Exosomal circRNA-miRNA Expression profile from plasma in Alzheimer's Disease Patients by Bioinformatics and Integrative Analysis

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Abstract

Alzheimer's disease (AD) is an age-dependent neurodegenerative ailment globally. Compelling evidence suggests the function of exosomal non-coding RNAs has been associated with the progression of AD but whose exosomal-linked non-coding RNAs mediated regulatory mechanisms are broadly unlit. This study, therefore, set out with the aim of exploring the exosomal circRNA-miRNA networks in the plasma of AD patients. Data of 3 samples from each group (healthy, mild cognitive impairment (MCI), and AD) were fetched from ArrayExpress. The MCI and AD groups were compared with the healthy group by screening for differentially expressed miRNAs (DEmiRs) and circRNAs (DEcircRs) in plasma exosomes. Subsequently, common DEmiRs and DEcircRs for both MCI and AD groups were evaluated to identify gene ontologies, pathways, and networks. Lastly, the analysis of the PPI (protein–protein interaction) network and hub genes selection were performed. A total of common 19 (7 upregulated and 12 downregulated) DEmiRs and 24 DEcircRs were identified. It was predicted 4559 target genes for upregulated DemiRs, while 6504 target genes for downregulated DEmiRs and most of the target genes were associated with the PI3K-Akt pathway and that they were mostly regulated by hsa-mir-615-3p, hsa-mir-196a-5p, hsa-let-7c-5p, hsa-let-205-5p, hsa-mir-185-3p, hsa-mir-185-5p, hsa-mir-374a-5p, hsa-mir-374a-3p. Also, 9 hub genes (CCNE2, CCND1, CDK6, ACTB, MAPK1, AKT1, GSK3B, IGF1R, HSP90AA) were uncovered as the genes most associated with AD by a PPI network using Cytoscape plug-in cytohubba. Our outcomes exhibit a new outlook on a possible exosomal-linked miRNA-circRNA network in the pathogenesis of AD.

Introduction

Alzheimer's disease (AD) is observed as the most frequent case of dementia, and an incurable globally with the prevalence continuing to increase in part inasmuch as the aging society. Increasingly recognized as a serious, worldwide public health concern, AD is a chronic disorder characterized by misfolding of beta-amyloid peptides resulting in plaque formation and neurofibrillary tangles of hyperphosphorylated tau (Weller and Budson 2018; Busche and Hyman 2020). In the condition of diseases alike AD, where cognitive impairments take place much later in life than the real circumstances of disease progression, the search for identification of early screening biomarkers is gaining importance (Rastogi et al. 2021).

Exosomes are small vesicles and variable-membrane structures of endosomal origin shuttling in the extracellular area, levied an existing way of intercellular linkage (Xu et al. 2021). Since it is well-known that the ingredient of exosomes is to alter as per their originating and receiver cells, these vesicles can be employed as a biomarker for early AD diagnosis (Rastogi et al. 2021). Interestingly, exosomes depended biomarker screening is a current and quickly developing area in the diagnoses of neurodegenerative disease seeing that they mostly cross the blood-brain barrier (Rastogi et al. 2021). Hence, exosomes are considered to act a significant role as a drug delivery vehicle as well.

Given the progressions in the methods of microarray and next-generation sequencing (NGS), a few recent types of non-coding RNAs (ncRNAs) including long non-coding RNAs, Micro RNAs (miRNAs) and circular

RNAs (circRNAs) have appeared in several tissues as well as in exosomes (Gong and Jiang 2020). However, Ribonucleases (RNases), a big group of hydrolytic enzymes, are capable of degrading plasma ribonucleic acids (RNAs) and therefore may not precisely provide a clue for pathological variations; exosomes can preserve the plasma RNAs from the degradation (Ge et al. 2014).

miRNAs that powerful molecules that regulated the gene silencing process by preventing the translation of protein or inducing degradation by targeting specific binding site regions of the messenger RNA (mRNA) (Saliminejad et al. 2019). The increasing amount of miRNAs inside the nervous system, where they are pivotal regulators of processes including neurogenesis, synaptic plasticity, neuronal differentiation and neurite growth, helps to understand the hypothesis that miRNAs possess likely a function in neurodegenerative diseases, particularly in AD (Femminella et al. 2015; Moradifard et al. 2018). Besides, circRNAs are a special group of noncoding single-stranded highly stable ribonucleic acid and conspicuously augmented in the parts of brain tissues, where they are an important player in the development of neurodegenerative diseases like AD (Akhter 2018). Recent evidence suggests that circRNAs also exhibit vital roles in critical biological processes via functioning as a miRNA sponge, competing endogenous RNA (ceRNA), regulators of transcription, or insomuch by translating themselves to generate proteins (Kristensen et al. 2019; Chen 2020). Despite much new knowledge about the role of circRNAs, little progress has been made in elucidating the molecular regulatory mechanisms by interacting with miRNAs.

In the current research, to assist in better comprehending the fundamental molecular regulatory mechanisms of AD and to offer novel insights into potential therapeutic targets, we sought to address the following intents; to identify differentially expressed the plasma exosomal miRNAs and circRNAs, to predict whether or not the expression of the RNAs in exosomes from the part of the brain, to utilize the RNAs to construct an integrative network to conduct the functional enrichment analysis as in pathway and Gene Ontology (GO).

Methods

Data Collection

A pipeline of the study is represented in Fig. 1. The data set was fetched from the ArrayExpress (Athar et al. 2019) (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-11222), which has been deposited for RNA-seq data by using the link to transfer to galaxy platform (Afgan et al. 2018). The E-MTAB-11222 data set, which was extracted from the study of xiaohuan et al. (Xiaohua et al. 2022), composed of a total of 9 male samples, containing 3 samples from each group respectively (Healthy group, MCI patients, and AD patients). Regarding nucleic acid sequencing protocol, the library preparations were sequenced on an Illumina Hiseq 2500/2000 platform and 50 bp single-end reads were generated. The data set in fastq format was processed for the differentially expressed miRNAs (DEmiRs) and circRNAs (DEcircRs).

Identification of Differentially Expressed RNAs

In summary in Fig. 1, to reach out to the lists of differentially expressed circRNAs (DEcircRs) and miRNAs (DEmiRs), up/down-regulated genes between healthy group and patients with MCI and AD were generated by following the bioinformatics tools that FASTQC (Andrews and others 2010), Trimmomatic (Bolger et al. 2014), HISAT2 (Kim et al. 2015), FeatureCounts (Liao et al. 2014), and Limma (Law et al. 2014) in the Galaxy platform. The value of |log2(foldchange)|> 0 and adjusted *p*-value (adj.P.Val) < 0.05 were filtered as cutoff criteria. Following, common DEmiRs and DEcircRs for both MCI and AD groups were opted.

