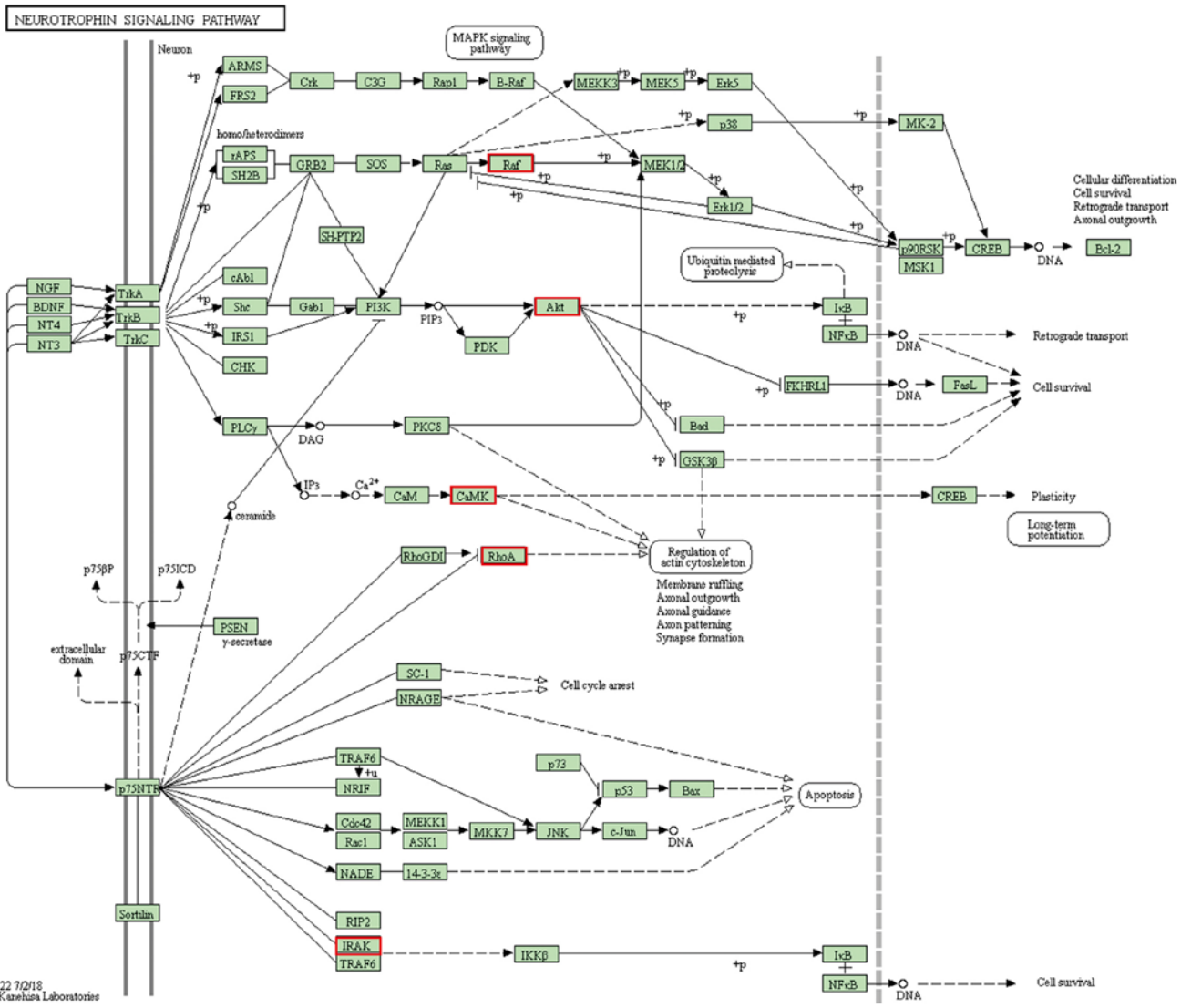

Figure 4. Neurotrophin signaling pathway is enriched in target mRNAs of differentially expressed microRNAs in Alzheimer's disease. Red rectangles represent the target mRNAs that are enriched in the neurotrophin signaling pathway. The image was obtained from the Kyoto Encyclopedia of Genes and Genomes database (https://www.kegg.jp/kegg-bin/show_pathway?map=hsa04722&show_description=show).

Table II. Top five nodes in the downregulated miRNA-mRNA network according to the score of network topology property indictors.

