

Gene co-expression network for analysis of plasma exosomal miRNAs in the elderly as markers of aging and cognitive decline

Zheng Ye, Bo Sun, Xue Mi and Zhongdang Xiao

State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu, China

ABSTRACT

Background: Evidence has shown that microRNA (miRNAs) are involved in molecular pathways responsible for aging and age-related cognitive decline. However, there is a lack of research linked plasma exosome-derived miRNAs changes with cognitive function in older people and aging, which might prove a new insight on the transformation of miRNAs on clinical applications for cognitive decline for older people.

Methods: We applied weighted gene co-expression network analysis to investigated miRNAs within plasma exosomes of older people for a better understanding of the relationship of exosome-derived miRNAs with cognitive decline in elderly adults. We identified network modules of co-expressed miRNAs in the elderly exosomal miRNAs dataset. In each module, we selected vital miRNAs and carried out functional enrichment analyses of their experimentally known target genes and their function.

Results: We found that plasma exosomal miRNAs hsa-mir-376a-3p, miR-10a-5p, miR-125-5p, miR-15a-5p have critical regulatory roles in the development of aging and cognitive dysfunction in the elderly and may serve as biomarkers and putative novel therapeutic targets for aging and cognitive decline.

Subjects Bioengineering, Bioinformatics, Geriatrics

Keywords WGCNA, Plasma, Exosome, miRNA, Eldly, Aging, Cognitive decline, MoCA score, Biomarker

INTRODUCTION

Aging is a complex biological process associated with variable dysfunction and chronic disease susceptibility (*Wagner et al., 2016*); for example, aging in human and animal is closely associated with changes in cognitive processes. As people age, their memory and the cognitive response rate will gradually decline (*Alexander et al., 2012; Febo & Foster, 2016; Nissim et al., 2017; O'Shea et al., 2016; Wagner et al., 2016; Woods, Cohen & Pahor, 2013*). Age-related cognitive declines are uneven; meanwhile, many environmental and biological factors including genes, exercise, diet, inflammation, and stress, were thought to be affect the age of onset and cognitive decline (*Barrientos et al., 2010; Cai et al., 2014; Craft et al., 2012; Fan, Wheatley & Villeda, 2017; Foster, 2006; Kumar et al., 2013; Speisman et al., 2013*).

Submitted 28 August 2019
Accepted 29 November 2019
Published 6 January 2020

Corresponding author
Zhongdang Xiao, zdxiao@seu.edu.cn

Academic editor
Walter de Azevedo Jr

Additional Information and
Declarations can be found on
page 14

DOI [10.7717/peerj.8318](https://doi.org/10.7717/peerj.8318)

© Copyright
2020 Ye et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

By 2050, at least 25% of the population will be over 60 years of age in developed countries. Considering that aging is a significant risk factor for many neurodegenerative diseases and cognitive impairment, there is an urgent need to develop a cost-effective and minimally invasive method for identifying individuals with cognitive decline who, thus could benefit from preventive interventions or early treatment ([Burkle et al., 2015](#)). A tremendous effort has been made in last decades to validate unbiased blood-based protein for detecting the process of aging, though results have been markedly inconsistent likely due to pre-analytical factors, assay sensitivity, and affinity of the antibodies ([Toledo et al., 2011](#)). Furthermore, the limited trafficking of solutes from the brain into the bloodstream through the blood–brain barrier makes that the blood protein levels are challenging to interpret brain changes ([Barbu et al., 2009](#)). Therefore, the identification of more accessible accessed, non-protein biological molecules in body fluids was thought to help promote the prevention of aging-related brain diseases and, finally, increasing life expectancy.

Exosomes, membrane-bound vesicles of 40–100 nm in diameter, are presented in biological fluids. They are released from many cell types into the extracellular space. Lipids and proteins are the main components of exosome membranes, where enriched lipid rafts ([Gross et al., 2012](#); [Mathivanan, Ji & Simpson, 2010](#); [Schneider & Simons, 2013](#)). Various nucleic acids have recently been identified in exosomes, including mRNA, microRNAs (miRNAs), and other non-coding RNAs ([Sato-Kuwabara et al., 2015](#)). During circulation in the body, the RNA carried by exosomes can be taken up by adjacent or distant cells and thus regulate the activity of recipient cells. Exosomes can transport nucleic acids through the blood–brain barrier, enabling exosome carried nucleic acids as a promising candidate for identifying pathological changes in the brain ([El Andaloussi et al., 2013](#); [Tominaga et al., 2015](#); [Wood, O’Loughlin & Lakhali, 2011](#)).

MicroRNAs are a class of 17–24 nt small RNAs, which mediate post-transcriptional gene silencing by binding to the 3′-untranslated region or open reading frame region of target mRNAs ([Bartel, 2007](#)). The miRNAs involve in many biological activities, including cell proliferation, cell differentiation, cell migration, disease initiation, and disease progression ([Liu et al., 2017](#); [Ma, Teruya-Feldstein & Weinberg, 2007](#); [Png et al., 2012](#); [Tay et al., 2008](#)). miRNAs can stably exist in body fluids, including saliva, urine, breast milk, and blood ([Arroyo et al., 2011](#); [Gallo et al., 2012](#); [Hu et al., 2010](#); [Lv et al., 2013](#); [Michael et al., 2010](#); [Zhou et al., 2012](#)); they also can be packed into extracellular vesicles such as exosomes ([Tabet et al., 2014](#); [Vickers et al., 2015](#); [Arroyo et al., 2011](#)), which help protecting miRNAs from degradation and further guarantee their stability. Given the transportability of vesicles, the role of miRNAs in exosomes is gaining increasing attention ([Harvey et al., 2015](#); [Impey et al., 2010](#); [Jovasevic et al., 2015](#); [Rajman et al., 2017](#); [Serafini et al., 2014](#)).

Indeed, exosomes and their carried mRNAs were detected in body fluids, including serum, urine, and saliva in many studies. Notably, studies suggested that circulating levels of miRNAs in plasma ([Kumar et al., 2013](#)) or exosomes ([Cheng et al., 2015](#); [Lugli et al., 2015](#)) were capable of identifying Alzheimer’s disease (AD). Several blood-based miRNAs have been reported to be differentially expressed in AD ([Cosin-Tomas et al., 2017](#);

Dong et al., 2015; Galimberti et al., 2014; Geekiyanage et al., 2012; Kiko et al., 2014; Kumar et al., 2013; Leidinger et al., 2013; Tan et al., 2014). Alzheimer's syndrome is a neurodegenerative disease whose typical manifestation is a decrease in cognitive function. Studies have shown that miRNA-146a is associated with high-risk Alzheimer's syndrome and cognitive decline (*Cui et al., 2014*) and that hippocampal miRNA-132 can receive stress-induced cognitive impairment (*Shaltiel et al., 2013*). In this study, we investigated whether plasma exosome derived miRNAs are associated with changes in age-related cognitive functioning dysfunction. Our study may contribute to understanding the mechanisms beneath the age-related cognitive decline and selecting mRNAs as a biomarker for identifying this kind of disease.

MATERIALS AND METHODS

Data collection and preprocessing

The miRNA-seq based expression dataset *GSE97644* was downloaded from the NCBI Gene Expression Omnibus (*Rani et al., 2017*). Data analyses were performed using the R programming language (v3.5.0), Bioconductor packages (v3.7), and R functions in the weighted gene co-expression network analysis (WGCNA) package (*Langfelder & Horvath, 2008*). The CPM normalized expression data were quantile normalized for subsequent data analysis.

Weighted gene co-expression networks and their modules

To explore the relationship between changes in miRNA expression with age and The Montreal Cognitive Assessment (MoCA) scores, we used WGCNA. WGCNA is a useful method to reveal the relationship between linking clustered genes and phenotypic traits. In vivo, miRNAs act in the same way as mRNA and are also consistent with the scale-free topology. Therefore, we use the WGCNA method to analyze the relationship between link clustered miRNAs and phenotypic traits (*Giulietti et al., 2017; Yepes et al., 2016*). The MoCA is a 10-min cognitive screening tool to assist first-line physicians in the detection of mild cognitive impairment (MCI), a clinical state that often progresses to dementia. The MoCA is a brief cognitive screening tool with high sensitivity and specificity for detecting MCI (*Nasreddine et al., 2004*).

Select the optimal soft threshold

The research shows that the co-expression network conforms to the scale-free network (*Langfelder & Horvath, 2008*), that is, the logarithm $\log(k)$ of the node with the connection degree k is negatively correlated with the logarithm $\log(p(k))$ of the probability of the node, and the correlation coefficient is higher than 0.8. R software package WGCNA was used to build a weighted co-expression network. To ensure that the network is a scale-free network, we choose a soft threshold of $\beta = 10$.

Construct co-expression modules

Convert the expression matrix into an adjacency matrix, and then convert the adjacency matrix into a topological matrix. Based on TOM, the average-linkage hierarchical

clustering method was used to cluster the miRNAs according to the criteria of the mixed dynamic cut tree and set each miRNA. The network module has a minimum base number of 20. After using the dynamic shear method to determine the miRNA module, we calculate the eigengenes of each module in turn, then cluster the modules to merge the closer modules into new modules, and set height = 0.2.

Phenotypic correlation analysis of modules

According to the feature vector of each module, we calculate the correlation between these modules and each phenotype separately. The module-trait relationship was estimated using the correlation between the module eigengene and the phenotype (gender, age, and MoCA score). For each expression profile, miRNA significance was calculated as the absolute value of the correlation between expression profile and each trait; module membership (MM) was defined as the correlation of expression profile and each module eigengene (*Shi et al., 2015*).

