American Journal of Pharmacology

Research Article
Published: 25 Nov, 2019

Microarray Profile of Long Noncoding RNA and Messenger RNA Expression in an Alzheimer's Mouse Model

Linlin W, Jiang H, Wang Q, Zeng L, Rui L and Zhuorong L**

Department of Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College, China

Abstract

Alzheimer's disease (AD) is progressive and irreversible neurodegenerative disease characterized by a deficiency in memory, language, problem solving, and other cognitive skills. Nevertheless, the mechanisms underlying the etiology of AD are still not very well understood. Long noncoding RNAs (lncRNAs) have been shown to play an important role in biological processes, such as cell differentiation, invasion, and immune responses, as well as synaptic plasticity and memory. In this study, we selected 1-month-old, 3-month-old, 6-month-old, and 9-month-old APP/PS1 transgenic mice, along with their corresponding age-matched Wild-Type (WT) mice, which were used as controls to carry out lncRNA microarray analysis. Our results show that there were 554 upregulated lncRNAs and 346 downregulated lncRNAs in 1-month-old APP/PS1 mice, 635 upregulated lncRNAs and 362 downregulated lncRNAs in 3-month-old APP/PS1 mice, 362 upregulated lncRNAs and 643 downregulated lncRNAs in 6-month-old APP/PS1 mice, and 1145 upregulated lncRNAs and 649 downregulated lncRNAs in 9-month-old APP/PS1 mice, when compared to their respective age-matched WT control mice. In addition, we constructed an lncRNA-messenger RNA (mRNA)-microRNA (miRNA) network and found that the IGF-1, *P2RX7*, TSPO, serpin E1, EGFR, *HMOX1*, and *NFE212* genes may play important role in the development of AD. In conclusion, our study provides a database and the basis for further molecular mechanism and functional studies of lncRNA expression in AD and may provide potential targets for the diagnosis of the disease and novel treatment options.

Keywords: Alzheimer's disease; Long noncoding RNA; Messenger RNA; Microarray; Gene ontology analysis; Kyoto encyclopedia of genes and genomes pathways

OPEN ACCESS

Correspondence:

Rui L, Department of Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, PR China, Tel: + 86-10-67087731;

E-mail: liurui@imb.pumc.edu.cn

Zhuorong L, Department of Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, PR China, Tel: + 86-10-83152017;

E-mail: lizhourong@imb.pumc.edu.cn

Received Date: *26 Sep 2019*

Accepted Date: *18 Nov 2019*

Published Date: *25 Nov 2019*

Citation:

Linlin W, Jiang H, Wang Q, Zeng L, Rui L, Zhuorong L. Microarray Profile of Long Noncoding RNA and Messenger RNA Expression in an Alzheimer's Mouse Model. Am J Pharmacol. 2019; 2(3): 1021.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the accumulation of amyloid-β (Aβ), neurofibrillary tangles consisted of phosphorylated Tau protein, and neuronal loss [1]. Most of the types of dementia that affect adults over 65 years of age are caused by AD, and as the disease advances, affected patients gradually lose their ability to communicate and self-care [2]. Nonetheless, the underpinning mechanisms of AD are poorly understood and currently there are no effective drugs or treatments to prevent the progress of the disease. Given that AD presents a complex genetic architecture with a high expression of Amyloid Precursor Protein (APP), Presenilin1 (*PSEN1*), among other genes, it is of pivotal importance to elucidate gene changes mediating the development of AD [3]. Long noncoding RNAs (lncRNAs) are RNAs, which are more than 200 nucleotides (nt) in length with little or no transcription [4,5]. Once considered to have no function, lncRNAs are now proven to participate in essential biological processes, such as cell differentiation, cell cycle regulation, and inflammatory responses. More recently, lncRNAs have been confirmed to be tightly connected with AD development, through their interaction with chromatin, protein, and other RNAs. Importantly, lncRNAs appear to participate in neural development and maintenance, synaptic plasticity, cognitive function, and memory [6]. The lncRNA, β-site APP cleaving enzyme 1-antisense strand (*BACE1-AS*) and *BACE1* mRNA are two transcriptions of the *BACE1* gene, with *BACE1-AS* being able to regulate the expression of *BACE1* mRNA by forming an RNA duplex. Moreover, *BACE1-AS* was found to be upregulated in AD brains coupled with increased levels of *BACE1* mRNA and Aβ overproduction and accumulation [7]. The Brain Cytoplasmic 200 (BC200) lncRNA, a neuron-specific non-coding RNA, has been shown to be specifically upregulated in the hippocampus of AD patients, and is of vital importance to dendritic protein expression [8]. Notably, overexpression of BC200 lncRNA causes inadequate RNA delivery to the synapses and results in neurodegenerative processes that lead to AD [7,9].