Construction of the Networks and Module Analysis

The target genes of potential DEmiRs and circRNAs were predicted using miRNet (https://www.mirnet.ca), which is a comprehensive tool that integrated data from 14 databases related to miRNA (Chang et al. 2020). Two up/down regulated DEmiR gene lists were separately submitted to the miRNet. However, DEcircRs and DEmiRs were submitted as multiple query types to monitor possible integrations in the network. The intersection of predicted target genes by miRNet was obtained to sort using the online tool (https://molbiotools.com/listcompare.php). Subsequently, the protein-protein interaction (PPI) network of target genes was constructed using the StringApp (Szklarczyk et al. 2016), one of the most well-known data sources of networks being a Cytoscape application for both visualization and the analysis of protein networks on Cytoscape 3.9.1. (Shannon et al. 2003). Then, the hub genes in the network were identified by analyzing the MCC model of connectivity using Cytoscape plug-in cytohubba (Chin et al. 2014).

Expression Validation of DEcircRs and DEmiRs Genes

To confirm the expression of DEcircRs and DEmiRs in the brain, DEcircRs, DEmiRs, and their target genes in the networks were screened and filtered out using the circBase database (Glažar et al. 2014) and TissueAtlas (Keller et al. 2022). In the TissueAtlas, the searching criteria were set as NGS, Normalization RPMM (Reads Per Million Reads, and Min Expression >=2.

Functional Enrichment and Pathway Analyses

The Database for Annotation Visualization and Integrated Discovery (DAVID) (Huang et al. 2009) online tool (version 2021; http://david.abcc.ncifcrf.gov) was utilized to perform Gene Ontology (GO) (Ashburner et al. 2000) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for predicted target genes of potential up/down-regulated miRNAs and circRNAs. A *p*-value < 0.05 was set as a significant enrichment threshold for all the analyses.

Results

Identification of Exosomal DEmiRs and DEcircRs and Predicted Target Genes in Networks

In the current research, the differences in the miRNA expression profiles between three AD - MCI samples (60-year-old males) and three age-matched healthy samples (60-year-old men) of plasma exosomes were analyzed. It can be seen from the data in Table 1 that a total of seven upregulated and twelve

downregulated DEmiRs were acquired after a preliminary analysis of the E-MTAB-11222 data set. miRNet online tool predicted 4559 target genes for upregulated DEmiRs (Fig. 2a), whereas 6504 target genes for downregulated DEmiRs (Fig. 2b). On the other hand, 433 circRnas are among 6504 target genes of upregulated miRNAs in the network, but further analysis of these genes using a Venn diagram appeared that 21 out of 6504 target genes are exosomal-upregulated circRna genes in Fig. 2b. Additionally, 122 genes out of 4559 target genes of upregulated miRNAs are circRnas in Fig. 2a.

Table 1 Common differentially expressed miRNAs (DEmiRs) between both MCI and AD groups versus the healthy group. The statistical values belong to DEmiRs in AD group. *adj.P.Val* < 0.05

Upregulated DEmiRs	**log_2FC**	**Adj.P.Val**
hsa-mir-148a	0.78	4.29E-03
hsa-mir-196a-1	2.46	1.89E-03
hsa-mir-205	5.48	1.42E-02
hsa-mir-4731	2.70	4.29E-03
hsa-mir-615	6.39	1.89E-03
hsa-mir-6812	2.46	1.89E-03
hsa-let-7c	0.99	2.61E-02
Downregulated DEmiRs	**log_2FC**	**Adj.P.Val**
hsa-mir-889	-4.87	1.28E-02
hsa-mir-654	-3.78	1.46E-04
hsa-mir-6515	-5.33	1.52E-03
hsa-mir-5698	-4.57	8.48E-03
hsa-mir-504	-4.50	4.51E-02
hsa-mir-493	-3.85	3.53E-02
hsa-mir-485	-2.41	3.00E-04
hsa-mir-4446	-2.27	1.28E-02
hsa-mir-409	-2.95	1.60E-09
hsa-mir-374a	-4.90	1.89E-03
hsa-mir-3177	-4.77	3.64E-02
hsa-mir-185	-1.97	5.97E-03

As can be seen from Table 2 (below), DEcircRs in the AD group possessed lower *P values* compared to those in the MCI group by performing statistical analysis of both groups compared to the healthy group. The fold change value of AD group is mostly higher than MCI group compared to healthy group. By screening the circBase platform, 24 of the 34 common DEcircRs were found to be of probable neuronal origin in Fig. 2b.

Table 2 Common differentially expressed neuronal circRNAs (DEcircRs) between both MCI and AD groups compared to the healthy group. Neuronal cirRNAs that bold fonts are regulated by downregulated miRNAs. *adj.P.Val* < 0.05. MCI; Mild cognitive impairment, AD; Alzheimer`s disease. DemiRs; differentially expressed miRNAs

		MCI group		AD group	
Gene symbol	**Adj.P.Val**	**log_2FC**	**Adj.P.Val**	**log_2FC**	**DemiRs**
COL14A1	4,57E-02	0.74	3,54E-02	2.87	hsa-mir-654-5p
ATP6V1A	4,57E-02	0.74	1,33E-02	2.82	hsa-mir-485-3p/5p hsa-mir-654-5p hsa-mir-409-3p
CAPNS1	4,57E-02	3.48	2,34E-04	6.24	hsa-mir-185-5p
CGGBP1	4,57E-02	0.74	1,82E-04	6.10	
EXPH5	4,57E-02	0.74	1,90E-03	2.46	
FLNA	7,74E-03	1.63	1,10E-04	6.18	hsa-mir-196a-5p
RGS22	4,57E-02	0.74	1,90E-03	2.46	
NAMPT	4,57E-02	1.75	2,73E-03	5.56	hsa-mir-145-5p
NXN	4,57E-02	0.74	4,29E-03	2.70	hsa-mir-409-3p
PFKL	1,66E-02	1.49	1,90E-03	2.46	hsa-mir-654-3p hsa-mir-654-5p
PDPR	4,57E-02	2.62	1,90E-03	2.46	hsa-mir-185-5p
RIN2	4,57E-02	0.74	1,90E-03	2.46	hsa-mir-485-5p hsa-mir-485-3p
SAMD4B	4,57E-02	2.56	1,90E-03	2.46	hsa-mir-185-5p
SEC22C	4,57E-02	0.74	1,90E-03	2.46	hsa-mir-185-3p, hsa-mir-485-5p
UBN1	4,57E-02	0.97	1.35E-17	8.12	hsa-mir-485-5p
CNTN4	4,57E-02	0.74	4,29E-03	2.70	
ADAMTS13	4,57E-02	0.74	1,90E-03	2.46	
NUDC	4,57E-02	0.74	1,33E-02	2.82	
HDAC1	4,57E-02	0.74	4,29E-03	2.71	
PC	1,67E-02	1.49	3,54E-02	2.87	
PDK3	4,57E-02	0.744	1,90E-03	5.65	
SH3BGRL	4,57E-02	0.744	4,29E-03	2.71	

THOC2	4,57E-02	0.744	1,90E-03	2.46
EIF3E	4,57E-02	0.744	1,33E-02	2.82

Functional Enrichment Analysis of the Predicted Target Genes of Up/Down Regulated miRNAs

After submitting up/down regulated miRNAs to mirNet, to analyze the two networks with better performance, the degree cutoff was set as 2.0, so two networks formed smaller with 625 genes and 250 genes.