Analysis of miRNAs and phenotype correlation of modules

Based on the expression levels of miRNAs of each sample, we calculated the correlation between the miRNAs in these modules and each phenotype to measure the degree of association between miRNAs and phenotypes. The larger the value, the more biologically meaningful. The correlation equal to 0 indicates that the miRNA is not related to the phenotype.

Correlation between integration modules and phenotypes and miRNA and phenotypic correlation in modules

Our integration module and phenotypic correlation results and miRNA- and phenotypic correlation results in the module further analyze the correlation between the two, and then speculate the relationship between miRNAs and phenotype, the higher the correlation, the phenotype more relevant to the module.

Module-related miRNA correlation analysis

According to the eigenvectors of each module, we calculated the correlation between the expression of these modules and the miRNAs in their modules. Among them, the miRNAs with a correlation higher than 0.85 in each module (Hub miRNAs) were selected for subsequent target gene prediction and functional annotation.

Hub miRNAs and their functional annotations

To explain the biological functions of the miRNAs in these modules, we performed functional enrichment analysis of the targets of these miRNAs. We imported the selected miRNAs into the miRNet web tool (*Fan & Xia, 2018*) to enrich the corresponding miRNA target genes in biological pathways, processes, and molecular functions. In particular, this tool can perform functional enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) and REACTOME pathways based on experimentally validated miRNA targets. Therefore, the signal path enriched by this tool has higher reliability.

Sample clustering to detect outliers

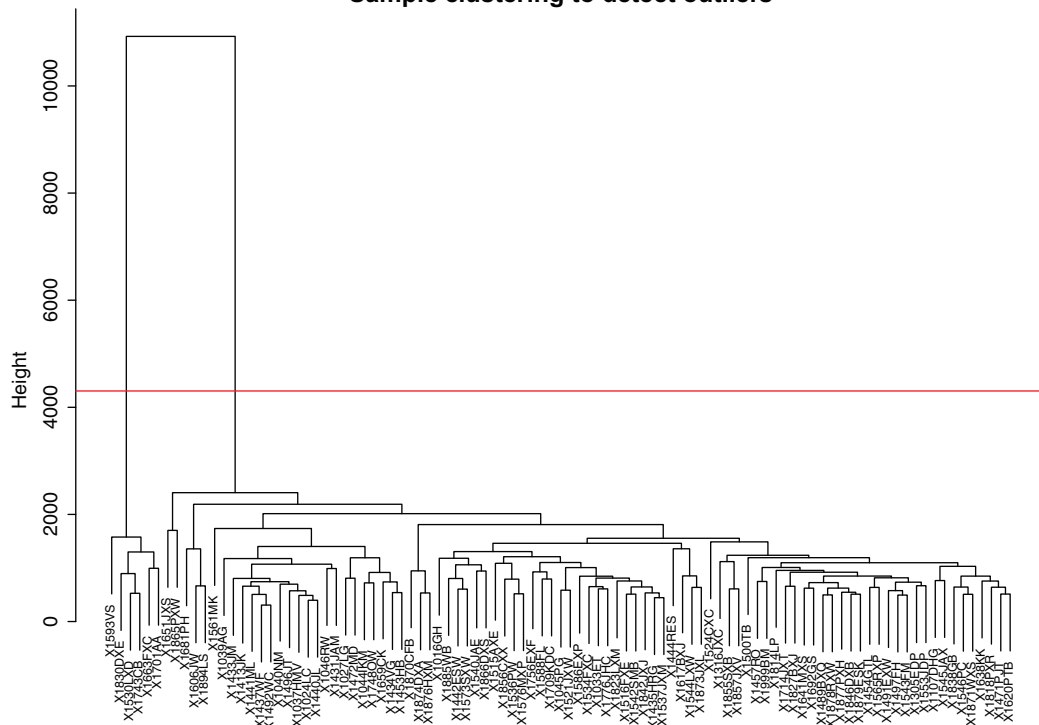


Figure 1 Sample clustering to detect outliers. In order to make the dataset more reasonable, The hierarchical clustering method was used to cluster the expression profile of the samples and calculated the distance between the samples. X1593VS, X1830DX, X1530LXD, X1743CB, X1663FXC, X1701AA, these six samples were removed according to the cluster distance, as shown in the red line in the figure.

Full-size DOI: 10.7717/peerj.8318/fig-1

RESULTS

Weighted gene co-expression network analysis

Firstly, we obtained the expression profile and the phenotypic database, which contained 97 samples, 2,588 miRNAs, and three phenotypes. We removed the miRNAs with NA expressing records according to the expression profile, calculated the variance of each miRNA in each sample, and selected the miRNAs with a standard deviation higher than 0.5. All the samples further clustered, as shown in Fig. 1. We removed the unreasonable samples according to the cluster distance, as shown in the red line in the figure. Finally, we got a new data expression spectrum, containing a total of 91 samples, 302 miRNAs. We chose a soft threshold of $\beta = 10$ in order to ensure building the scale-free characteristics of the network (Fig. 2).

After Computational co-expression modules, a total of five modules are shown in Fig. 3, where the gray module is a collection of miRNAs that could not be aggregated into other modules.

The miRNA statistics in each module are shown in Table 1, and it can be seen that these miRNAs are assigned to five modules. The blue, brown, turquoise, yellow, and gray module contains 40, 33, 44, 27, and 158 miRNAs respectively.

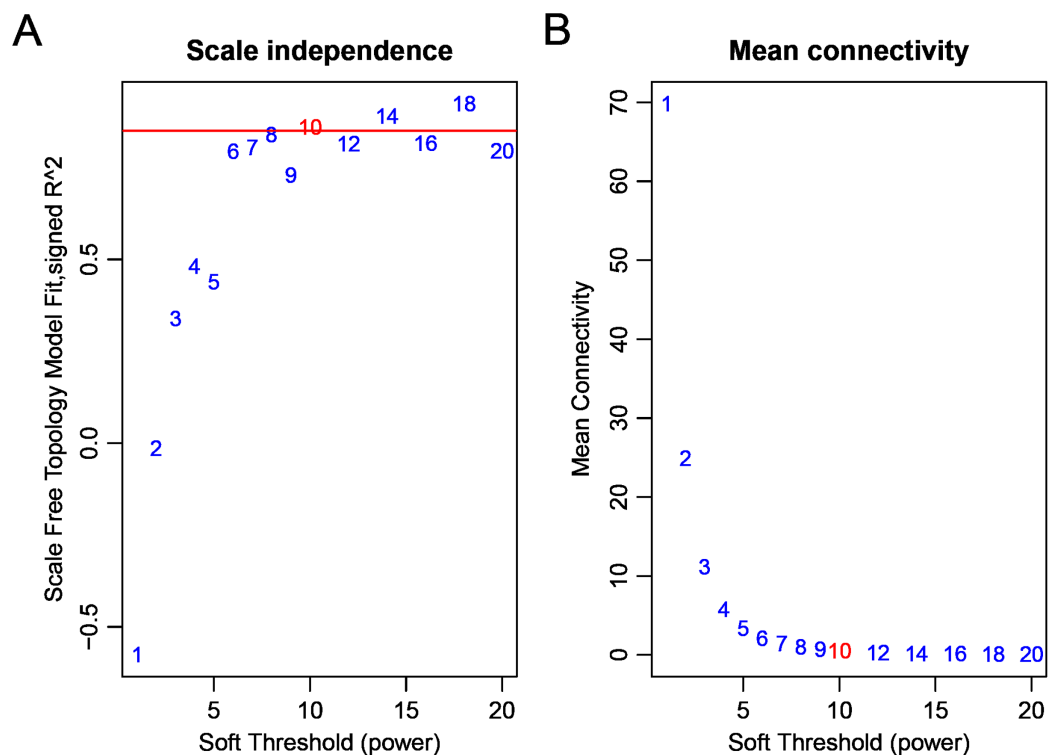


Figure 2 Analysis of network topology for various soft-thresholding powers β and γ . (A) The scale-free fit index (y-axis) as a function of the soft-thresholding power (x-axis). (B) The mean connectivity (degree, y-axis) as a function of the soft-thresholding power (x-axis). [Full-size !\[\]\(1679558f37f6db0dd8360a2a7e913e90_img.jpg\) DOI: 10.7717/peerj.8318/fig-2](https://doi.org/10.7717/peerj.8318/fig-2)

For the Phenotypic correlation analysis of modules, we analyze the relationship between the feature vectors of each module with the gender, age, and MoCA score.

The correlation between the phenotype and each module showed in Fig. 4. The blue module and the turquoise module have a positive correlation with age. Thorough Analysis of miRNA and phenotype correlation of modules, we separately calculated the distribution of GS of each phenotype in each module. Figures 5A–5C illustrated the overall correlation between the phenotype and the miRNAs in each module.

By integrating the analytical data of correlation between integration modules and phenotypes, as well as miRNA and phenotypic correlation in modules, we further analyzed the relationship between MM and miRNA significance for each module in each phenotype. The corresponding analysis results for each phenotype are as shown in Figs. 5D–5O. In the gender feature, the correlation between MM and miRNA significance of each module is very low, proving there is no significant relationship between the four modules and gender. In the age feature, we find that the blue module is involved in the regulation of this feature ($\text{cor} = 0.57$, $p = 0.00012$). In the MoCA score feature, we found that the brown module was involved in the adjustment of this feature ($\text{cor} = 0.31$, $p = 0.079$). Through Module-related miRNA correlation analysis, we selected miRNAs with module correlation greater than 0.85 in each module from each module. These miRNAs were used for target gene prediction and functional enrichment analysis.