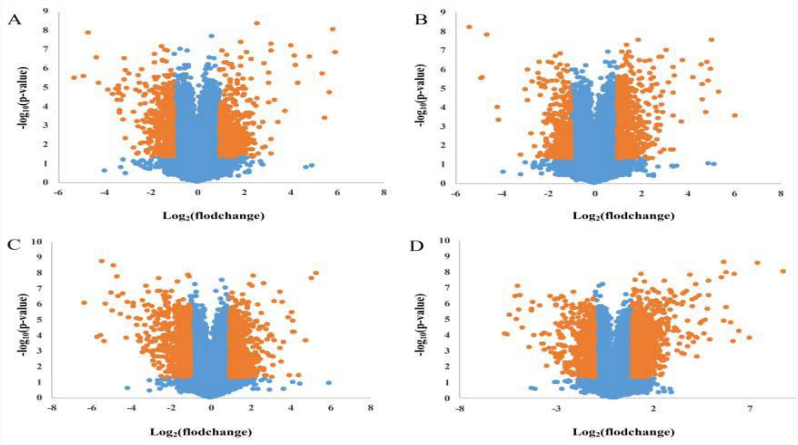

Figure 1: Volcano plots analysis of differentially expressed LncRNAs. (A)1-month-old, (B) 3-month-old, (C) 6-month-old, and (D) 9-month-old APP/PS1 mice, compared to their respective age-matched WT control mice. Red points stand for lncRNAs that significantly changed, presented as the fold change ≥ 2 and with *P*-values ≥ 0.5.

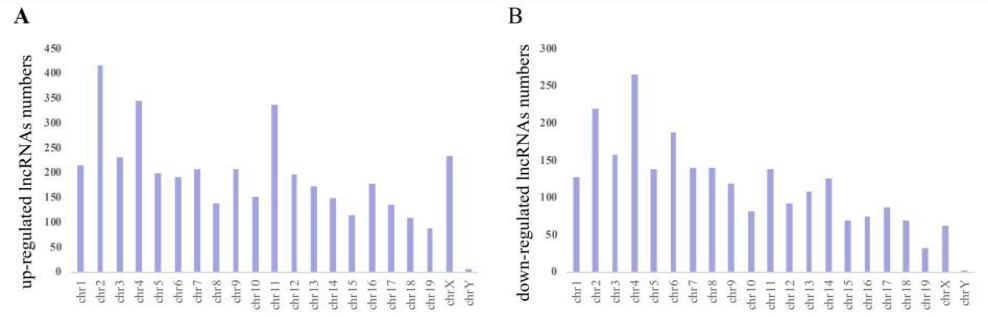

Figure 2: Chromosome location distribution of lncRNAs. (A) Upregulated lncRNAs and (B) downregulated lncRNAs.

In order to identify the changes in lncRNA and mRNA underlying AD process and detect the time-sequencing of altered genes in AD, we employed lncRNA microarray analysis of 1-month-old, 3-month-old, 6-month-old, and 9-month-old APP/PS1 transgenic mice, which express a chimeric mouse/human Amyloid Precursor Protein (APP) bearing the Swedish mutation (APPswe) and a mutant human presenilin1 (PS1ΔE9), known to be associated with early-onset AD, as well as Wild-Type (WT) mice as control [10].

Materials and Methods

Animal and tissue preparation

Heterozygous APPswe/PS1Δ9 transgenic founder mice and age-matched WT littermates were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). In our study, we chose 1-month-old, 3-month-old, 6-month-old, and 9-month-old APP/PS1 mice and their respective age-matched WT control mice to accomplish our analysis. Each age group contained three male mice as well as the WT controls. All the mice were given food and water *ad libitum* and kept in a constant temperature and humidity environment according to the Guide for the Care and Use of Laboratory Animals. The mice were then sacrificed by cervical dislocation and their brains were quickly removed and preserved *via.* Flash freezing in liquid nitrogen.

RNA extraction

The total RNA was extracted from brain tissues using Invitrogen Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, 0.2 ml of chloroform was added per 1 ml of Trizol Reagent and the aqueous phase was transferred into a fresh tube. 0.5 ml of isopropyl alcohol was used per 1 ml of Trizol Reagent for RNA precipitation following RNA washing with 1 ml of 75% ethanol. The RNA pellet was air-dried for 5 min to 10 min. The RNA was then dissolved in RNase-free water by passing the solution a few times through a pipette tip and incubating it for 10 minutes at a temperature of 55°C to 60°C. The resulting RNA concentration was detected using the NanoDrop ND-1000 technology (Thermo Fisher Scientific).

RNA labeling and array hybridization

Our array experiment employed the Agilent Mouse LncRNA Microarray technology, performed according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA, USA). In brief, we used the Quick Amp Labeling Kit (Agilent Technologies) to label Cyanine-3-CTP to cDNAs synthesized from total RNA. Then, the labeled cDNAs were purified by RNeasy Mini Kit (QIAGEN GmBH, Düsseldorf, Germany) and hybridized onto the lncRNA microarray using the Agilent Gene Expression Hybridization Kit (Agilent Technologies). After washing, the Agilent Microarray Scanner was employed to scan the raw data of the microarray, and the Agilent Feature Extraction Software was used to extract the resulting data (Agilent Technologies).