To investigate the biological functions and pathways of the genes, the enrichment analyses of the genes were assessed using the DAVID, GO category and KEGG analysis outcomes for each of the two networks as up-down regulated micro RNAs are summed up in Table 3 and 4. In addition, target genes in the two networks were associated with Alzheimer's disease through DISGENET database.

Table 3 Functional enrichment analysis of the target genes of DEmiRs. The selected enriched GO terms related to AD are listed. GO, Gene Ontology; MF, molecular function; BP, biological process; CC, cellular component

Target Genes of Downregulated miRNAs				
GO	**Term**	**Gene Count**	**P-Value**	**Benjamini**
BP	Neuron migration	11	2,00E-03	1,10E-01
BP	Response to endoplasmic reticulum stress	10	7,60E-04	7,00E-02
BP	Negative regulation of neuron apoptotic process	12	6,40E-03	1,90E-01
BP	Positive regulation of neuron apoptotic process	6	3,30E-02	4,70E-01
BP	Cellular response to beta-amyloid	7	2,60E-03	1,20E-01
BP	Central nervous system neuron axonogenesis	4	8,50E-04	7,00E-02
BP	Negative regulation of autophagy	7	4,00E-03	1,40E-01
MF	Tau protein binding	8	4,30E-04	1,80E-02
MF	Calmodulin binding	16	1,90E-03	4,70E-02
MF	Transcription factor activity, sequence-specific DNA binding	43	8.5E-8	1.4E-5
MF	Transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific binding	36	1.7E-6	1.7E-4
MF	RNA polymerase II transcription factor activity, sequence-specific DNA binding	63	2.8E-4	1.3E-2
CC	Nucleus	283	1,30E-23	7,70E-21
CC	Nucleoplasm	211	1,60E-22	4,70E-20
CC	Cytoplasm	251	6,10E-18	1,20E-15
CC	Endoplasmic reticulum	45	2,30E-02	2,20E-01
CC	Extracellular exosome	106	2,50E-07	1,50E-05
Target Genes of Upregulated miRNAs				
BP	Negative regulation of transcription from RNA polymerase II promoter	35	1,46E+01	9,50E-09
BP	Negative regulation of apoptotic process	24	1,00E+01	5,90E-08
BP	Cell division	19	7,40E-07	4,70E-04
BP	Regulation of cell cycle	16	5,60E-06	2,60E-03
BP	Positive regulation of transcription, DNA-templated	23	1,40E-05	5,50E-03
MF	RNA binding	52	9,40E-12	3,80E-09
MF	Protein binding	202	1,50E-11	3,80E-09

MF	SMAD binding	10	1,20E-08	2,10E-06
MF	DNA binding	40	1,90E-07	2,40E-05
MF	Protein kinase binding	22	1,30E-06	1,30E-04
CC	Nucleus	142	7,10E-24	2,90E-21
CC	Nucleoplasm	110	3,80E-22	7,70E-20
CC	Membrane	65	5,20E-10	7,00E-08
CC	Cytosol	104	5,80E-09	5,90E-07
CC	Cytoplasm	103	1,10E-08	9,00E-07

Table 4 Funtional enrichment analysis of the target genes of DEmiRs. The selected enriched GO terms related to AD are listed. KEGG; Kyoto Encyclopedia of Genes and Genomes, DISGENET; Disease gene net.

Target Genes of downregulated miRNAs					
Category	**Term**		**Gene Count**	**P-Value**	**Benjamini**
KEGG Pathway	Cellular senescence		18	5,50E-05	1,50E-03
KEGG Pathway	PI3K-Akt signaling pathway		29	9,80E-05	1,80E-03
KEGG Pathway	Apoptosis		12	1,10E-02	5,80E-02
KEGG Pathway	Insulin signaling pathway		12	1,20E-02	5,80E-02
KEGG Pathway	mTOR signaling pathway		13	1,20E-02	5,80E-02
Target Genes of Upregulated miRNAs					
KEGG Pathway	PI3K-Akt signaling pathway		14	4,20E-03	6,20E-02
KEGG Pathway	p53 signaling pathway		7	1,00E-03	2,80E-02
Target Genes of Up/downregulated miRNAs		Enrichment Score: 1.69			
DISGENET	Alzheimer Disease, Late Onset	ADAMTS1, CALM1, DPYSL2, ENO1,GSK3B, IGF1R,PRNP,SLC30A6, SOD2,VEGFA	10	2,00E-02	4,00E-01
DISGENET	Alzheimer Disease, Early Onset		10	2,00E-02	4,00E-01
DISGENET	Alzheimer's Disease, Focal Onset		10	2,00E-02	4,00E-01
DISGENET	Familial Alzheimer Disease (FAD)		10	2,10E-02	4,00E-01
DISGENET	Alzheimer's Disease		10	2,30E-02	4,00E-01

Two subnetworks of the top 20 hub genes are STRING protein-protein interaction (PPI) network of 250 nodes of upregulated miRNAs and 625 nodes of downregulated miRNAs identified from differential gene analysis using Cytoscape software in Fig. 3. To validate expression of the DEmiRs in the parts of the brain, as can be seen in Fig. 4, some of the DEmiRs were found as mature miRNAs of precursor miRNAs in the DEmiRs list screening by TissuAtlas.

Discussion

The molecular complexity of Alzheimer's pathophysiology is bound to several molecular factors including multiple pathways and mechanisms. Besides, to date, there has been no reliable evidence that any cure for AD, which has existed as a health problem for many years. Yet, it might be predicted thanks to some molecular clue prior to reaching late AD level, thereby can be developed the quality of life in patients with AD. Hereby, in the complexity of molecular machinery, intercellular shuttled molecules may provide insight in enlightening the mechanism of AD (Rastogi et al. 2021). To contribute to the scope of that issue, the current paper explores the plasma exosomal non-coding RNAs including circRNA and miRNA expression profiles in AD patients by screening experimentally validated miRNA-target interaction databases.