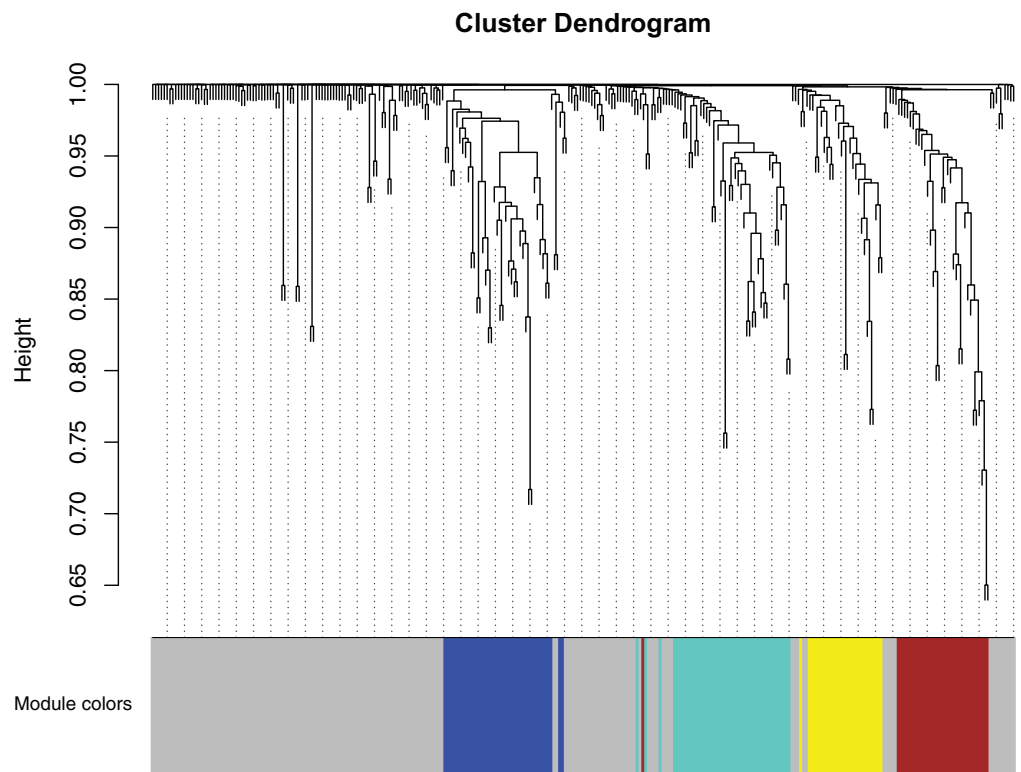


Figure 3 Clustering dendrogram of miRNAs. MiRNAs with higher topology similarity were clustered into different modules and assigned different colors. The expression profiles of miRNAs in each module had similar structural features in the network topology. Four co-expression modules were constructed and shown in different colors. These modules were ranged from large to small by the number of genes they included. The number of miRNAs in the four modules were listed in Table 1.

Full-size DOI: 10.7717/peerj.8318/fig-3

Table 1 The co-expression modules and hub miRNAs in each module (Correlation coefficient > 0.85).

Module	Blue module	Brown module	Turquoise module	Yellow module
Total miRNAs	40	33	158	27
hub miRNAs	hsa-miR-376c-3p	hsa-miR-125b-5p	hsa-let-7i-5p	hsa-miR-1307-3p
	hsa-miR-376a-3p	hsa-miR-10b-5p	hsa-miR-340-5p	hsa-miR-378a-3p
	hsa-miR-369-5p	hsa-miR-99a-5p	hsa-miR-15a-5p	hsa-miR-320b
	hsa-miR-654-3p	hsa-miR-100-5p	hsa-miR-374b-5p	hsa-miR-671-5p
	hsa-miR-487b-3p	hsa-miR-483-3p	hsa-let-7g-5p	hsa-miR-23a-5p
	hsa-miR-495-3p	hsa-miR-214-3p	hsa-miR-454-3p	hsa-miR-1908-5p
	hsa-miR-496	hsa-miR-342-3p	hsa-miR-30b-5p	
	hsa-miR-329-3p	hsa-miR-10a-5p	hsa-miR-590-3p	
	hsa-miR-136-3p	hsa-miR-125a-5p	hsa-let-7f-5p	
	hsa-miR-431-5p			

Hub miRNAs and their functional annotations

To clarify the link between each module and the trait, we selected the miRNAs with the highest MM scores in each module for functional enrichment. We selected 10 miRNAs

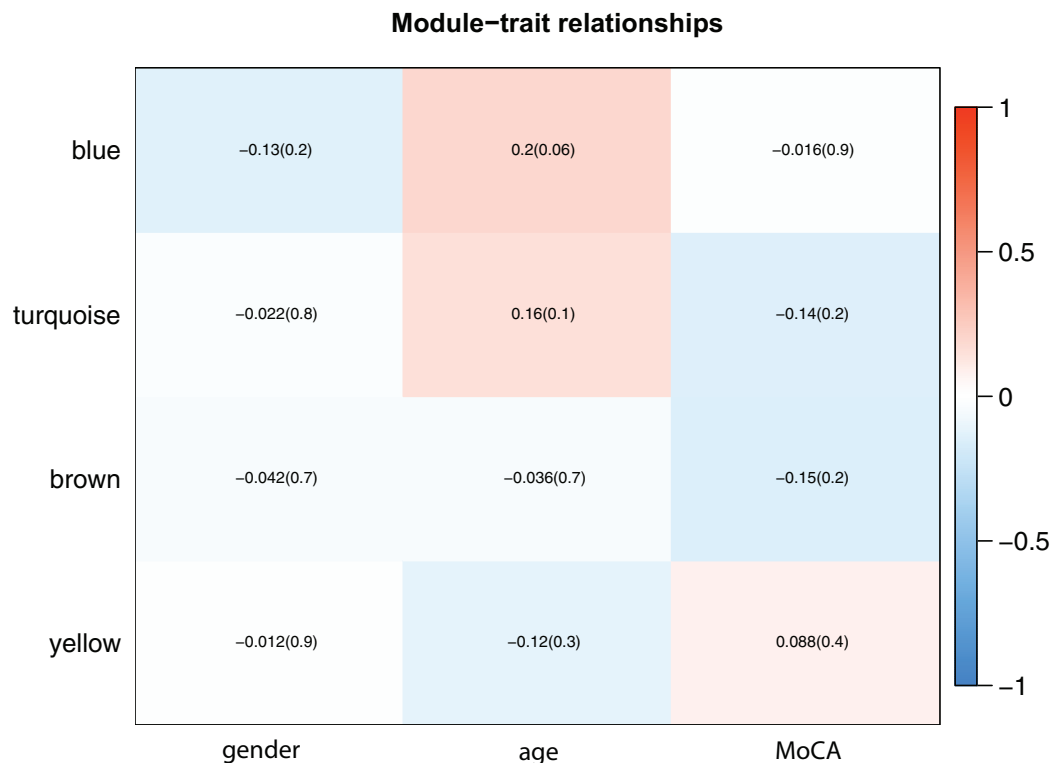


Figure 4 Module-trait associations. Each row corresponds to a module eigengene, column to a trait. Each cell contains the corresponding correlation and p -value. The table is color-coded by correlation according to the color legend. The results showed that the blue module was more correlated with age ($p = 0.06$), and each module was less correlated with gender. [Full-size !\[\]\(fcc3264021d438d9732560e78099f674_img.jpg\) DOI: 10.7717/peerj.8318/fig-4](https://doi.org/10.7717/peerj.8318/fig-4)

from the blue module, nine miRNAs in the brown module, nine miRNAs in the turquoise module, and six miRNAs in the yellow module (Table 1). We performed functional enrichment analysis and were limited to experimentally confirmed miRNA target genes. The enriched KEGG and REACTOME pathways were identified by analyzing the hub miRNAs of each module using the miRNet network tool. The hub miRNAs in the blue module are mainly enriched in aging-related signaling pathways such as Aging, Cellular responses to stress, and Oncogene induced Senescence (Figs. 6A and 6B). The hub miRNAs of the Brown module are mainly enriched in Pathways in cancer, Gene Expression, disease, aging, protein complex disassembly (Figs. 6C and 6D). The hub miRNAs in the Turquoise module are mainly enriched in cell cycle, TGF-beta signaling pathway, apoptosis, immune system (Figs. 6E and 6F). The hub-miRNAs in the Yellow module are mainly enriched in miRNA synthesis-regulated, senescence-related pathways (Figs. 6G and 6H).