Gene Ontology (GO) and pathway analysis

GO analysis consisted of Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MF); in order to identify the function of the differentially expressed genes. A Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was used to detect biological pathways. A False Discovery Rate (FDR) <0.05 was

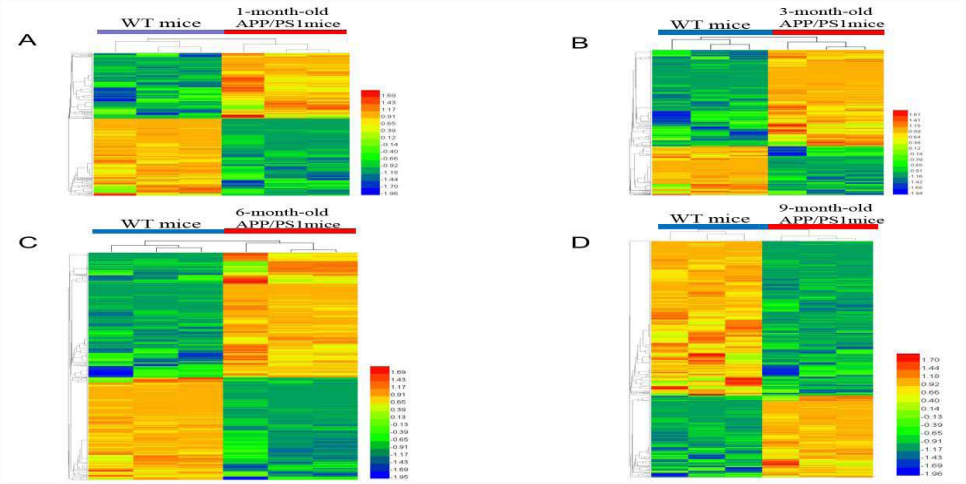

Figure 3: Hierarchical cluster analysis of differentially expressed mRNAs in APP/PS1 mice. (A) 1-month-old, (B) 3-month-old, (C) 6-month-old, and (D) 9-month-old APP/PS1 mice, compared to WT control mice. A Hierarchical cluster showing the differentially expressed genes indicating their differences from blue to red (the bigger the value is, the redder the cluster is).

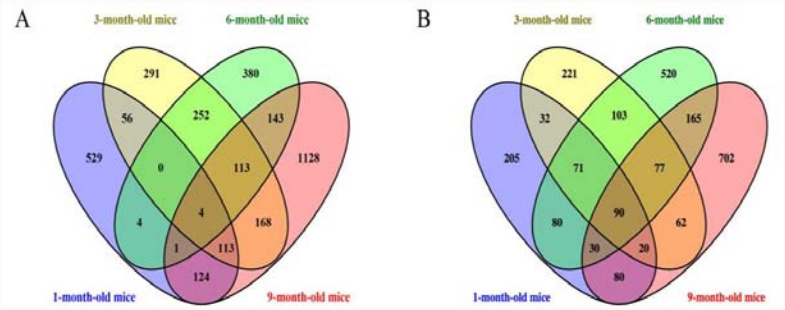

Figure 4: Venn diagram analysis of differentially expressed lncRNAs and mRNAs. (A) Expressed lncRNAs and (B) expressed mRNAs, among 1-month-old, 3-month-old, 6-month-old, and 9-month-old APP / PS1 mice, respectively.

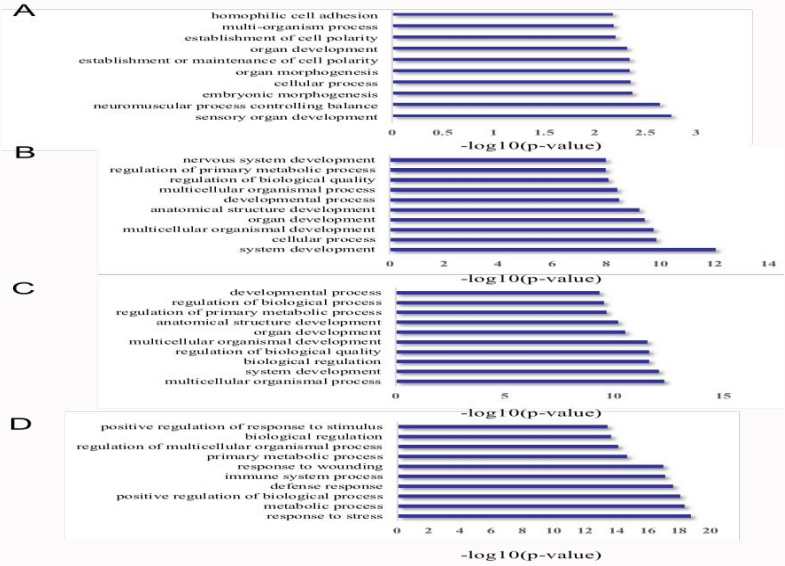

Figure 5: Top 10 GO biological processes of upregulated mRNAs in APP/PS1 mice. (A) 1-month-old, (B) 3-month-old, (C) 6-month-old, and (D) 9-month-old APP/PS1 mice.

considered as significant.

LncRNA-mRNA-miRNA co-expression networks

In order to draw a network between significantly changed lncRNA, mRNA, and miRNA, the Pearson Correlation Coefficient (PCC) statistic measurement was employed to calculate each different expressed lncRNA-mRNA-miRNA pair. A PCC value greater than 0.99 was considered as statistically significant.