Rank	Node	Degree	Betweenness	Closeness
1	hsa-miR-93	87	197.28	46,730.89
2	hsa-miR-26b	87	194.30	25,361.43
3	hsa-miR-34a	66	180.78	32,132.43
4	hsa-miR-98-5p	58	173.52	21,099.35
5	hsa-miR-15b-5p	48	170.25	20,266.93

Table III. Significantly enriched Gene Ontology terms of the target mRNAs of differentially expressed microRNAs identified by The Database for Annotation, Visualization and Integrated Discovery.

GO ID	GO terms	FDR
Biological process		
GO:0006355	Regulation of transcription, DNA-dependent	4.41×10^{-5}
GO:0006915	Apoptotic process	6.38×10^{-5}
GO:0006954	Inflammatory response	7.75×10^{-3}
GO:0007165	Signal transduction	2.34×10^{-5}
GO:0006468	Protein phosphorylation	4.63×10^{-8}
GO:0008360	Regulation of cell shape	4.84×10^{-2}
GO:0008283	Cell proliferation	9.85×10^{-4}
GO:0044419	Interspecies interaction between organisms	1.64×10^{-7}
GO:0006810	Transport	1.62×10^{-2}
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	5.45×10^{-6}
Molecular function		
GO:0046872	Metal ion binding	1.12×10^{-9}
GO:0003677	DNA binding	1.53×10^{-4}
GO:0008270	Zinc ion binding	4.04×10^{-4}
GO:0003700	Sequence-specific DNA binding transcription factor activity	4.85×10^{-4}
GO:0016740	Transferase activity	3.39×10^{-2}
GO:0005524	ATP binding	1.81×10^{-12}
GO:0005515	Protein binding	1.42×10^{-35}
GO:0000166	Nucleotide binding	1.12×10^{-15}
GO:0043565	Sequence-specific DNA binding	2.33×10^{-3}
GO:0016301	Kinase activity	6.54×10^{-4}
Cellular component		
GO:0005634	Nucleus	1.54×10^{-21}
GO:0005622	Intracellular	1.40×10^{-6}
GO:0016607	Nuclear speck	2.21×10^{-3}
GO:0016021	Integral to membrane	8.15×10^{-5}
GO:0005625	Soluble fraction	6.85×10^{-3}
GO:0005794	Golgi apparatus	7.52×10^{-5}
GO:0016020	Membrane	7.88×10^{-16}
GO:0005783	Endoplasmic reticulum	3.40×10^{-5}
GO:0005737	Cytoplasm	5.10×10^{-26}
GO:0005739	Mitochondrion	7.82×10^{-5}

GO, Gene Ontology; FDR, false discovery rate.

and miR-146a, may be used to discriminate AD from vascular dementia. Takashima *et al* (34) reported that hsa-miR-93 may be a potential prognostic biomarker in primary central nervous system lymphoma. GAPDH is a family of abundantly

Table IV. Significantly enriched KEGG pathways of the target mRNAs of differentially expressed miRNAs identified by The Database for Annotation, Visualization and Integrated Discovery.

KEGG ID	KEGG pathway	FDR	Gene count	Genes	Related key miRNAs
Kegg:04722	Neurotrophin signaling pathway	1.05×10^{-9}	15	MAPK1, PRKCD, PLCG2, NFKBIA, RHOA, IRAK1, MAP3K5, AKT1, NFKB1, RAF1, SORT1, RPS6KA4, CAMK2G, PIK3CG, RPS6KA1	hsa-miR-34a, hsa-miR-93, hsa-miR-26b, hsa-miR-15b-5p, hsa-miR-98-5p
Kegg:04910	Insulin signaling pathway	2.43×10^{-8}	14	EXOC7, MAPK1, PYGB, FOXO1, PTPN1, PHKA2, FLOT2, SREBF1, MKNK2, AKT1, RAF1, MKNK1, PIK3CG, CBL	hsa-miR-34a, hsa-miR-93, hsa-miR-26b, hsa-miR-15b-5p, hsa-miR-98-5p
Kegg:04010	MAPK signaling pathway	1.54×10^{-6}	16	MAPK1, IKBKG, DUSP1, RELB, TNFRSF1A, MAP3K11, MKNK2, MAP3K5, AKT1, NFKB1, RAF1, MKNK1, MAPK8IP3, RPS6KA4, RPS6KA1, STK4	hsa-miR-34a, hsa-miR-93, hsa-miR-26b, hsa-miR-15b-5p, hsa-miR-98-5p
Kegg:04142	Lysosome	2.62×10^{-6}	11	GGA3, CTSS, TPP1, PSAP, LAMP1, ATP6AP1, GNS, SORT1, IDS, IGF2R, CTNS	hsa-miR-34a, hsa-miR-93, hsa-miR-26b, hsa-miR-15b-5p, hsa-miR-98-5p
Kegg:04210	Apoptosis	8.35×10^{-6}	9	IKBKG, NFKBIA, TNFRSF1A, CAPN1, IRAK1, AKT1, NFKB1, PIK3CG, CFLAR	hsa-miR-93, hsa-miR-26b, hsa-miR-34a
Kegg:05010	Alzheimer's disease	1.43×10^{-4}	10	MAPK1, ATP2A2, NDUFS8, TNFRSF1A, CAPN1, ERN1, PLCB2, GAPDH, CDK5R1, ATP5B	hsa-miR-15b-5p, hsa-miR-26b, hsa-miR-93, hsa-miR-34a
Kegg:04150	mTOR signaling pathway	1.85×10^{-4}	6	MAPK1, AKT1, DDIT4, ULK1, PIK3CG, RPS6KA1	hsa-miR-15b-5p, hsa-miR-26b, hsa-miR-93, hsa-miR-34a
Kegg:05016	Huntington's disease	1.20×10^{-3}	9	DCTN1, NDUFS8, CREB1, AP2A2, PLCB2, CREBBP, REST, SP1, ATP5B	hsa-miR-93, hsa-miR-26b, hsa-miR-34a
Kegg:04310	Wnt signaling pathway	1.54×10^{-3}	8	RHOA, PLCB2, FRAT2, NFAT5, CAMK2G, CCND3, CREBBP, DVL3	hsa-miR-34a, hsa-miR-93, hsa-miR-26b, hsa-miR-15b-5p, hsa-miR-98-5p
Kegg:04330	Notch signaling pathway	3.83×10^{-2}	3	NOTCH2, CREBBP, DVL3	hsa-miR-34a, hsa-miR-93, hsa-miR-26b, hsa-miR-15b-5p, hsa-miR-98-5p

KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNA/miR, microRNA.

expressed oxidoreductases that are known for their role in glucose metabolism (35). It has been reported that GAPDH is able to interact with various small molecules (proteins and membranes) that serve key roles in normal and pathological cell functions, including AD-associated proteins and the β-amyloid precursor protein (36). It was therefore hypothesized that decreased hsa-miR-93 expression may serve vital roles in the pathological process of AD by targeting GADPH, ATP5B and MAPK1.

hsa-miR-26b was another hub node identified in the present study that negatively regulated 80 DEmRNAs in the AD downregulated miRNA-mRNA network, including cyclin-dependent kinase 5 regulatory subunit 1 (CDK5R1), ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2, AKT1 and NF-kB1, which were enriched in the AD pathway and 'neurotrophin signaling pathway'. Consistent with previous findings, the results of the present study demonstrated that hsa-miR-26b was significantly downregulated in patients with AD compared with the normal control samples, whereas the corresponding mRNAs were upregulated in patients with AD (25,37). CDK5R1 encodes for p35, a protein required for the main activation of CDK5 (38). The active p35/CDK5 complex has been reported to be involved in several aspects of brain development and function, and its deregulation is closely associated with AD onset and progression (39). Taken together, the results of the present study suggested that hsa-miR-26b may negatively regulate CDK5R1 expression in AD.

The 'neurotrophin signaling pathway' and 'insulin signaling pathway' were the most significantly enriched pathways of the target mRNAs of DEmiRNAs between patients with AD and normal control samples. The neurotrophin signaling pathway is activated by neurotrophins through binding to the tyrosine protein kinase receptor family, which results in a series of neuronal functions, such as axonal growth, cell survival, cell differentiation, dendritic arborization, synapse formation, plasticity and axonal guidance (40,41). The insulin signaling pathway, which is the main signal transduction pathway in insulin physiological function, serves a vital role in the metabolism, nerve protection and regulation of cognitive dysfunction (42). Increasing evidence has demonstrated that the symptoms of patients with AD are consistently accompanied with a disordered insulin signaling pathway or other symptoms, suggesting that the insulin signaling pathway may be closely associated with the pathogenesis of AD (43).

In conclusion, availably representative miRNA and mRNA expression profiling datasets of the blood from patients with AD were collected and subjected to comprehensive analysis though a series of bioinformatics methods in the present study. The AD-specific miRNA-mRNA crosstalk network was constructed and several key dysregulated miRNAs, mRNAs and signaling pathways affecting the pathogenesis of AD were identified. However, further experimental studies testing these results would be desirable. Taken together, the results of the present study provided a valuable resource for depicting the complexity of AD, and may contribute to the development of diagnostic biomarkers and therapeutic targets for AD.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SH and ZW conceived and designed the study; SH, ZW and LS analyzed and interpreted the data; YW acquired the data and wrote the manuscript; and SH, ZW and LS reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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