We used miRNet web tools to build miRNA-target interaction networks. The hub miRNAs (module and miRNA p -value > 0.85) in each module were used as the input node to calculate the node degree and betweenness, both of which indicators for node centrality. We found that hsa-mir-329-3p in the blue (Fig. 7A) has the highest 538 Degree, and the target genes in this module are AGO2, BTF3L4, WDR17, BMI1, UHMK1, TIPRL, AKT1, ARPP19, IGF1R with three degrees. In the brown module (Fig. 7B), hsa-mir-10a-5p, has-mir-125b-5p have 463,432 degrees, respectively, among the target

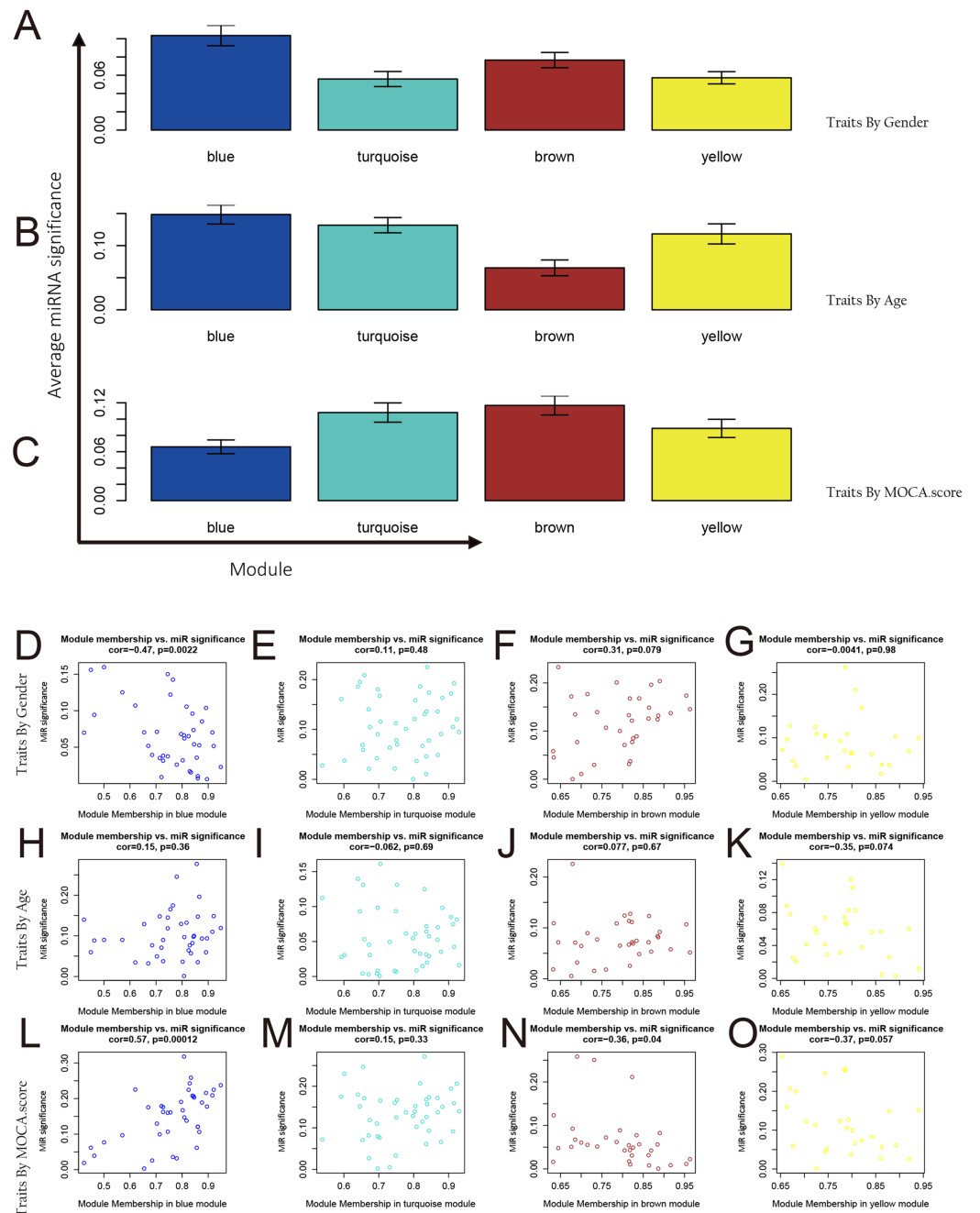


Figure 5 MiRNAs and phenotype correlation of modules. (A–C) Correlation between integration modules and phenotypes and miRNA and phenotypic correlation in modules. The average miRNA significance in the blue module, the turquoise module, and the yellow module was highly correlated with age; the average miRNA significance in the turquoise module and the brown module was highly correlated with the MOCA score. (D–O) Analysis of miRNAs and phenotype correlation of modules. From the analysis results in the figure, it was known that age has a high correlation with the miRNA in the blue module ($p < 0.001$) and the brown module ($p < 0.05$). The MOCA score was highly correlated with the miRNA in the blue module ($p < 0.01$). [Full-size !\[\]\(fcc3264021d438d9732560e78099f674_img.jpg\) DOI: 10.7717/peerj.8318/fig-5](https://doi.org/10.7717/peerj.8318/fig-5)

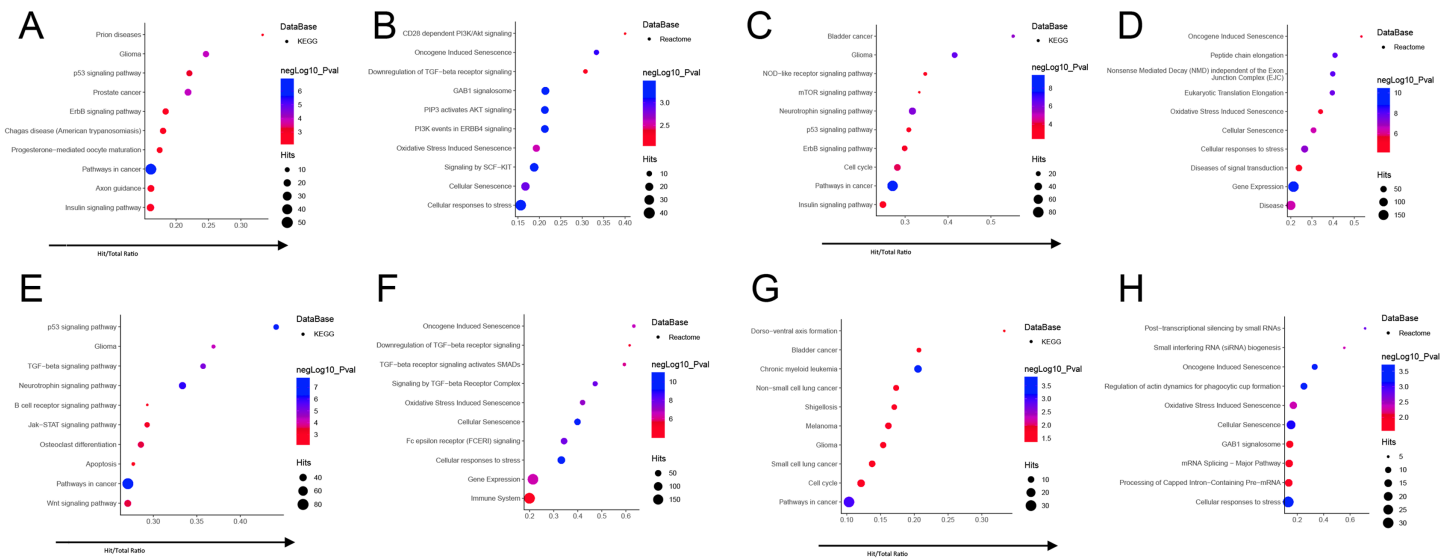


Figure 6 Functional annotation of hub miRNAs in each module ((A and B) blue module; (C and D) brown module; (E and F) turquoise module; (G and H) yellow module): enriched KEGG pathways (A, C, E, G) and REACTOME pathways (B, D, F, H). The y-axis represents the metabolic pathway in which the target genes of the hub miRNAs are involved, and the x-axis represents the ratio of Hits value of the mRNA enriched in each metabolic pathway to all mRNAs in this metabolic pathway. The size of the circle represents the Hits value of the target gene of the microRNA, and the color of the circle represents the significance of the signal pathway in which the target gene is involved ($-\log_{10}(p)$), and the redder color indicates a significantly higher value. The hub miRNAs in each module were shown in Table 1.

Full-size DOI: 10.7717/peerj.8318/fig-6

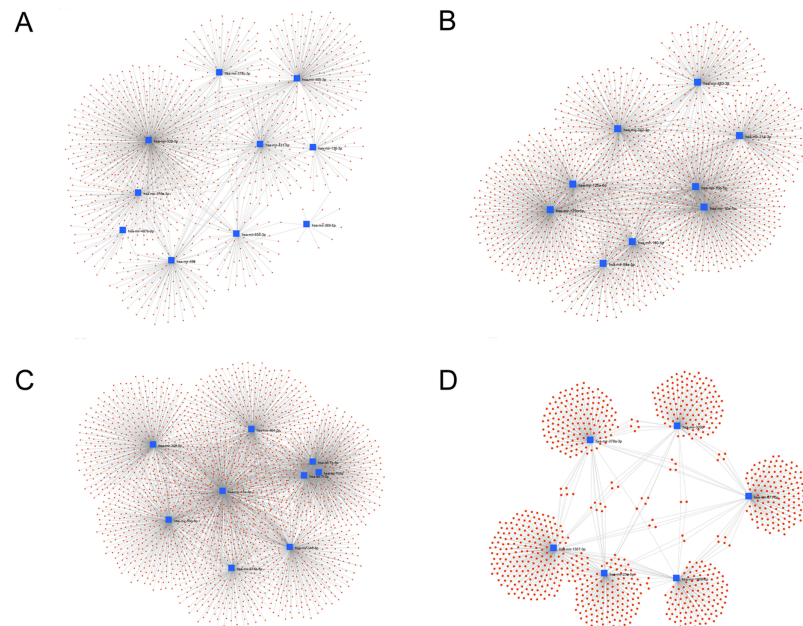


Figure 7 Interaction network of hub miRNAs. Interaction network of hub miRNAs in blue (A) and brown (B) and turquoise (C) and yellow (D) with their experimentally validated target genes using the miRNet tool. This network graphically highlights which miRNAs exhibit a large number of interactions.