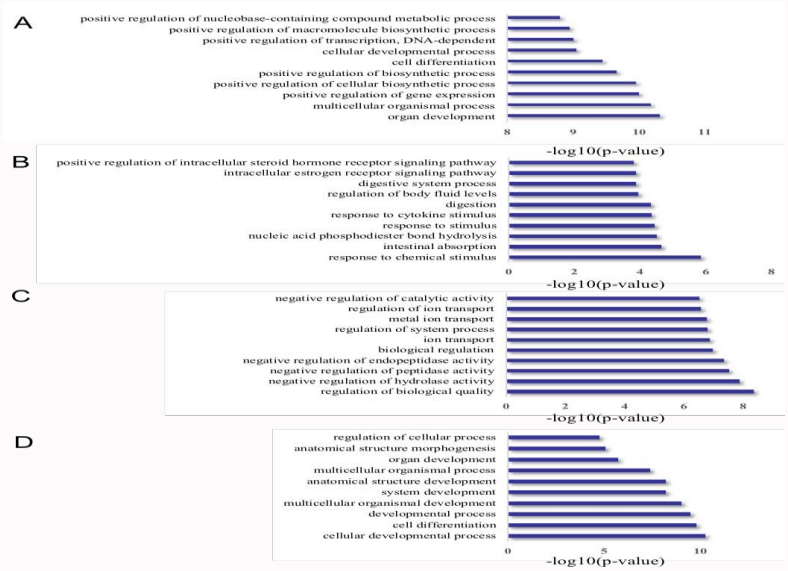

Figure 6: Top 10 GO biological processes of downregulated mRNAs in APP/PS1 mice. (A) 1-month-old, (B) 3-month-old, (C) 6-month-old, and (D) 9-month-old APP/PS1 mice.

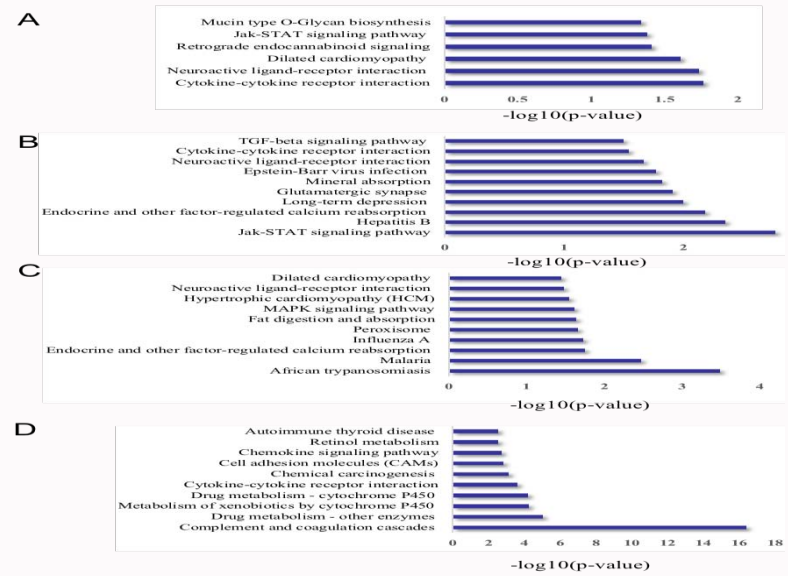

Figure 7: Top 10 KEGG pathways of upregulated mRNAs in APP/PS1 mice. (A) 1-month-old, (B) 3-month-old, (C) 6-month-old, and (D) 9-month-old APP/PS1 mice.

Statistical analysis

Replicate probes were averaged. A Fold Change ≥ 2.0 and a P-value ≤ 0.05 were chosen to distinguish the differentially expressed genes that were significant between the two groups. Finally, a hierarchical clustering was performed to show the distinguishable gene expression profiles among samples.

Results

The different expression profile of lncRNA and mRNA

The Agilent LncRNA Microarray is designed to perform global profiling of human lncRNAs and protein-coding transcripts with a capacity to detect approximately 30,586 lncRNAs and 26,109 coding transcripts. Using a Volcano plot, we were able to demonstrate the different expression profiles of lncRNAs as shown in Figure 1 (fold change ≥ 2, P-value ≤ 0.05). Our results showed 554 upregulated lncRNAs and 346 downregulated lncRNAs in the 1-month-old APP/PS1 mice, when compared to the age-matched WT mice that were used as controls. Moreover, the expression of 635 lncRNAs increased, whereas 362 lncRNAs decreased in the 3-month-old APP/PS1 mice, when compared to their WT counterparts. Similarly, the expression of 362 lncRNAs was elevated, but reduced in 643 lncRNAs in the 6-month-old APP/PS1 mice, while the expression of 1145 lncRNAs was augmented, but diminished in 649 lncRNAs in the 9-month-old APP/PS1 mice, when compared to their WT controls (Tables 1-4). The distribution of the differentially expressed lncRNAs is shown in Figure 2. Our data showed that significant expression changes were found in 619 mRNAs from APP/PS1 mice aged 1-month and 694 mRNAs from APP/PS1 mice aged 3-months. Prominently altered expression changes were also observed in 1191 mRNAs obtained from the 6-month-old APP/PS1 mice and in 1265 mRNAs obtained