In the present study, common 19 DEmiRs and 34 circRNAs in both AD and MCI groups were found in the plasma exosome compared to the healthy group based on NGS from ArrayExpress dataset. Theoretically, assuming that exosomes are secreted from AD brain tissue into blood, it was discerned that 15 of the commonly upregulated circRNAs in Table 2 were of neuronal origin by screening the circBase database, and were regulated by some downregulated miRNAs (see Fig. 5). Furthermore, we interrogated the circRNA genes in the Single-cell Atlas of the Entorhinal Cortex in Human Alzheimer's Disease database (Grubman et al. 2019) to assign which cell type expressed the upregulated circRNAs, and 26 out of 34 circRNA genes were found in the dataset (Supplementary Fig.1).

The priority in AD physiology approaches is to focus on the dysfunction and misregulation of beta-amyloid and tau protein (Weller and Budson 2018; Rastogi et al. 2021). However, little is known about AD and it is not clear what factors cause AD pathology. In this context, 10 AD-associated genes mentioned in Table 4 with an enrichment score of 1.69 were regulated by hsa-mir-185, hsa-mir-3177-3p, hsa-mir-374a, hsa-mir-409, hsa-mir-4446-5p, hsa-mir-485, hsa-mir-493-3p, hsa-mir-504, hsa-mir-5698 in the DEmiRs (Supplementary Fig.3). In the sub-network, VEGFA, IGF1R and GSK3B genes are also related to Phosphoinositide 3-kinases-Akt (PI3K-Akt) signaling pathway and regulated by hsa-mir-504, hsa-mir-5698, and hsa-mir-374a-5p respectively.

CCND1, CCND2, CDK6, CDKN1A, MDM2 and CCNE2 are common regulated target genes of upregulated miRNAs in both PI3K-Akt and p53 signaling pathway, and CCNE2 from these genes is a hub gene that downregulated by hsa-mir-196a-5p, hsa-mir-205-5p and hsa-let-7c-5p. As related to AD, in the biological process of negative regulation of apoptosis, CCND2, CDKN1A, IGF1R, MDM2, MYC and PRLR genes in PI3K-Akt were regulated by hsa-mir-615-5p, hsa-mir-615-3p, hsa-mir-196a-5p, hsa-let-7c-5p in the downregulated DEmiRs. We uncovered the genes of CDKN1A, IGF1R, MDM2, and MYC as hub genes in the network of target genes of downregulated miRNA. This finding was unexpected and suggests that the same genes may be found in both networks of up/down regulated DEmiRs, as previous studies have found that a gene is regulated by more than one miRNA in several pathologic conditions (Catalanotto et al. 2016). The hub genes of CCND1, CCNE2, and CDK6 in PI3K-Akt pathway are commonly associated with regulation of cell cycle and cell division process. Besides, occurring evidence demonstrates that one of the complicated roles of circRNAs in biological functions is regulating transcription (Kristensen et al. 2019). Among target genes of upregulated miRNAs, 17 genes are involved in the negative regulation of transcription from RNA polymerase II promoter, while 12 genes from circRNAs are associated with the

positive regulation of transcription in the DNA-templated. On the other hand, hsa-mir-615-3p, hsa-mir-205-5p, hsa-mir-196a-5p, hsa-let-7c-5p regulated XBP1 (Wolter et al. 2014), TUBB, PSMD8, CSNK2A1 and APP (Helwak et al. 2013), WIPI1 and NRAS (Johnson et al. 2005), DVL3 (Memczak et al. 2013), CALM1 (Kishore et al. 2011) in the main pathway of AD (Supplementary Fig.4). These miRNAs are the mature form of hsa-mir-196a-1, hsa-mir-205, hsa-mir-615, and hsa-let-7c into plasma exosomal statistically significant found in the upregulated DEmiRs in AD group compared to healthy group (see Table 1).

It is well known that there is a contrary relationship between the expression of miRNA and the expression of the target gene. Considering this view, KEGG pathway enrichment analysis displayed that the targets of downregulated were enhanced in pathways related to PI3K-Akt, p53 signaling pathway, cellular senescence, insulin signaling, and mTOR signaling pathway while targets of downregulated solely were enriched PI3K-Akt and p53 signaling pathway. Involving in apoptosis and cell senescence, for the 14 downregulated DEmiRs, hsa-mir-185-5p and hsa-mir-374a-5p had the most target genes that number is eight (Supplementary Fig.5). From downregulated DEmiRs, hsa-mir-485-5p is regulated TP53 in cell senescence pathway (see Fig. 5) and noteworthily the some neuronal circRNAs in Table 2. BCL2L11 and BBC3 genes in apoptosis pathway associated to response to ER stress, BCL2L11 also related cellular response to beta-amyloid, and AKT1 in negative regulation of autophagy, which is highest degree hub gene in MCC model, were regulated by commonly downregulated hsa-mir-185-3p, hsa-mir-185-5p, whilst hub genes of ACTB and MAPK1 in tau protein binding were regulated by hsa-mir-5698. More importantly, in our statistical results, we merely found the hsa-mir-5698 from DEmiRs is downregulated in AD group despite being upregulated in MCI group compared to healthy group. Interestingly, from these circRNAs, a remarkable correlation was discerned between hub gene TP53 and UBN1in cellular senescence pathway, since UBN1 takes a role as a novel regulator of aging and resides in the formation of aging-related heterochromatin foci (SAHF), which suppresses the expression of proliferation-promoting genes (Banumathy et al. 2009). Moreover, although UBN1 is predominantly localized in the nucleoplasm, our output suggests that it is the most statistically significant of the DEcircRs in plasma exosomes (Adj.P.Val =1.35E-17).

The hub genes of AKT1, GSK3B and IGF1R in the insulin signaling, mTOR and PI3K-Akt signaling pathway were regulated by commonly hsa-mir-185-3p, hsa-mir-185-5p, and hsa-mir-409-3p. Very recently, previous studies have revealed that exosomal hsa-miR-185-5p is that acts on APP dysregulation (Ding et al. 2022) and neurofibrillary pathology (Sabaie et al. 2022) by discovering reduced amounts of hsa-miR-185-5p in AD patients' plasma samples. As mentioned before, among several AD types related to 10 genes, AKT1 and GSK3B in three pathways are commonly activated in the negative regulation of neuron death, IGF1R and GSK3B in the mTOR pathway are taken part in the cellular response to beta-amyloid. Likewise, by involving in the PI3K-Akt signaling pathway, BCL2L11, CREB3L2, HSP90B1, and THBS1 are associated with the biological process of response to ER stress, HSP90AA1, HSP90AB1 and GSK3B related to positive regulation of tau protein kinase activity, and BCL2L11, GSK3B, and IGF1R take place in the cellular response to beta-amyloid. Overall, possible target genes of downregulated DEmiRs related to mentioned pathways in Table 3-4 represent in supplementary Fig.5.