Full-size DOI: 10.7717/peerj.8318/fig-7

genes in this module, AKT1, ANKRD33B, PLA2G4F, TMEM101, IGF1R, EIF1AD, GSS, SREBF1, TP53, EXTL3, RUNDC3B, MKNK2, CCNG1, CSNK2A1, XIAP, NCOR2, PAFAH1B1, SNX4 have at least four degrees. In the turquoise module (Fig. 7C), hsa-mir-15a-5p has the highest 717 degrees, of which KEMEN1, CCND1, KLHL15, PPP1R15B, ZNF264, ZNF460, YOD1, NOM1, WASL, TNFSF9, NCOA3 have more than five degrees. In the yellow module (Fig. 7D), hsa-mir-1307-3p has the highest 240 degrees, of which PEX26, ZNF385A has more than three degrees. From these results, we can think that miRNAs in exosomes can play a regulatory role in inter-tissue signaling. In particular, miR-329-3p, miR-10a-5p, miR-125b-5p, miR-15a-5p play an important role in the regulation of exocytosis transmission, and these functions are closely related to the decline of aging and cognitive functions (Bottoni et al., 2005; Tan et al., 2014).

DISCUSSION

In this study, data came from 97 elderly patients. Four co-expression modules were analyzed by WGCNA analysis. WGCNA can be used to explore the relationship between each module and clinical traits. WGCNA analysis focuses on the Analysis of the link between co-expression modules and clinical traits. Therefore, compared to other analytical methods, the analysis results of WGCNA have higher credibility and biological significance (Chou et al., 2014). Genes in the same block are considered to be functionally related. Therefore, miRNAs in modules that are highly correlated with biological traits can be considered as biomarkers for clinical testing and treatment. We analyzed four co-expression modules. In these modules, we found that the blue module has a specific correlation with age. Although the expression profiles of these miRNAs are relatively low in covariance correlation with Age and MoCA score, exosomes can be used as an indicator of brain health as a membrane molecule that can cross the blood-brain barrier (Andras & Toborek, 2016). At the same time, exosomes in the blood can transport functional macromolecules such as proteins, mRNAs, and miRNAs to the brain through the blood-brain barrier, thus affecting the function of the brain. In particular, it is considered that miRNAs can be enriched in exosomes so that the concentration of miRNA in exosomes is much higher than the concentration of miRNA in normal blood. Therefore, exosomes achieve regulation of the brain, which may be an important means of regulating the brain's function through humoral regulation (Bastos et al., 2017). At the same time, recent research has also shown that the hypothalamic-mediated aging process is accompanied by dysregulation of nutrient sensing, altered intercellular communication, stem cell exhaustion, loss of proteostasis, and epigenetic alterations. This suggests that the process of aging is strictly regulated by the central nervous system of the brain. From these perspectives, exosomes in the blood may be important hubs for the regulation of aging and cognitive decline in the blood circulatory system and brain, and miRNAs in exosomes may play an important role. In order to further explore the relationship between the co-expression module of miRNAs in exosomes and the age and MoCA scores, we used KEGG and Reactome to perform the enrichment analysis for the hub miRNAs in each module. From the results, the antigen of blue module hub miRNAs, mir-376a-3p, is mainly involved in tumor-associated metabolic pathways (Gao et al., 2010; Tu et al., 2016) and

muscular dystrophy. However, Thalyana Smith-Vikos et al. found that the plasma content of hsa-mir-376a-3p in long-lived people was significantly different from that in short-lived people, indicating that mir-376a-3p is strongly associated with age. Mir-369-5p is involved in the regulation of senescence of mesenchymal stem cells (Wagner et al., 2008) and participates in Duchenne muscular dystrophy (Bottoni et al., 2005), Nemaline myopathy (NM) and other muscular dystrophy associated disease (Xia et al., 2008). Muscle atrophy is a typical feature of aging. Yao et al. (2019) found that the up-regulation of miR-496 can reduce cerebral ischemia-reperfusion injury. SP Ross et al. found that miRNA-431 can prevent synaptic loss in amyloid- β -induced cell culture models of AD by silencing the expression of Kremen1 protein (Ross et al., 2018). Among the brown module, the target genes of hub miRNAs are mainly involved in cancer-related pathways. Mir-125b-5p is involved in Alzheimer Disease (Sethi & Lukiw, 2009), Cerebellar neurodegeneration disease metabolic pathway, and it is also related to the occurrence of vascular diseases and heart failure (Dai et al., 2009), and there is a strong correlation between the two diseases with the life expectancy of the elderly. Mir-342-3p is involved in Neurodegeneration and Prion diseases (Sayed et al., 2007). Among the turquoise modules, hub miRNAs are mainly involved in cancer-related signaling pathways. The let7 family of miRNAs (let-7i-5p, let-7g-5p, let-7f-5p) are thought to be highly correlated with the development of cancer and are important mechanisms for the body to suppress cancer (Pobezinsky & Wells, 2018). Among the yellow module, the target genes of hub miRNAs are also mainly enriched in cancer-related pathways. We obtained five miRNAs from these modules, miR-376a, miR-125a, miR-10a, miR-15a. Among them, miR-37a is up-regulated in T lymphocytes of patients with multiple sclerosis (Lindberg et al., 2010), miR125a is up-regulated in PBMC of patients with multiple sclerosis (Yang et al., 2014), miR-10a and miR-125 are down-regulated in cerebrospinal fluid of patients with Alzheimer's syndrome (Bekris et al., 2013), miR-15a Up-regulation of activated lesions in brain lesions in patients with multiple sclerosis is an age-related disease (Junker et al., 2009). These findings support the role of our selected hub miRNAs in Aging and age-related diseases. The hub miRNAs in these modules are associated with aging and neurodegenerative diseases. However, their expression profiles are relatively low in correlation coefficients between age and MoCA scores, making it difficult to find definitive connections. This also shows that the aging and cognitive impairment of the body are the results of many factors.

Furthermore, we identified enriched KEGG and REACTOME pathways through functional enrichment analysis of hub miRNA target genes. In this way, we found the relationship between the four module-related signaling pathways and the aging and MoCA scores. Interesting, we found that these modules are all related to cancer-related signaling pathways. Cancer is an important factor affecting the longevity of the elderly (Leshno et al., 2016; Paskett et al., 2018).

We also identified the highest number of genes targeted by hub miRNAs in each module (Fig. 7). In the blue module, the ARPP19 was found to be highly targeted. The 19-kD cAMP-regulated phosphoprotein (ARPP19) plays a role in regulating mitosis by inhibiting protein phosphatase-2A (PP2A) (Gharbi-Ayachi et al., 2010). Decreased

levels of ARPP-19 and PKA in brains of Down syndrome and AD (Kim et al., 2001). Interesting, ARPP19 expression in brain tissue is significantly higher than other tissues (Fagerberg et al., 2014). Within the brown module, we found that the AKT1, RUN domain containing 3B (RUNDC3B), cyclin G1 (CCNG1), platelet-activating factor acetylhydrolase 1b regulatory subunit 1 (PAFAH1B1), tumor protein p53 (TP53), sorting nexin 4 (SNX4) The expression levels of RUNDC3B and PAFAH1B1 in brain tissue are significantly higher than in other tissues (Fagerberg et al., 2014), where PAFAH1B1 gene duplication is associated with developmental, behavioral and brain abnormalities (Curry et al., 2013). SNX4 gene encodes a member of the sorting nexin family. Study shows that SNX4-mediated regulation of the steady-state levels and trafficking of BACE1, as well as the subsequent increase in BACE1-mediated cleavage, may be relevant to AD progression (Kim et al., 2017). These genes are primarily involved in the regulation of cancer signaling (Cruz-Garcia et al., 2018; Ko & Kim, 2019; Ma, Lu & Gu, 2019). Within the turquoise module, the protein phosphatase 1 regulatory subunit 15B (PPP1R15B), TNF superfamily member 9 (TNFSF9), nuclear receptor coactivator3 (NCOA3) were found to be highly targeted. The TNFSF9 is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. This transmembrane cytokine is a bidirectional signal transducer, acts as a ligand for TNFRSF9/4-1BB, which is a costimulatory receptor molecule in T lymphocytes (Tsuda et al., 2017). The NCOA3 is a nuclear receptor coactivator that interacts with nuclear hormone receptors to enhance their transcriptional activator functions. Data suggest that over-stimulating the steroid receptor coactivators SRC-1, SRC-2, and SRC-3 oncogenic program can be an effective strategy to kill cancer cells (Wang et al., 2015). Within the yellow module, we found that the zinc finger protein 385A (ZNF385A) was highly targeted. Brain gene expression of ZNF385A and DNA methylation dysregulations are implicated in the Alteration of brain tissue properties associated with late-life cognitive decline above and beyond the influence of common neuropathologic conditions (Yu et al., 2017). We analyzed the experimentally validated interactions by miRNA-target network and eliminated the network. The node with degree = 1 intensifies the interaction of the internal interactions in each module and, of course, also loses much information about the possible interactions between miRNA-mRNA.

CONCLUSIONS

In summary, we found four linking clusters miRNA expression profiles from the plasma exosome of human peripheral blood and analyzed target gene prediction and functional enrichment for hub miRNAs in these four clusters. The relationship between modules and age and cognitive function. Some of these hub miRNAs have been experimentally validated for their relationship with age and cognitive function, and some new candidate miRNAs that may be exploited as biomarkers or therapeutic targets of aging or cognitive decline. Although there is overlap between known candidate biomarkers and the ones that we identified here, more research needs to be used to verify the performance of these possible biomarkers.