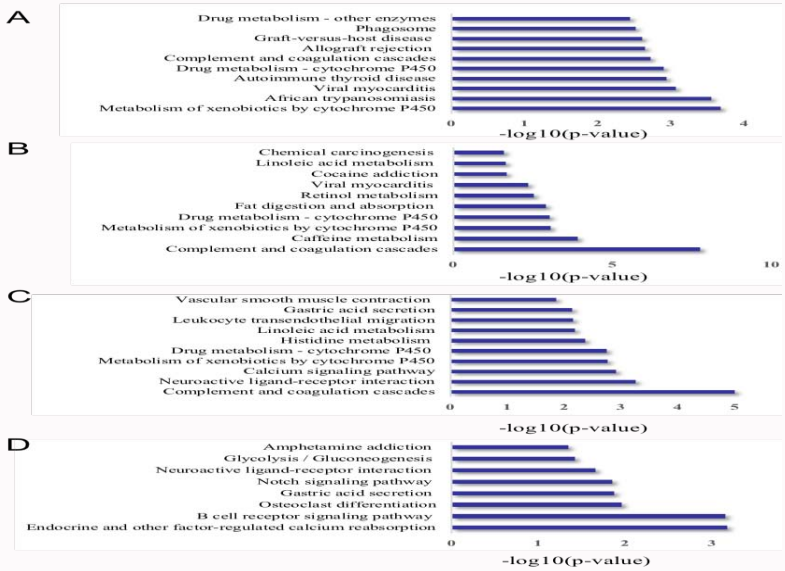

Figure 8: The top 10 KEGG pathways of downregulated mRNAs in APP/PS1 mice. (A) 1-month-old, (B) 3-month-old, (C) 6-month-old, and (D) 9-month-old APP/PS1 mice.

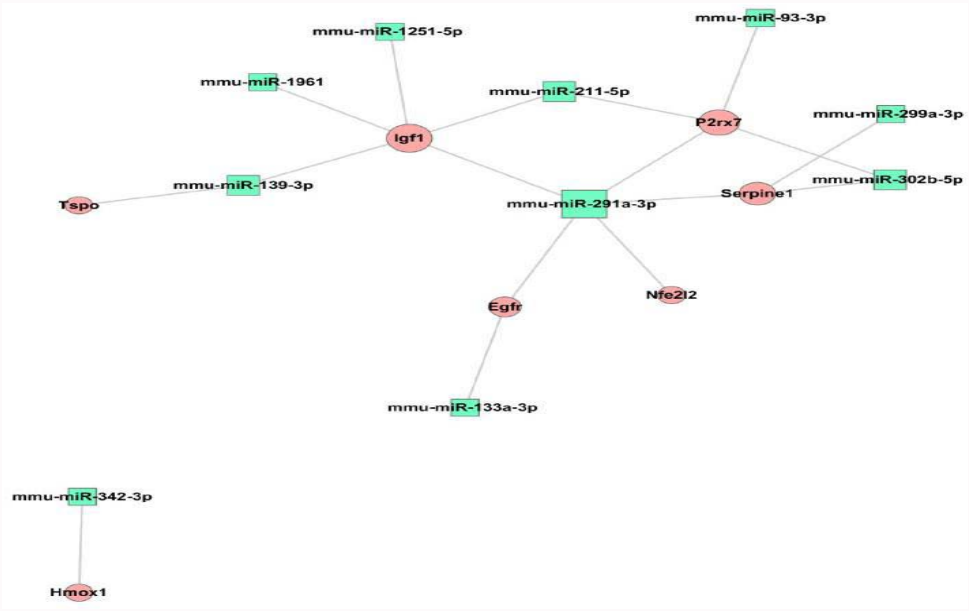

Figure 9: The miRNA-mRNA network of miRNAs and mRNAs expressed at different developmental stages.

from the 9-month-old APP/PS1 mice, when compared to their WT control counterparts (Figure 3 and Tables 5-8). Furthermore, a Venn diagram was utilized to analyze the changes in lncRNA and mRNA expression levels between the different four age groups (Figure 4). Markedly, there were 4 lncRNAs and 90 mRNAs that significantly changed at the different developmental stages of the APP/PS1 mice analyzed.

GO and KEGG pathway analysis

GO pathway analysis was employed to analyze the biological functions of the different expressed mRNAs found. In addition, a KEGG pathway analysis was also performed to identify enriched pathways of the differentially expressed mRNAs obtained. The top 10 biological processes of GO terms and KEGG pathways are described in Figures 5-8. In this study, we focused on the GO biological process terms and found that the variable mRNAs identified, had a key role in organ development, system development, and nervous system development. In addition, our KEGG pathway analysis demonstrated a centralized JAK-ATAT signaling pathway, long-term depression, and neuroactive ligands-receptor interaction.

The LncRNA-mRNA-miRNA co-expression network

In our previous analysis, we detected the expression of miRNAs in 1-month-old, 3-month-old, 6-month-old, and 9-month-old APP/PS1 mice and found that 58 miRNAs were significantly changed [11]. We then established a miRNA-mRNA co-expression network as shown in Figure 9. Connected with the lncRNA data obtained in this study, we then constructed the lncRNA-mRNA-miRNA network as shown in Figure 10.