Briefly, the comprehensive structure of the study is to investigate the plasma exosomal circRNAs and miRNAs expression patterns performed the functional enrichment analysis correlated to Alzheimer's Disease. In this manner, based on the statistically significant miRNA and circRNA network, it was determined that most of the target genes were associated with the PI3K-Akt pathway and that they were mostly regulated by hsa-mir-615-3p, hsa-mir-196a-5p, hsa-let-7c-5p hsa-let-205-5p, hsa-mir-185-3p, hsa-mir-185-5p, hsa-mir-374a-5p, hsa-mir-374a-3p. From these micro RNAs, has-mir-615-3p (Liu et al. 2022), mir-196 and mir-185 (Zeng et al. 2021), and mir-374a (Bian et al. 2019) are consistent with those of other studies with AD, and in this study, they were found in the plasma exosomes. Mir-let-7c and mir-205 were not associated with AD before to the best of our knowledge and might be a novel biomarker candidate. However, there are some limitations to scientifically influencing the results of the study. Firstly, the reader should bear in mind that the study was based on prediction in part of DEmiRs and circRNAs analysis by bioinformatic tools, so it needs to be validated by further experimental investigations. Secondly, due to the lack of adequate data in the databases, all DEmiRs and circRNAs could not be integrated into associated with AD. Third, the sample size of the NGS employed here was not large sufficiently, exclusively comprising 9 tissue samples.

Conclusion

Here, we implemented exosomal DEG analysis from plasma in AD patients and then, integrative and bioinformatics analysis. Our current in silico analysis points out several potential miRNA-mRNA pathways and integration miRNA-circRNA of contributing to the pathology of Alzheimer's disease. We desire that our findings based on consequences will broaden the horizon of our understanding of the pathology of AD.

Declarations

Funding Not Applicable.

Data Availability Analysis data is ready to be shared upon request. Entire regarding data analyzed during the present study are open source in the ArrayExpress.

Declaration

Ethics Approval and Consent to Participate Not Applicable.

Consent for Publication The authors consents to publication of this paper.

Competing Interests The authors declare that they have no conflicts of interest.

References

1. Afgan E, Baker D, Batut B, et al (2018) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537–W544. https://doi.org/10.1093/nar/gky379
2. Akhter R (2018) Circular RNA and Alzheimer's disease. Circ RNAs 239–243. https://doi.org/10.1007/978-981-13-1426-1_19
3. Andrews S, others (2010) FastQC: a quality control tool for high throughput sequence data. https://doi.org/10.1093/bioinformatics/bty560
4. Ashburner M, Ball CA, Blake JA, et al (2000) Gene ontology: tool for the unification of biology. Nat Genet 25:25–29. https://doi.org/10.1038/75556
5. Athar A, Füllgrabe A, George N, et al (2019) ArrayExpress update–from bulk to single-cell expression data. Nucleic Acids Res 47:D711–D715. https://doi.org/10.1093/nar/gky964
6. Banumathy G, Somaiah N, Zhang R, et al (2009) Human UBN1 is an ortholog of yeast Hpc2p and has an essential role in the HIRA/ASF1a chromatin-remodeling pathway in senescent cells. Mol Cell Biol 29:758–770. https://doi.org/10.1128/MCB.01047-08
7. Bian H, Zhou Y, Zhou D, et al (2019) The latest progress on miR-374 and its functional implications in physiological and pathological processes. J Cell Mol Med 23:3063–3076. https://doi.org/10.1111/jcmm.14219
8. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170
9. Busche MA, Hyman BT (2020) Synergy between amyloid-$β$ and tau in Alzheimer's disease. Nat Neurosci 23:1183–1193
10. Catalanotto C, Cogoni C, Zardo G (2016) MicroRNA in control of gene expression: an overview of nuclear functions. Int J Mol Sci 17:1712. https://doi.org/10.3390/ijms17101712
11. Chang L, Zhou G, Soufan O, Xia J (2020) miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. Nucleic Acids Res 48:W244–W251. https://doi.org/10.1093/nar/gkaa467
12. Chen L-L (2020) The expanding regulatory mechanisms and cellular functions of circular RNAs. Nat Rev Mol cell Biol 21:475–490. https://doi.org/10.1038/s41580-020-0243-y
13. Chin C-H, Chen S-H, Wu H-H, et al (2014) cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 8:1–7. https://doi.org/10.1186/1752-0509-8-S4-S11
14. Ding L, Yang X, Xia X, et al (2022) Exosomes Mediate APP Dysregulation via APP-miR-185-5p Axis. Front Cell Dev Biol 10:793388. https://doi.org/10.3389/fcell.2022.793388
15. Femminella GD, Ferrara N, Rengo G (2015) The emerging role of microRNAs in Alzheimer's disease. Front Physiol 6:40. https://doi.org/10.3389/fphys.2015.00040
16. Ge Q, Zhou Y, Lu J, et al (2014) miRNA in plasma exosome is stable under different storage conditions. Molecules 19:1568–1575. https://doi.org/10.3390/molecules19021568

17. Glažar P, Papavasileiou P, Rajewsky N (2014) circBase: a database for circular RNAs. Rna 20:1666–1670. https://doi.org/10.1261/rna.043687.113
18. Gong R, Jiang Y (2020) Non-coding RNAs in pancreatic ductal adenocarcinoma. Front Oncol 10:309. https://doi.org/10.3389/fonc.2020.00309
19. Grubman A, Chew G, Ouyang JF, et al (2019) A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. Nat Neurosci 22:2087–2097. https://doi.org/10.1038/s41593-019-0539-4
20. Helwak A, Kudla G, Dudnakova T, Tollervey D (2013) Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. Cell 153:654–665. https://doi.org/10.1016/j.cell.2013.03.043
21. Huang DW, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57. https://doi.org/10.1038/nprot.2008.211
22. Johnson SM, Grosshans H, Shingara J, et al (2005) RAS is regulated by the let-7 microRNA family. Cell 120:635–647. https://doi.org/10.1016/j.cell.2005.01.014
23. Keller A, Gröger L, Tschernig T, et al (2022) miRNATissueAtlas2: an update to the human miRNA tissue atlas. Nucleic Acids Res 50:D211–D221. https://doi.org/10.1093/nar/gkab808
24. Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12:357–360. https://doi.org/10.1038/nmeth.3317
25. Kishore S, Jaskiewicz L, Burger L, et al (2011) A quantitative analysis of CLIP methods for identifying binding sites of RNA-binding proteins. Nat Methods 8:559–564. https://doi.org/10.1038/nmeth.1608
26. Kristensen LS, Andersen MS, Stagsted LVW, et al (2019) The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet 20:675–691. https://doi.org/10.1038/s41576-019-0158-7
27. Law CW, Chen Y, Shi W, Smyth GK (2014) voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 15:1–17. https://doi.org/10.1186/gb-2014-15-2-r29
28. Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923–930. https://doi.org/10.1093/bioinformatics/btt656
29. Liu Y, Xu Y, Yu M (2022) MicroRNA-4722-5p and microRNA-615-3p serve as potential biomarkers for Alzheimer's disease. Exp Ther Med 23:1–10. https://doi.org/10.3892/etm.2022.11166
30. Memczak S, Jens M, Elefsinioti A, et al (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495:333–338. https://doi.org/10.1038/nature11928
31. Moradifard S, Hoseinbeyki M, Ganji SM, Minuchehr Z (2018) Analysis of microRNA and gene expression profiles in Alzheimer's disease: a meta-analysis approach. Sci Rep 8:1–17. https://doi.org/10.1038/s41598-018-20959-0
32. Rastogi S, Sharma V, Bharti PS, et al (2021) The evolving landscape of exosomes in neurodegenerative diseases: exosomes characteristics and a promising role in early diagnosis. Int J Mol Sci 22:440. https://doi.org/10.3390/ijms22010440