ACKNOWLEDGEMENTS

We thank Doulatunnisa Jaffar Ali for help in honing the manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81671807) and Fundamental Research Funds for the Central Universities (2242018K3DN05, 2242017K40236). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
National Natural Science Foundation of China: 81671807.
Central Universities: 2242018K3DN05 and 2242017K40236.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Zheng Ye analyzed the data, conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Bo Sun analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Xue Mi performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Zhongdang Xiao conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:
The raw measurements are available in the [Supplemental Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.8318#supplemental-information>.

REFERENCES

- Alexander GE, Ryan L, Bowers D, Foster TC, Bizon JL, Geldmacher DS, Glisky EL. 2012. Characterizing cognitive aging in humans with links to animal models. *Frontiers in Aging Neuroscience* 4:21 DOI 10.3389/fnagi.2012.00021.
- Andras IE, Toborek M. 2016. Extracellular vesicles of the blood-brain barrier. *Tissue Barriers* 4(1):e1131804 DOI 10.1080/21688370.2015.1131804.

- Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogossova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. 2011. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proceedings of the National Academy of Sciences of the United States of America* **108**(12):5003–5008 DOI [10.1073/pnas.1019055108](https://doi.org/10.1073/pnas.1019055108).
- Barbu E, Molnár É, Tsibouklis J, Górecki DC. 2009. The potential for nanoparticle-based drug delivery to the brain: overcoming the blood-brain barrier. *Expert Opinion on Drug Delivery* **6**(6):553–565 DOI [10.1517/17425240902939143](https://doi.org/10.1517/17425240902939143).
- Barrientos RM, Frank MG, Watkins LR, Maier SF. 2010. Memory impairments in healthy aging: role of aging-induced microglial sensitization. *Aging and Disease* **1**:212–231.
- Bartel DP. 2007. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **131**:11–29 DOI [10.1016/S0092-8674\(04\)00045-5](https://doi.org/10.1016/S0092-8674(04)00045-5).
- Bastos P, Ferreira R, Manadas B, Moreira PI, Vitorino R. 2017. Insights into the human brain proteome: disclosing the biological meaning of protein networks in cerebrospinal fluid. *Critical Reviews in Clinical Laboratory Sciences* **54**(3):185–204 DOI [10.1080/10408363.2017.1299682](https://doi.org/10.1080/10408363.2017.1299682).
- Bekris LM, Lutz F, Montine TJ, Yu CE, Tsuang D, Peskind ER, Leverenz JB. 2013. MicroRNA in Alzheimer's disease: an exploratory study in brain, cerebrospinal fluid and plasma. *Biomarkers* **18**(5):455–466 DOI [10.3109/1354750X.2013.814073](https://doi.org/10.3109/1354750X.2013.814073).
- Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, Uberti ECD. 2005. miR-15a and miR-16-1 down-regulation in pituitary adenomas. *Journal of Cellular Physiology* **204**(1):280–285 DOI [10.1002/jcp.20282](https://doi.org/10.1002/jcp.20282).
- Burkle A, Moreno-Villanueva M, Bernhard J, Blasco M, Zondag G, Hoeijmakers JHJ, Toussaint O, Grubeck-Loebenstein B, Mocchegiani E, Collino S, Gonos ES, Sikora E, Gradinaru D, Dolle M, Salmon M, Kristensen P, Griffiths HR, Libert C, Grune T, Breusing N, Simm A, Franceschi C, Capri M, Talbot D, Caiafa P, Friguet B, Slagboom PE, Hervonen A, Hurme M, Aspinall R. 2015. MARK-AGE biomarkers of ageing. *Mechanisms of Ageing and Development* **151**:2–12 DOI [10.1016/j.mad.2015.03.006](https://doi.org/10.1016/j.mad.2015.03.006).
- Cai LY, Chan JSY, Yan JH, Peng KP. 2014. Brain plasticity and motor practice in cognitive aging. *Frontiers in Aging Neuroscience* **6**:43 DOI [10.3389/fnagi.2014.00031](https://doi.org/10.3389/fnagi.2014.00031).
- Cheng L, Doecke JD, Sharples RA, Villemagne VL, Fowler CJ, Rembach A, Martins RN, Rowe CC, Macaulay SL, Masters CL, Hill AF, Biomarkers AI. 2015. Prognostic serum miRNA biomarkers associated with Alzheimer's disease shows concordance with neuropsychological and neuroimaging assessment. *Molecular Psychiatry* **20**(10):1188–1196 DOI [10.1038/mp.2014.127](https://doi.org/10.1038/mp.2014.127).
- Chou WC, Cheng AL, Brotto M, Chuang CY. 2014. Visual gene-network analysis reveals the cancer gene co-expression in human endometrial cancer. *BMC Genomics* **15**(1):300 DOI [10.1186/1471-2164-15-300](https://doi.org/10.1186/1471-2164-15-300).
- Cosin-Tomas M, Antonell A, Llado A, Alcolea D, Fortea J, Ezquerra M, Lleo A, Martí MJ, Pallas M, Sanchez-Valle R, Molinuevo JL, Sanfeliu C, Kaliman P. 2017. Plasma miR-34a-5p and miR-545-3p as early biomarkers of Alzheimer's disease: potential and limitations. *Molecular Neurobiology* **54**(7):5550–5562 DOI [10.1007/s12035-016-0088-8](https://doi.org/10.1007/s12035-016-0088-8).
- Craft S, Foster TC, Landfield PW, Maier SF, Resnick SM, Yaffe K. 2012. Session III: mechanisms of age-related cognitive change and targets for intervention: inflammatory, oxidative, and metabolic processes. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences* **67**(7):754–759 DOI [10.1093/gerona/gls112](https://doi.org/10.1093/gerona/gls112).
- Cruz-Garcia L, O'Brien G, Donovan E, Gothard L, Boyle S, Laval A, Testard I, Ponge L, Wozniak G, Miszczuk L, Candeias SM, Ainsbury E, Widlak P, Somaiah N, Badie C. 2018.

- Influence of confounding factors on radiation dose estimation using in vivo validated transcriptional biomarkers. *Health Physics* **115**(1):90–101 DOI [10.1097/HP.0000000000000844](https://doi.org/10.1097/HP.0000000000000844).
- Cui L, Li Y, Ma G, Wang Y, Cai Y, Liu S, Chen Y, Li J, Xie Y, Liu G, Zhao B, Li K. 2014.** A functional polymorphism in the promoter region of microRNA-146a is associated with the risk of Alzheimer disease and the rate of cognitive decline in patients. *PLOS ONE* **9**(2):e89019 DOI [10.1371/journal.pone.0089019](https://doi.org/10.1371/journal.pone.0089019).
- Curry CJ, Rosenfeld JA, Grant E, Gripp KW, Anderson C, Aylsworth AS, Saad TB, Chizhikov Dybose VV, Fagerberg G, Falco C, Fels M, Fichera C, Graakjaer M, Greco J, Hair D, Hopkins J, Huggins E, Ladda M, Li R, Moeschler C, Nowaczyk J, Ozmore JR, Reitano S, Romano C, Roos L, Schnur RE, Sell S, Suwannarat P, Svaneby D, Szybowska M, Tarnopolsky M, Tervo R, Tsai AC, Tucker M, Vallee S, Wheeler FC, Zand DJ, Barkovich AJ, Aradhya S, Shaffer LG, Dobyns WB. 2013.** The duplication 17p13.3 phenotype: analysis of 21 families delineates developmental, behavioral and brain abnormalities, and rare variant phenotypes. *American Journal of Medical Genetics* **161A**:1833–1852 DOI [10.1002/ajmg.a.35996](https://doi.org/10.1002/ajmg.a.35996).
- Dai Y, Sui WG, Lan HJ, Yan Q, Huang H, Huang YS. 2009.** Comprehensive analysis of microRNA expression patterns in renal biopsies of lupus nephritis patients. *Rheumatology International* **29**(7):749–754 DOI [10.1007/s00296-008-0758-6](https://doi.org/10.1007/s00296-008-0758-6).
- Dong H, Li JL, Huang L, Chen X, Li DH, Wang T, Hu CY, Xu J, Zhang CN, Zen K, Xiao SF, Yan Q, Wang C, Zhang CY. 2015.** Serum MicroRNA profiles serve as novel biomarkers for the diagnosis of Alzheimer's disease. *Disease Markers* **2015**:11 DOI [10.1155/2015/625659](https://doi.org/10.1155/2015/625659).
- El Andaloussi S, Lakhil S, Mager I, Wood MJ. 2013.** Exosomes for targeted siRNA delivery across biological barriers. *Advanced Drug Delivery Reviews* **65**(3):391–397 DOI [10.1016/j.addr.2012.08.008](https://doi.org/10.1016/j.addr.2012.08.008).
- Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpour S, Danielsson A, Edlund K, Asplund A, Sjostedt E, Lundberg E, Szgyarto CA, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, Von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, Uhlen M. 2014.** Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Molecular & Cellular Proteomics* **13**(2):397–406 DOI [10.1074/mcp.M113.035600](https://doi.org/10.1074/mcp.M113.035600).
- Fan X, Wheatley EG, Villeda SA. 2017.** Mechanisms of hippocampal aging and the potential for rejuvenation. *Annual Review of Neuroscience* **40**(1):251–272 DOI [10.1146/annurev-neuro-072116-031357](https://doi.org/10.1146/annurev-neuro-072116-031357).
- Fan Y, Xia J. 2018.** miRNet-functional analysis and visual exploration of miRNA-target interactions in a network context. *Methods in Molecular Biology* **1819**(2):215–233 DOI [10.1007/978-1-4939-8618-7_10](https://doi.org/10.1007/978-1-4939-8618-7_10).
- Febo M, Foster TC. 2016.** Preclinical magnetic resonance imaging and spectroscopy studies of memory, aging, and cognitive decline. *Frontiers in Aging Neuroscience* **8**(e17):158 DOI [10.3389/fnagi.2016.00158](https://doi.org/10.3389/fnagi.2016.00158).
- Foster TC. 2006.** Biological markers of age-related memory deficits – treatment of senescent physiology. *CNS Drugs* **20**(2):153–166 DOI [10.2165/00023210-200620020-00006](https://doi.org/10.2165/00023210-200620020-00006).
- Galimberti D, Villa C, Fenoglio C, Serpente M, Ghezzi L, Cioffi SMG, Arighi A, Fumagalli G, Scarpini E. 2014.** Circulating miRNAs as potential biomarkers in Alzheimer's disease. *Journal of Alzheimer's Disease* **42**(4):1261–1267 DOI [10.3233/JAD-140756](https://doi.org/10.3233/JAD-140756).