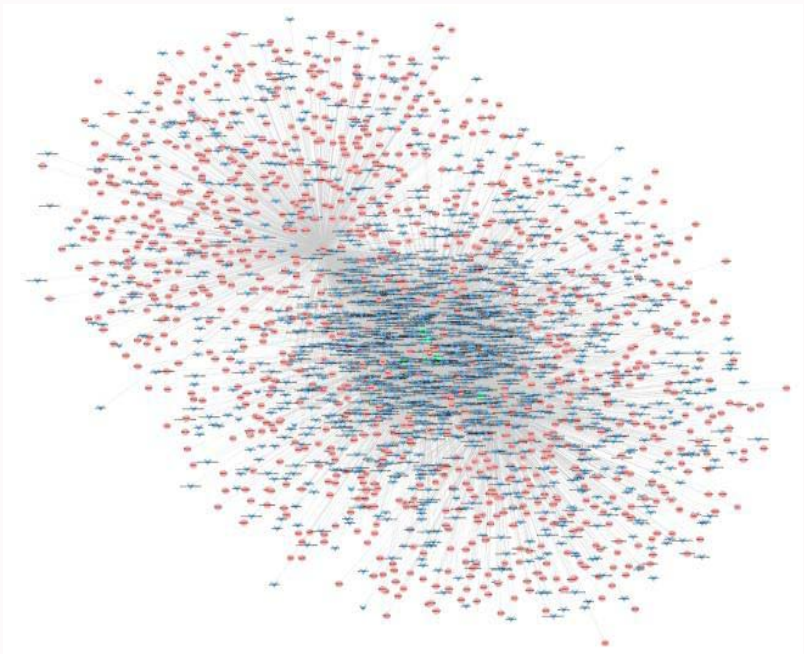

Figure 10: The lncRNA-mRNA-miRNA co-expression network.

Discussion

AD is progressive and irreversible neurodegenerative disease that causes a number of gene expression changes that are still not fully understood [12]. Markedly, our research study is the first report evaluating the expression changes of lncRNA and mRNA in mouse brains aged 1 to 9 months using the APP/PS1 transgenic animal model of AD. Our analysis was performed at different stages of disease progression with the aim to identify miRNA-mRNA-lncRNA networks involved in the processes of AD formation. Cerebral amyloidosis is present as early as 6 weeks old mice, which suggest that 1-month-old APP/PS1 mice not demonstrate any symptom of AD and serve as incubation stage of AD. 3-month-old APP/PS1 mice showed reduced brain and intracranial volumes but did not show cerebral Aβ plaques compared with controls [13]. Pet imaging studies showed that amyloid related signals could be found in the cortex, hippocampus and striatum in 6-month-old APP/PS1 mice and readily recognizable in 9-month-old APP/PS1 mice which suggested that the Aβ symptom became significant with increasing age [14]. The 6-month-old APP/PS1 mice were impaired in short-term memory deficits but not at 3-month age [15]. The 9-month-old APP/PS1 showed hypoactivity in the open field and deficits in spatial memory [16]. Thus, we chose 3-month-old APP/PS1 mice, 6-month-old mice and 9-mongth-old mice to mimics early, medium and late stages of AD symptom and investigated the gene changes.

A total of 3306 lncRNAs were found altered during different age stages of the APP/PS1 transgenic mouse. Notably, there are 4 lncRNAs that continuously changed at every age stage of this mouse model: two of them, AK081040 and ENSMUST00000119471, were significantly upregulated, while the remaining two, AK142586 and ENSMUST00000117578, were significantly downregulated. To the best of our knowledge, there are no previous reports in the literature associating these lncRNAs to the pathology of AD. Importantly, a total of 2458 mRNAs were found altered throughout the four age stages analyzed, with 90 mRNAs presenting significant changes during the progression of AD, of which some were shown to be closely associated with AD, such as DNA methyltransferase 1 (*DNMT1*). *DNMT1* is a vital methyltransferase enzyme mediating epigenetic functions that influence cell processes, such as cell proliferation and invasion [17]. Several studies have now proven that the hypermethylation of the APP gene in the hippocampus and cortex is linked to the overproduction and accumulation of Aβ [18]. In addition, DNA methylation could regulate the expression of genes, such as *BACE1* and PS1, and thus modulate Aβ related processes as well [19]. Combined with the miRNA microarray data, we were able to construct a miRNA-mRNA network. Our results demonstrated that there are 10 miRNAs and 7 mRNAs that significantly change in the APP/PS1 transgenic mouse. Particularly, miRNAs are well known to regulate the expression of mRNA during post-transcription processes [20]. It is, therefore, speculated that these miRNAs might play important roles in the development of AD through regulating the differential expression patterns of mRNAs.

Notably, the Insulin-like Growth Factor 1 (IGF-1) mRNA that significantly increased in APP/PS1 mice aged 9-months is the target of miR-139-3p, miR-1961, miR-1251-5p, miR-291a-3p, and miR-211-5p miRNAs. The IGF-1 protein is of vital importance in brain development during embryogenesis, as well as during the process of aging, when circulating IGF-1 and brain IGF-1 receptors decline [21]. An insufficiency of IGF-1 can be also found in cognitive deficiencies and dementia [22]. IGF-1 has been also shown to inhibit Aβ-triggered cell death by activating PI3K/Akt signaling pathways and thereby inhibiting the expression of the P53 Upregulated Modulator of Apoptosis (PUMA) protein, thus facilitating Aβ clearance from the brain to the circulating system [23]. Based on this, the regulation of IGF-1 expression by miRNAs may be a potential target of AD. The P2X purinoceptor 7 (*P2RX7*) is the mRNA of the P2X7 receptor (*P2X7R*). *P2X7R*s are ATP-gated, non-selected channels, and key regulators of the inflammasome molecular complex [24,25]. Notably, inhibition of *P2X7R* in transgenic mice expressing a mutant form of the human APP was found to reduce amyloid plaques in the hippocampus [26].