33. Sabaie H, Talebi M, Gharesouarn J, et al (2022) Identification and Analysis of BCAS4/hsa-miR-185-5p/SHISA7 Competing Endogenous RNA Axis in Late-Onset Alzheimer's Disease Using Bioinformatic and Experimental Approaches. Front Aging Neurosci 14: https://doi.org/10.3389/fnagi.2022.812169
34. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH (2019) An overview of microRNAs: biology, functions, therapeutics, and analysis methods. J Cell Physiol 234:5451–5465. https://doi.org/10.1002/jcp.27486
35. Shannon P, Markiel A, Ozier O, et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504. https://doi.org/10.1101/gr.1239303
36. Szklarczyk D, Morris JH, Cook H, et al (2016) The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. Nucleic Acids Res gkw937. https://doi.org/10.1093/nar/gkw937
37. Weller J, Budson A (2018) Current understanding of Alzheimer's disease diagnosis and treatment. F1000Research 7: https://doi.org/10.12688/f1000research.14506.1
38. Wolter JM, Kotagama K, Pierre-Bez AC, et al (2014) 3' LIFE: a functional assay to detect miRNA targets in high-throughput. Nucleic Acids Res 42:e132–e132. https://doi.org/10.1093/nar/gku626
39. Xu Y-Z, Cheng M-G, Wang X, Hu Y (2021) The emerging role of non-coding RNAs from extracellular vesicles in Alzheimer's disease. J Integr Neurosci 20:239–245. https://doi.org/10.31083/j.jin.2021.01.360
40. Zeng L, Jiang H-L, Ashraf GM, et al (2021) MicroRNA and mRNA profiling of cerebral cortex in a transgenic mouse model of Alzheimer's disease by RNA sequencing. Neural Regen Res 16:2099. https://doi.org/10.4103/1673-5374.308104

Figures

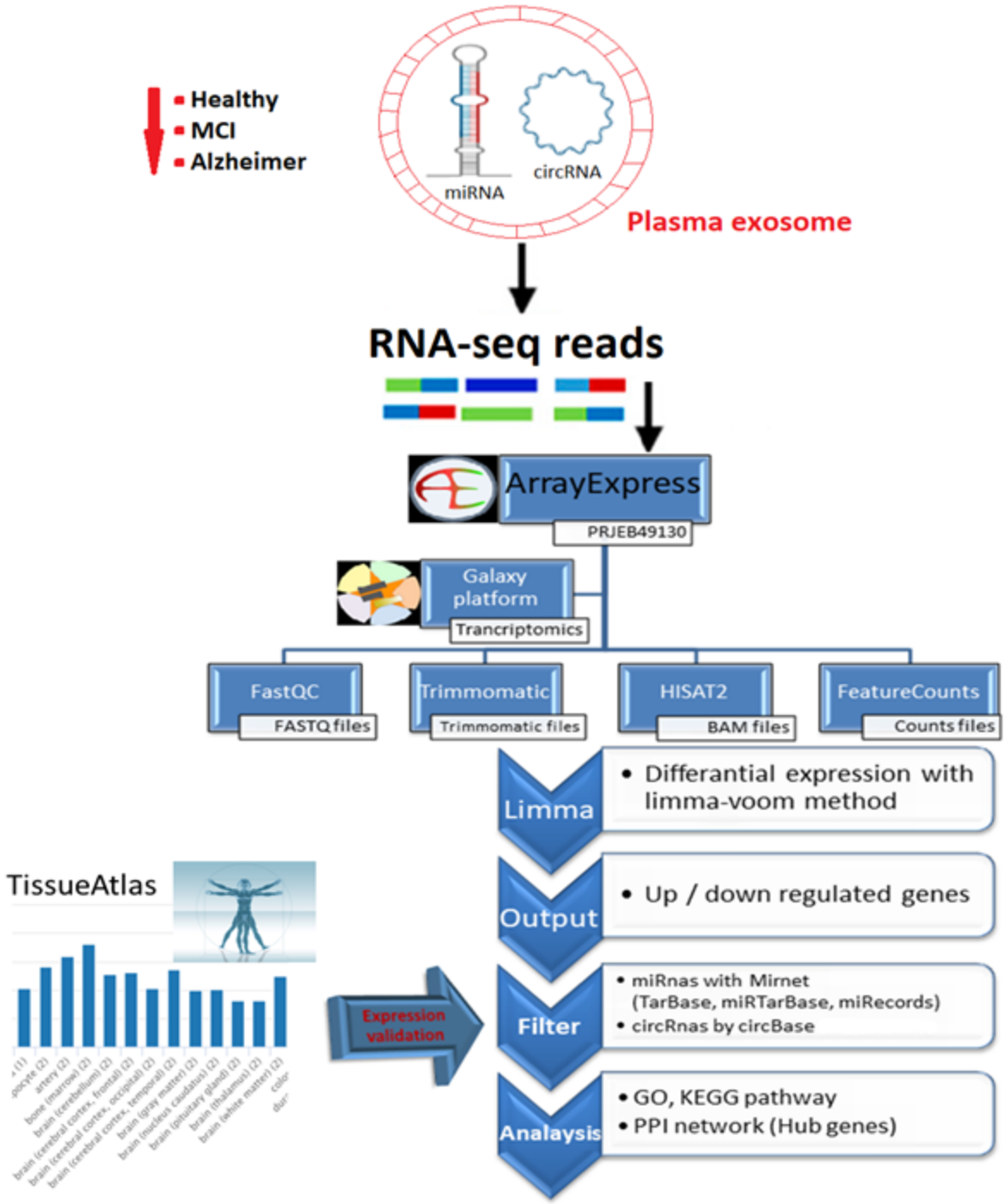

Figure 1

The schema of the current study is composed of three major steps. First, differential expression gene (DEG) analysis of exosomal RNA-seq data in AD Patients. Second, the integrative analysis of up/down-regulated genes. Third, functional enrichment analysis of the gene groups. MCI: Mild cognitive impairment, GO:Gene Ontology, KEGG:Kyoto Encyclopedia of Genes and Genomes, PPI:protein–protein interaction.