- Gallo A, Tandon M, Alevizos I, Illei GG. 2012.** The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLOS ONE* 7(3):e30679 DOI 10.1371/journal.pone.0030679.
- Gao Y, Wang C, Shan Z, Guan H, Mao J, Fan C, Wang H, Zhang H, Teng W. 2010.** miRNA expression in a human papillary thyroid carcinoma cell line varies with invasiveness. *Endocrine Journal* 57(1):81–86 DOI 10.1507/endocrj.K09E-220.
- Geekiyana H, Jicha GA, Nelson PT, Chan C. 2012.** Blood serum miRNA: non-invasive biomarkers for Alzheimer's disease. *Experimental Neurology* 235(2):491–496 DOI 10.1016/j.expneurol.2011.11.026.
- Gharbi-Ayachi A, Labbe JC, Burgess A, Vigneron S, Strub JM, Brioudes E, Van-Dorselaer A, Castro A, Lorca T. 2010.** The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. *Science* 330(6011):1673–1677 DOI 10.1126/science.1197048.
- Giulietti M, Occhipinti G, Principato G, Piva F. 2017.** Identification of candidate miRNA biomarkers for pancreatic ductal adenocarcinoma by weighted gene co-expression network analysis. *Cellular Oncology* 40(2):181–192 DOI 10.1007/s13402-017-0315-y.
- Gross JC, Chaudhary V, Bartscherer K, Boutros M. 2012.** Active Wnt proteins are secreted on exosomes. *Nature Cell Biology* 14(10):1036–1045 DOI 10.1038/ncb2574.
- Harvey S, Martinez-Moreno CG, Luna M, Aramburo C. 2015.** Autocrine/paracrine roles of extrapituitary growth hormone and prolactin in health and disease: an overview. *General and Comparative Endocrinology* 220:103–111 DOI 10.1016/j.ygcen.2014.11.004.
- Hu ZB, Chen X, Zhao Y, Tian T, Jin GF, Shu YQ, Chen YJ, Xu L, Zen K, Zhang CY, Shen HB. 2010.** Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. *Journal of Clinical Oncology* 28(10):1721–1726 DOI 10.1200/JCO.2009.24.9342.
- Impey S, Davare M, Lesiak A, Fortin D, Ando H, Varlamova O, Obrietan K, Soderling TR, Goodman RH, Wayman GA. 2010.** An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. *Molecular and Cellular Neuroscience* 43(1):146–156 DOI 10.1016/j.mcn.2009.10.005.
- Jovasevic V, Corcoran KA, Leaderbrand K, Yamawaki N, Guedea AL, Chen HJ, Shepherd GM, Radulovic J. 2015.** GABAergic mechanisms regulated by miR-33 encode state-dependent fear. *Nature Neuroscience* 18(9):1265–1271 DOI 10.1038/nn.4084.
- Junker A, Krumbholz M, Eisele S, Mohan H, Augstein F, Bittner R, Lassmann H, Wekerle H, Hohlfeld R, Meinel E. 2009.** MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47. *Brain* 132(12):3342–3352 DOI 10.1093/brain/awp300.
- Kiko T, Nakagawa K, Tsuduki T, Furukawa K, Arai H, Miyazawa T. 2014.** MicroRNAs in plasma and cerebrospinal fluid as potential markers for Alzheimer's disease. *Journal of Alzheimer's Disease* 39(2):253–259 DOI 10.3233/JAD-130932.
- Kim NY, Cho MH, Won SH, Kang HJ, Yoon SY, Kim DH. 2017.** Sorting nexin-4 regulates beta-amyloid production by modulating beta-site-activating cleavage enzyme-1. *Alzheimers Research & Therapy* 9(1):4 DOI 10.1186/s13195-016-0232-8.
- Kim SH, Nairn AC, Cairns N, Lubec G. 2001.** Decreased levels of ARPP-19 and PKA in brains of down syndrome and Alzheimer's disease. *Journal of Neural Transmission. Supplementum* 14:263–272 DOI 10.1007/978-3-7091-6262-0_21.
- Ko H, Kim MM. 2019.** TP53 tumor-suppressor gene plays a key role in IGF1 signaling pathway related to the aging of human melanocytes. *Anticancer Research* 39(5):2447–2451 DOI 10.21873/anticancer.13363.

- Kumar P, Dezso Z, MacKenzie C, Oestreicher J, Agoulnik S, Byrne M, Bernier F, Yanagimachi M, Aoshima K, Oda Y. 2013. Circulating miRNA biomarkers for Alzheimer's disease. *PLOS ONE* 8(7):e69807 DOI 10.1371/journal.pone.0069807.
- Langfelder P, Horvath S. 2008. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9(1):559 DOI 10.1186/1471-2105-9-559.
- Leidinger P, Backes C, Deutscher S, Schmitt K, Mueller SC, Frese K, Haas J, Ruprecht K, Paul F, Stahler C, Lang CJG, Meder B, Bartfai T, Meese E, Keller A. 2013. A blood based 12-miRNA signature of Alzheimer disease patients. *Genome Biology* 14(7):R78 DOI 10.1186/gb-2013-14-7-r78.
- Leshno A, Shapira S, Liberman E, Kraus S, Srer M, Harlap-Gat A, Avivi D, Galazan L, David M, Maharshak N, Moanis S, Arber N, Moshkowitz M. 2016. The APC I1307K allele conveys a significant increased risk for cancer. *International Journal of Cancer* 138(6):1361–1367 DOI 10.1002/ijc.29876.
- Lindberg RL, Hoffmann F, Mehling M, Kuhle J, Kappos L. 2010. Altered expression of miR-17-5p in CD4⁺ lymphocytes of relapsing-remitting multiple sclerosis patients. *European Journal of Immunology* 40(3):888–898 DOI 10.1002/eji.200940032.
- Liu X, Guan Y, Wang L, Niu Y. 2017. MicroRNA-10b expression in node-negative breast cancer-correlation with metastasis and angiogenesis. *Oncology Letters* 14:5845–5852 DOI 10.3892/ol.2017.6914.
- Lugli G, Cohen AM, Bennett DA, Shah RC, Fields CJ, Hernandez AG, Smalheiser NR. 2015. Plasma exosomal miRNAs in persons with and without Alzheimer disease: altered expression and prospects for biomarkers. *PLOS ONE* 10(10):e0139233 DOI 10.1371/journal.pone.0139233.
- Lv LL, Cao YH, Liu D, Xu M, Liu H, Tang RN, Ma KL, Liu BC. 2013. Isolation and quantification of microRNAs from urinary exosomes/microvesicles for biomarker discovery. *International Journal of Biological Sciences* 9(10):1021–1031 DOI 10.7150/ijbs.6100.
- Ma Q, Lu Y, Gu Y. 2019. ENKUR is involved in the regulation of cellular biology in colorectal cancer cells via PI3K/Akt signaling pathway. *Technology in Cancer Research & Treatment* 18(6):1533033819841433 DOI 10.1177/1533033819841433.
- Ma L, Teruya-Feldstein J, Weinberg RA. 2007. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449(7163):682–688 DOI 10.1038/nature06174.
- Mathivanan S, Ji H, Simpson RJ. 2010. Exosomes: extracellular organelles important in intercellular communication. *Journal of Proteomics* 73(10):1907–1920 DOI 10.1016/j.jprot.2010.06.006.
- Michael A, Bajracharya SD, Yuen PST, Zhou H, Star RA, Illei GG, Alevizos I. 2010. Exosomes from human saliva as a source of microRNA biomarkers. *Oral Diseases* 16(1):34–38 DOI 10.1111/j.1601-0825.2009.01604.x.
- Nasreddine ZS, Chertkow H, Phillips N, Whitehead V, Collin I, Cummings JL. 2004. The Montreal Cognitive Assessment (MoCA): a brief cognitive screening tool for detection of mild cognitive impairment. *Neurology* 62(1):A132 DOI 10.1212/WNL.62.1.132.
- Nissim NR, O'Shea AM, Bryant V, Porges EC, Cohen R, Woods AJ. 2017. Frontal structural neural correlates of working memory performance in older adults. *Frontiers in Aging Neuroscience* 8(250):328 DOI 10.3389/fnagi.2016.00328.
- O'Shea A, Cohen RA, Porges EC, Nissim NR, Woods AJ. 2016. Cognitive aging and the hippocampus in older adults. *Frontiers in Aging Neuroscience* 8:298 DOI 10.3389/fnagi.2016.00298.
- Paskett ED, Caan BJ, Johnson L, Bernardo BM, Young GS, Pennell ML, Ray RM, Kroenke CH, Porter PL, Anderson GL. 2018. The women's health initiative (WHI) life and longevity after