Additionally, *P2X7R* is a target of the miRNAs, miR-93-3p, miR-211-5p, miR-291a-3p, and miR-302b-5p, and might play an important role during the late stages of AD.

The Epidermal Growth Factor Receptor (EGFR) belongs to the receptor tyrosine kinases family and its activation can trigger the activity of various signaling pathways involved in cell proliferation, differentiation, and survival [27]. Moreover, the Plasminogen Activator Inhibitor-1 (PAI-1) is a transcription product of the Serpin E1 gene. PAI-1 inhibits the tissue Plasminogen Activator (tPA) and urokinase-type Plasminogen Activator (uPA), which function to excite the conversion of plasminogen to plasmin, the key protease that cleaves Aβ [28]. Markedly, PAI-1 has been shown to be significantly reduced in the brain of AD patients, when compared to healthy controls [29]. The Translocator Protein (TSPO) is involved in mediating cell apoptosis by regulating the generation of Reactive Oxygen Species (ROS) and the release of cytochrome C. The inhibition of TSPO may in turn, delay the aging progress and decrease inflammation [30]. *HMOX1* gene and *Nef212* gene were initially found linked with AD.

In our analysis, we constructed an lncRNA-mRNA-miRNA network on the basis of the observed changes in the expression of miRNA-mRNA networks. IGF-1 is the competing endogenous RNA (ceRNA) to *P2RX7* and TSPO by targeting the same miRNA and *P2RX7* is the ceRNA to the Serpin E1 gene. The mRNA-circled lncRNA can also play a role through competitive binding. Considerably, these lncRNAs, mRNAs, and miRNAs may counteract each other in order to modulate the processes underlying the development of AD.

Naturally, there is a limitation in our research. For instance, the sample size used in our analysis is restricted and a higher number of samples are needed to validate our results. Additionally, more experiments such as RNA interference (RNAi) and RNA immunoprecipitation sequencing (RNAis) might be employed to better elucidate the molecular mechanisms and biological functions underlying the changes in lncRNAs and mRNAs levels observed here. Nevertheless, our study demonstrates compelling changes to lncRNA and mRNA levels during different stages of AD development. Particularly, the seven mRNAs identified in our analysis, are likely to play a precise role in AD progression and may be investigated as targets for the development of effective AD treatments.

In summary, lncRNA and mRNA microarray analyses were performed in different-stage AD mouse model brain, and through bioinformatics analysis, an lncRNA-mRNA-miRNA network was constructed. Many pathological genes, such as IGF-1, *P2RX7*, TSPO, Serpin E1, EGFR, *HMOX1*, and *NFE212* were shown plentiful altered expressions and conserved in evolution with possible target correlation to AD. Thus, our study provided a clue to understanding the pathological hallmarks and looking for effective therapeutic targets based on the altered lncRNA level of AD, which may be suggested to act as "markers" to distinguish the pathological changes of this disease.

Supplementary Materials

We provided the items of differently expressed lncRNAs in supplementary tables: Table 1 for 1-month-old APP/PS1, Table 2 for 3-month-old APP/PS1, Table 3 for 6-month-old APP/PS1, and Table 4 for 9-month-old APP/PS1 mice compared with their corresponding age-matched WT mice, respectively.

Similarly, we provided the items of differently expressed mRNAs in supplementary tables: Table 5 for 1-month-old APP/PS1, Table 6 for 3-month-old APP/PS1, Table 7 for 6-month-old APP/PS1, and Table 8 for 9-month-old APP/PS1 mice compared with their corresponding age-matched WT mice, respectively.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (No. 81673411 and U1803281), China; the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2018RC350013), China; and Chinese Academy Medical Sciences (CAMS) Innovation Fund for Medical Science (2017-I2M-1-016), China.