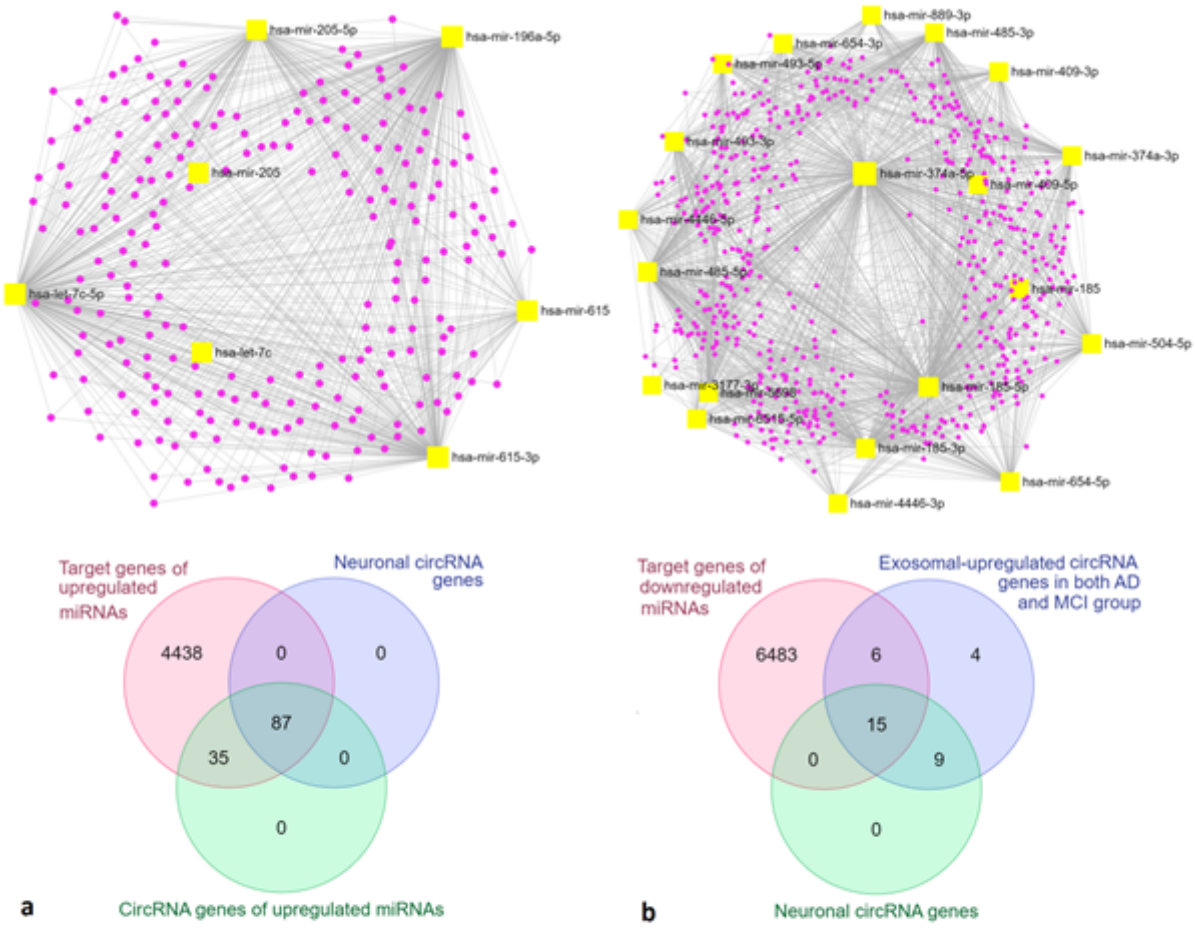

Figure 2

Venn diagrams of common DEmiRs and DEcircRs, and target genes of DEmiRs in the network for both MCI and AD groups from ArrayExpress, **(a)** the intersection of possible target genes of upregulated miRNAs, and **(b)** the target genes of downregulated miRNA.

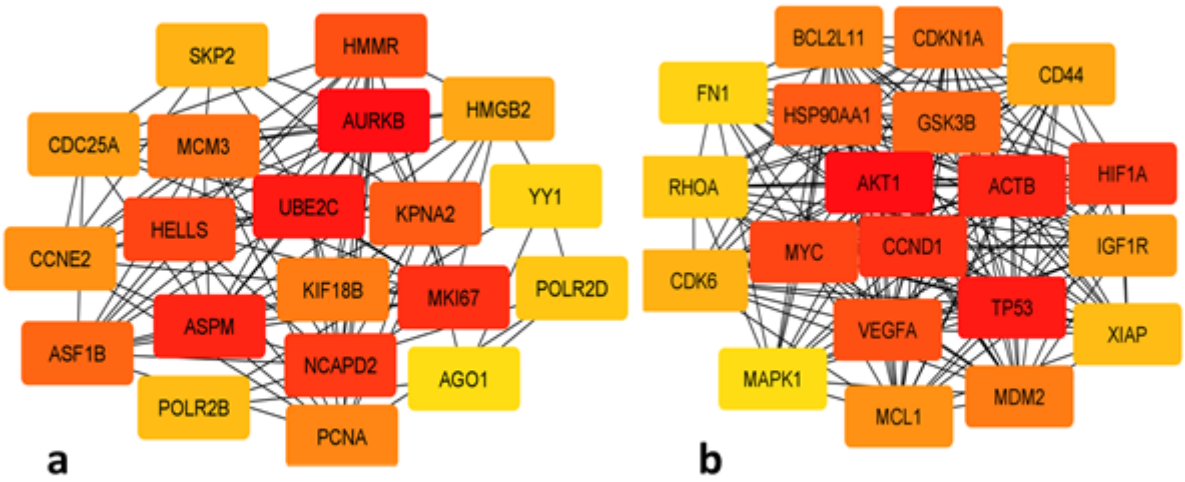

Figure 3

The figure provides sub-networks obtained from the networks of target genes of up/down-regulated miRNAs with MCC model in Cytoscape software. **(a)** Possible down-regulated hub genes in a network of protein-protein interaction (PPI). **(b)** Probable up-regulated hub genes in a network of PPI. The grade of the node color indicates a degree of connectivity. Red color indicates the highest degree, orange color shows the intermediate degree, and yellow color represents the lowest degree.