- cancer (LILAC) study: description and baseline characteristics of participants. *Cancer Epidemiology Biomarkers & Prevention* 27(2):125–137 DOI 10.1158/1055-9965.EPI-17-0581.
- Png KJ, Halberg N, Yoshida M, Tavazoie SF. 2012.** A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. *Nature* 481(7380):190–194 DOI 10.1038/nature10661.
- Pobezinsky LA, Wells AC. 2018.** Let's fight cancer: let-7 is a tool to enhance antitumor immune responses. *Future Oncology* 14(12):1141–1145 DOI 10.2217/fon-2018-0037.
- Rajman M, Metge F, Fiore R, Khudayberdiev S, Aksoy-Aksel A, Bicker S, Ruedell Reschke C, Raoof R, Brennan GP, Delanty N, Farrell MA, O'Brien DF, Bauer S, Norwood B, Veno MT, Kruger M, Braun T, Kjems J, Rosenow F, Henshall DC, Dieterich C, Schratt G. 2017.** A microRNA-129-5p/Rbfox crosstalk coordinates homeostatic downscaling of excitatory synapses. *EMBO Journal* 36(12):1770–1787 DOI 10.15252/embj.201695748.
- Rani A, O'Shea A, Ianov L, Cohen RA, Woods AJ, Foster TC. 2017.** miRNA in circulating microvesicles as biomarkers for age-related cognitive decline. *Frontiers in Aging Neuroscience* 9:323 DOI 10.3389/fnagi.2017.00323.
- Ross SP, Baker KE, Fisher A, Hoff L, Pak ES, Murashov AK. 2018.** miRNA-431 prevents amyloid- β -induced synapse loss in neuronal cell culture model of Alzheimer's disease by silencing Kremen. *Frontiers in Cellular Neuroscience* 12:87 DOI 10.3389/fncel.2018.00087.
- Sato-Kuwabara Y, Melo SA, Soares FA, Calin GA. 2015.** The fusion of two worlds: non-coding RNAs and extracellular vesicles – diagnostic and therapeutic implications. *International Journal of Oncology* 46(1):17–27 DOI 10.3892/ijco.2014.2712.
- Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M. 2007.** MicroRNAs play an essential role in the development of cardiac hypertrophy. *Circulation Research* 100(3):416–424 DOI 10.1161/01.RES.0000257913.42552.23.
- Schneider A, Simons M. 2013.** Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. *Cell and Tissue Research* 352(1):33–47 DOI 10.1007/s00441-012-1428-2.
- Serafini G, Pompili M, Hansen KF, Obrietan K, Dwivedi Y, Shomron N, Girardi P. 2014.** The involvement of microRNAs in major depression, suicidal behavior, and related disorders: a focus on miR-185 and miR-491-3p. *Cellular and Molecular Neurobiology* 34(1):17–30 DOI 10.1007/s10571-013-9997-5.
- Sethi P, Lukiw WJ. 2009.** Micro-RNA abundance and stability in human brain: specific alterations in Alzheimer's disease temporal lobe neocortex. *Neuroscience Letters* 459(2):100–104 DOI 10.1016/j.neulet.2009.04.052.
- Shaltiel G, Hanan M, Wolf Y, Barbash S, Kovalev E, Shoham S, Soreq H. 2013.** Hippocampal microRNA-132 mediates stress-inducible cognitive deficits through its acetylcholinesterase target. *Brain Structure & Function* 218(1):59–72 DOI 10.1007/s00429-011-0376-z.
- Shi K, Bing ZT, Cao GQ, Guo L, Cao YN, Jiang HO, Zhang MX. 2015.** Identify the signature genes for diagnose of uveal melanoma by weight gene co-expression network analysis. *International Journal of Ophthalmology* 8:269–274 DOI 10.3980/j.issn.2222-3959.2015.02.10.
- Speisman RB, Kumar A, Rani A, Foster TC, Ormerod BK. 2013.** Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. *Brain, Behavior, and Immunity* 28:25–43 DOI 10.1016/j.bbi.2012.09.013.
- Tabet F, Vickers KC, Torres LFC, Wiese CB, Shoucri BM, Lambert G, Catherinet C, Prado-Lourenco L, Levin MG, Thacker S, Sethupathy P, Barter PJ, Remaley AT, Rye KA. 2014.** HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. *Nature Communications* 5(1):3292 DOI 10.1038/ncomms4292.

- Tan L, Yu JT, Liu QY, Tan MS, Zhang W, Hu N, Wang YL, Sun L, Jiang T, Tan L. 2014.** Circulating miR-125b as a biomarker of Alzheimer's disease. *Journal of the Neurological Sciences* 336(1–2):52–56 DOI 10.1016/j.jns.2013.10.002.
- Tay Y, Zhang JQ, Thomson AM, Lim B, Rigoutsos I. 2008.** MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature* 455(7216):1124–1128 DOI 10.1038/nature07299.
- Toledo J, Alegre M, Lopez-Azcarate J, Guridi J, Iriarte J, Obeso J, Artieda J, Rodriguez-Oroz MC. 2011.** Relationship between subthalamic nucleus oscillatory activity and age, gender, and clinical features in Parkinson's disease. *Movement Disorders* 26:S275.
- Tominaga N, Kosaka N, Ono M, Katsuda T, Yoshioka Y, Tamura K, Lotvall J, Nakagama H, Ochiya T. 2015.** Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood–brain barrier. *Nature Communications* 6(1):6716 DOI 10.1038/ncomms7716.
- Tsuda Y, Tanikawa C, Miyamoto T, Hirata M, Yodsurang V, Zhang YZ, Imoto S, Yamaguchi R, Miyano S, Takayanagi H, Kawano H, Nakagawa H, Tanaka S, Matsuda K. 2017.** Identification of a p53 target, CD137L, that mediates growth suppression and immune response of osteosarcoma cells. *Scientific Reports* 7(1):10739 DOI 10.1038/s41598-017-11208-x.
- Tu L, Zhao EH, Zhao WY, Zhang ZZ, Tang DF, Zhang YQ, Wang CJ, Zhuang C, Cao H. 2016.** Regulates gastric tumor growth both in vitro and in vivo. *BioMed Research International* 2016:9604257 DOI 10.1155/2016/9604257.
- Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. 2015.** MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature Cell Biology* 17(1):104 DOI 10.1038/ncb3074.
- Wagner KH, Cameron-Smith D, Wessner B, Franzke B. 2016.** Biomarkers of aging: from function to molecular biology. *Nutrients* 8(6):338 DOI 10.3390/nu8060338.
- Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, Benes V, Blake J, Pfister S, Eckstein V, Ho AD. 2008.** Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PLOS ONE* 3(5):e2213 DOI 10.1371/journal.pone.0002213.
- Wang L, Yu Y, Chow DC, Yan F, Hsu CC, Stossi F, Mancini MA, Palzkill T, Liao L, Zhou SL, Xu JM, Lonard DM, O'Malley BW. 2015.** Characterization of a steroid receptor coactivator small molecule stimulator that overstimulates cancer cells and leads to cell stress and death. *Cancer Cell* 28(2):240–252 DOI 10.1016/j.ccell.2015.07.005.
- Wood MJA, O'Loughlin AJ, Lakhil S. 2011.** Exosomes and the blood–brain barrier: implications for neurological diseases. *Therapeutic Delivery* 2(9):1095–1099 DOI 10.4155/tde.11.83.
- Woods AJ, Cohen RA, Pahor M. 2013.** Cognitive frailty: frontiers and challenges. *Journal of Nutrition Health & Aging* 17(9):741–743 DOI 10.1007/s12603-013-0398-8.
- Xia L, Zhang DX, Du R, Pan YL, Zhao LN, Sun SR, Hong L, Liu J, Fan DM. 2008.** miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. *International Journal of Cancer* 123(2):372–379 DOI 10.1002/ijc.23501.
- Yang D, Wang W-Z, Zhang X-M, Yue H, Li B, Lin L, Fu J. 2014.** MicroRNA expression aberration in Chinese patients with relapsing remitting multiple sclerosis. *Journal of Molecular Neuroscience* 52(1):131–137 DOI 10.1007/s12031-013-0138-x.
- Yao X, Yao R, Yi J, Huang F. 2019.** Upregulation of miR-496 decreases cerebral ischemia/reperfusion injury by negatively regulating BCL2L14. *Neuroscience Letters* 696:197–205 DOI 10.1016/j.neulet.2018.12.039.

- Yepes S, Lopez R, Andrade RE, Rodriguez-Urrego PA, Lopez-Kleine L, Torres MM. 2016.** Co-expressed miRNAs in gastric adenocarcinoma. *Genomics* **108**(2):93–101 DOI [10.1016/j.ygeno.2016.07.002](https://doi.org/10.1016/j.ygeno.2016.07.002).
- Yu L, Dawe RJ, Boyle PA, Gaiteri C, Yang JY, Buchman AS, Schneider JA, Arfanakis K, De Jager PL, Bennett DA. 2017.** Association between brain gene expression, DNA methylation, and alteration of ex vivo magnetic resonance imaging transverse relaxation in late-life cognitive decline. *JAMA Neurology* **74**(12):1473–1480 DOI [10.1001/jamaneurol.2017.2807](https://doi.org/10.1001/jamaneurol.2017.2807).
- Zhou Q, Li MZ, Wang XY, Li QZ, Wang T, Zhu Q, Zhou XC, Wang X, Gao XL, Li XW. 2012.** Immune-related microRNAs are abundant in breast milk exosomes. *International Journal of Biological Sciences* **8**(1):118–123 DOI [10.7150/ijbs.8.118](https://doi.org/10.7150/ijbs.8.118).