References

1. Ulep GM, Saraon KS, McLea S. Alzheimer disease. J Nurse Pract. 2018;14(3):129-35.
2. Alzhermer's Association. 2018 Alzheimer's disease facts and figures, Alzheimer's Dement. 2018;14(3):367-429.
3. Grøntvedt GR, Schröder TN, Sando SB, White L, Bråthen G, Doeller CF. Alzheimer's disease. Curr Biol. 2018;28(11):R645-9.
4. Shi C, Zhang L, Qin C. Long non-coding RNAs in brain development, synaptic biology, and Alzheimer's disease. Brain Res Bull. 2017;132:160-9.
5. Wang LK, Chen XF, He DD, Li Y, Fu J. Dissection of functional lncRNAs in Alzheimer's disease by construction and analysis of lncRNA-mRNA networks based on competitive endogenous RNAs. Biochem Biophys Res Commun. 2017;485(3):569-76.
6. Wu P, Zuo X, Deng H, Liu X, Liu L, Ji A. Roles of long noncoding RNAs in brain development, functional diversification and neurodegenerative diseases. Brain Res Bull. 2013;97:69-80.
7. Liu T, Huang Y, Chen J, Chi H, Yu Z, Wang J, et al. Attenuated ability of BACE1 to cleave the amyloid precursor protein via silencing long noncoding RNA BACE1AS expression. Mol Med Rep. 2014;10(3):1275-81.
8. Li H, Zheng L, Jiang A, Mo Y, Gong Q. Identification of the biological affection of long noncoding RNA BC200 in Alzheimer's disease. Neuroreport. 2018;29(13):1061-7.
9. Sosińska P, Mikuła-Pietrasik J, Książek K. The double-edged sword of long non-coding RNA: The role of human brain-specific BC200 RNA in translational control, neurodegenerative diseases, and cancer. Mutat Res Rev Mutat Res. 2015;766:58-67.
10. Shen Z, Lei J, Li X, Wang Z, Bao X, Wang R. Multifaceted assessment of the APP/PS1 mouse model for Alzheimer's disease: Applying MRS, DTI, and ASL. Brain Res. 2018;1698:114-20.
11. Wang LL, Min L, Guo QD, Zhang JX, Jiang HL, Shao S, et al. Profiling microRNA from Brain by Microarray in a Transgenic Mouse Model of Alzheimer's Disease. Biomed Res Int. 2017;2017:8030369.
12. Guerreiro R, Hardy J. Genetics of Alzheimer's disease. Neurotherapeutics. 2014;11(4):732-7.
13. Delatour B, Guégan M, Volk A, Dhenain M. *In vivo* MRI and histological evaluation of brain atrophy in APP/PS1 transgenic mice. Neurobiol Aging. 2006;27(6):835-47.
14. Poisnel G, Dhilly M, Moustié O, Delamare J, Abbas A, Guilloteau D, et al. PET imaging with [18F] AV-45 in an APP/PS1-21 murine model of amyloid plaque deposition. Neurobiol Aging. 2012;33(11):2561-71.
15. Lagadec S, Rotureau L, Hémar A, Macrez N, Delcasso S, Jeantet Y, et al. Early temporal short-term memory deficits in double transgenic APP/PS1 mice. Neurobiol Aging. 2012;33(1):203.e1-11.
16. Ferguson SA, Sarkar S, Schmued LC. Longitudinal behavioral changes in the APP/PS1 transgenic Alzheimer's disease model. Behav Brain Res.

2013;242:125-34.

17. Bai J, Zhang X, Hu K, Liu B, Wang H, Li A, et al. Silencing DNA methyltransferase 1 (DNMT1) inhibits proliferation metastasis and invasion in ESCC by suppressing methylation of RASSF1A and DAPK. Oncotarget. 2016;7(28):44129-41.
18. West RL, Lee JM, Maroun LE. Hypomethylation of the Amyloid Precursor Protein Gene in the brain of an Alzheimer's disease patient. J Mol Neurosci. 1995;6(2):141-6.
19. Fuso A, Seminara L, Cavallaro RA, D'Anselmi F, Scarpa S. S-adenosylmethionine/homocysteine cycle alterations modify DNA methylation status with consequent deregulation of PS1 and BACE and beta-amyloid production. Mol Cell Neurosci. 2005;28(1):195-204.
20. Fransquet PD, Ryan J. Micro RNA as a potential blood-based epigenetic biomarker for Alzheimer's disease. Clin Biochem. 2018;58:5-14.
21. Frater J, Lie D, Bartlett P, McGrath JJ. Insulin-like Growth Factor 1 (IGF-1) as a marker of cognitive decline in normal ageing: A review. Ageing Res Rev. 2018;42:14-27.
22. Angelini A, Bendini C, Neviani F, Bergamini L, Manni B, Trenti T, et al. Insulin-like growth factor-1 (IGF-1): Relation with cognitive functioning and neuroimaging marker of brain damage in a sample of hypertensive elderly subjects. Arch Gerontol Geriatr. 2009;49(Suppl1):5-12.
23. Hou X, Jin Y, Chen J, Hong Y, Luo D, Yin Q, et al. IGF-1 protects against Abeta25-35-induced neuronal cell death *via* inhibition of PUMA expression and Bax activation. Neurosci Lett. 2017;637:188-94.
24. Sperlágh B, Illes P. P2X7 receptor: An emerging target in central nervous system diseases. Trends Pharmacol Sci. 2014;35(10):537-47.
25. Johnson JE. Numb and Numblike control cell number during vertebrate neurogenesis. Trends Neurosci. 2003;26(8):395-6.
26. Diaz-Hernandez JI, Gomez-Villafuertes R, León-Otegui M, Hontecillas-Prieto L, Del Puerto A, Trejo JL, et al. *In vivo* P2X7 inhibition reduces amyloid plaques in Alzheimer's disease through GSK3 and secretases. Neurobiol Aging. 2012;33(8):1816-28.
27. Komposch K, Sibilia M. EGFR Signaling in Liver diseases. Int J Mol Sci. 2015;17(1):E30.
28. Soeda S, Koyanagi S, Kuramoto Y, Kimura M, Oda M, Kozako T, et al. Anti-apoptotic roles of plasminogen activator inhibitor-1 as a neurotrophic factor in the central nervous system. Thromb Haemost. 2008;100(6):1014-20.
29. Barker R, Kehoe PG, Love S. Activators and inhibitors of the plasminogen system in Alzheimer's disease. J Cell Mol Med. 2012;16(4):865-76.
30. Repalli J. Translocator Protein (TSPO) Role in aging and Alzheimer's disease. Curr Aging Sci. 2014;7(3):168-75.