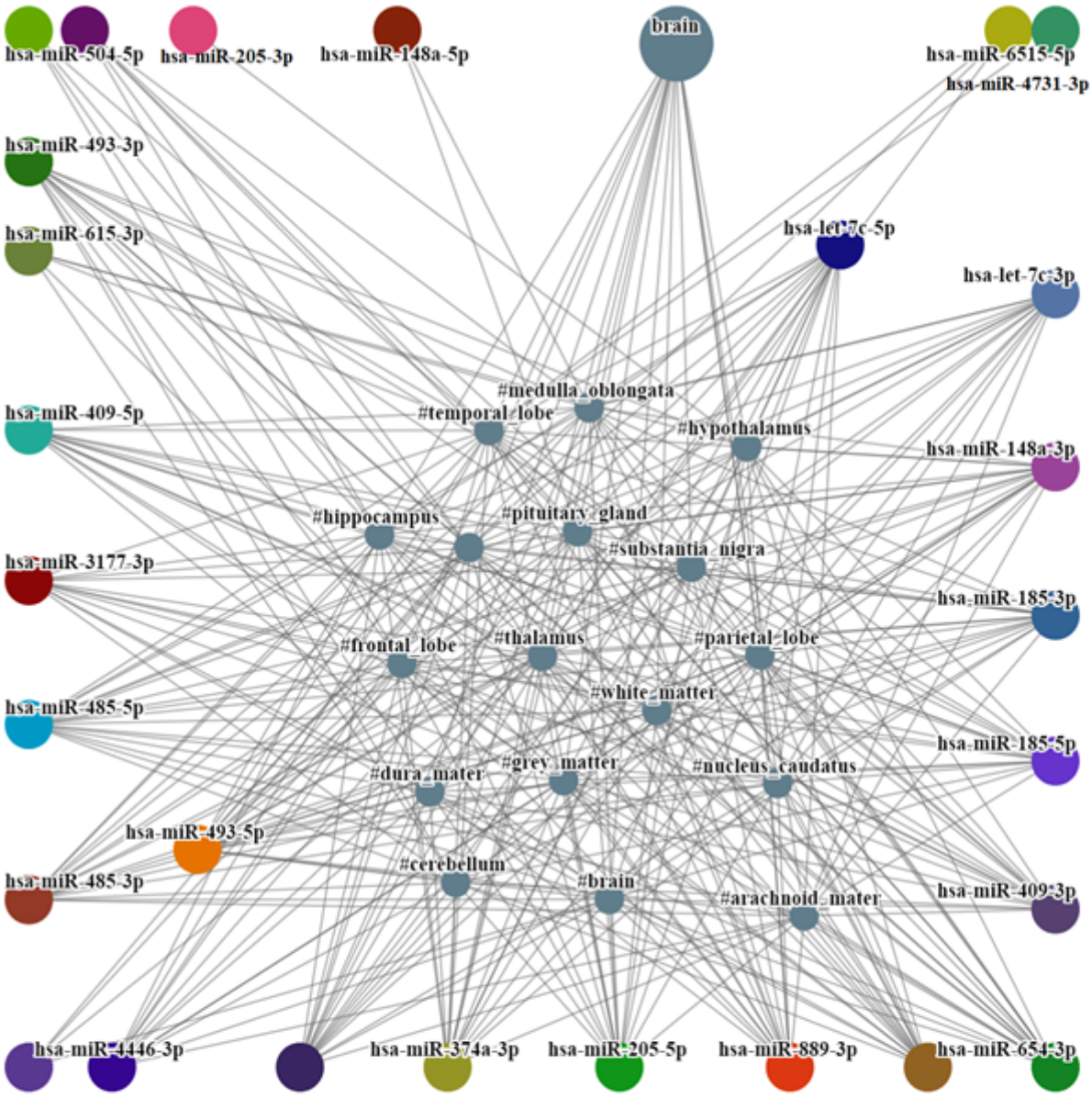

Figure 4

It presents the statistical validation of up/down-regulated miRNAs in exosomes expressed in parts of the brain from the TissueAtlas. RPMM (Reads Per Million Reads, and Min Expression >=2. DEmiRs are represented as distinct color nodes, and some of them are mature miRNAs of precursors in DEmiRs.

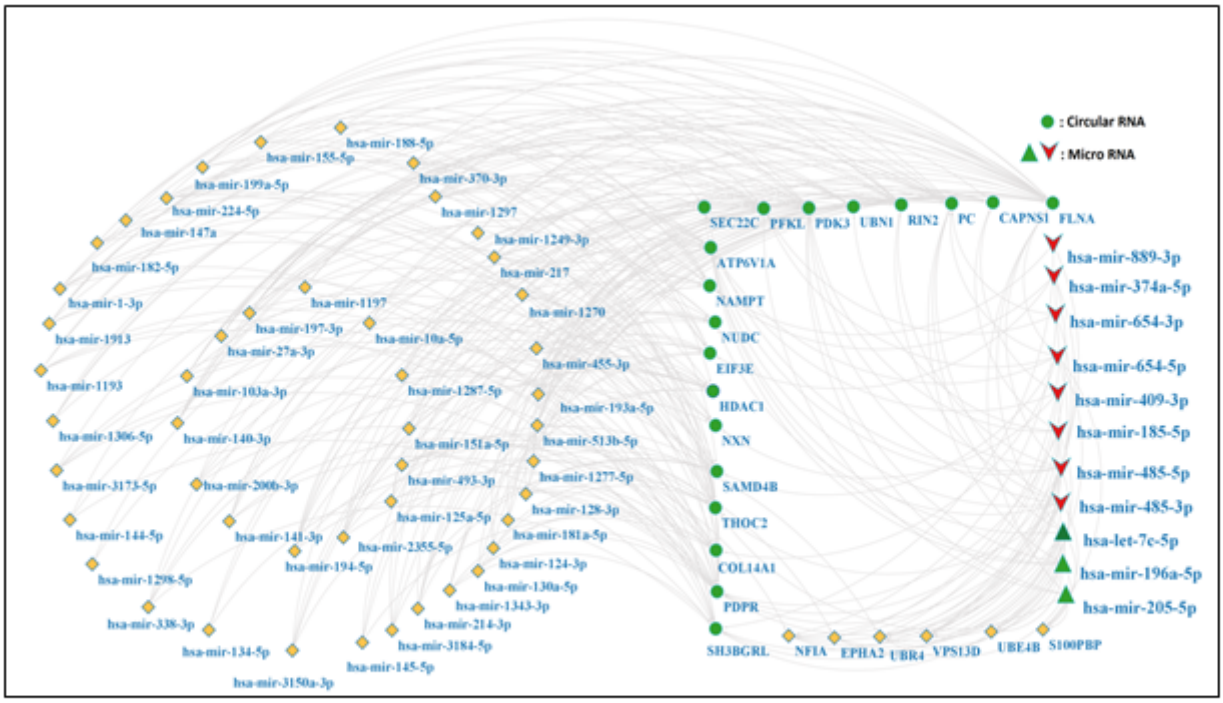

Figure 5

It represents, as a result of multiple query of DEcircRs and DEmiRs, the possible interaction in integrative network of in the miRNet tool. Downregulated miRNAs colored by red while upregulated miRNAs and circRnas colored by green. Orange nodes are possible interaction genes (miRNAs and mRNAs) by DEcircRs and DEmiRs from miRTarbase v8.0 database.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.pdf