Brain Research 1646 (2016) 139–151

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

# Expression of microRNA-34a in Alzheimer's disease brain targets genes linked to synaptic plasticity, energy metabolism, and resting state network activity

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ARTICLE INFO

*Article history:*
Received 15 March 2016
Received in revised form
6 May 2016
Accepted 13 May 2016
Available online 25 May 2016

*Keywords:*
Alzheimer's disease
MicroRNAs
MiR-34a
Oxidative phosphorylation
Glycolysis
Synaptic plasticity
Resting state network

ABSTRACT

Polygenetic risk factors and reduced expression of many genes in late-onset Alzheimer's disease (AD) impedes identification of a target(s) for disease-modifying therapies. We identified a single microRNA, miR-34a that is over expressed in specific brain regions of AD patients as well as in the 3xTg-AD mouse model. Specifically, increased miR-34a expression in the temporal cortex region compared to age matched healthy control correlates with severity of AD pathology. miR-34a over expression in patient's tissue and forced expression in primary neuronal culture correlates with concurrent repression of its target genes involved in synaptic plasticity, oxidative phosphorylation and glycolysis. The repression of oxidative phosphorylation and glycolysis related proteins correlates with reduced ATP production and glycolytic capacity, respectively. We also found that miR-34a overexpressed neurons secrete miR-34a containing exosomes that are taken up by neighboring neurons. Furthermore, miR-34a targets dozens of genes whose expressions are known to be correlated with synchronous activity in resting state functional networks. Our analysis of human genomic sequences from the tentative promoter of miR-34a gene shows the presence of NFκB, STAT1, c-Fos, CREB and p53 response elements. Together, our results raise the possibilities that pathophysiology-induced activation of specific transcription factor may lead to increased expression of miR-34a gene and miR-34a mediated concurrent repression of its target genes in neural networks may result in dysfunction of synaptic plasticity, energy metabolism, and resting state network activity. Thus, our results provide insights into polygenetic AD mechanisms and disclose miR-34a as a potential therapeutic target for AD.



## 1. Introduction

Late-onset Alzheimer's disease (AD) accounts for more than 90% of AD cases and is the most common form of dementia (Querfurth and LaFerla, 2010). Currently, there are no effective treatments for AD. Recently genome-wide association studies (GWAS) of AD have identified that nine novel risk factor genes (Seshadri et al., 2010) and their expression may be involved in AD (Allen et al., 2012). Furthermore, genome-wide transcriptome study indicate that many important genes necessary for energy metabolism and synapse activity are downregulated in AD (Liang et al., 2008). fMRI and PET imaging studies of AD patient's resting state network (RSN) revealed that with progressing AD severity, a decrease in glucose and oxygen metabolism correlates with increased Clinical Dementia Rating Scores (Brier et al., 2012). Although genes that cause loss of functional connections in intra- and inter-network resting state with AD progression remain unknown, many genes are recently reported to be correlated with functional RSN (Richiardi et al., 2015). Thus, in order to develop new therapeutic strategies to treat AD, there is a need to identify novel molecular target(s) that concomitantly dysregulate expression of multiple genes involved in synaptic plasticity, energy metabolism, and RSN. A single 22-nt non-coding microRNA targets many genes and concomitantly down regulates multiple biological pathways by repressing mRNA translation/or degradation (Bartel, 2009). As such, we sought to identify expression of novel microRNAs in AD brains that maximizes the number of target genes possibly involved in cognitive function. Although regulation of memory and synaptic plasticity via a single microRNAs has been previously reported in mice (Gao et al., 2010; Griggs et al., 2013), the causal effects of a single microRNAs involving polygenetic mechanism of AD are not known.

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http://dx.doi.org/10.1016/j.brainres.2016.05.026
0006-8993/

## 2. Results

### 2.1. Expression levels of selected miRNAs in human and mouse AD brains

Because a single microRNA targets many genes and concomitantly down regulates multiple biological pathways, we sought to identify expression of novel microRNAs in AD brains that maximizes the number of targets possibly involved in cognitive function. To this end, we selected five miRNAs after data mining of dozens of miRNA target genes from five different miRNA target gene databases (Vlachos and Hatzigeorgiou, 2013). These five miRNAs were hsa-miRNA-34a, 146a,-9, -132, and -15 whose target predicted by all five databases collectively have the potential to deregulate genes associated with synaptic plasticity, neuronal survival, energy metabolism, amyloid precursor protein (APP) metabolism, Aβ elimination, and RSN. We next quantified expression of these miRNAs *via* qRT-PCR from temporal cortex (TC), frontal cortex (FC), and cerebellum (CB) of subjects with AD and compared them to healthy age-matched control (AMC) (Table 1). Expression analysis showed that in the TC, miR-34a expression was significantly increased compared to AMC (Fig. 1A, left panel), but not in the FC (Fig. 1B), and CB (Fig. 1C). When differentiated by Braak and Braak (B&B) stages, miR-34a expression was significantly increased in the TC of both stage III and stage VI (Fig. 1A, middle panel) but not in the FC or CB. Pearson's test show significant positive correlation between miR-34a level and B&B stage as well as amyloid angiopathy in the TC (Fig. 1A, right panel), but not in FC or CB. Expression level the other four miRNAs in the same tissues are shown in Table 2. Expression of miR-146a was also increased in the TC and was B&B stage VI specific. There was no significant increase in expression of miR-132, miR-9, and miR-15a in TC region of AD. In case of the FC, changes in expression of miR-146a, -132, -9, and -15a were not significant. But B&B stage specific increased expression of miR-9in frontal cortex was significant. In the CB region, expression changes of miR-146a, -132 and -15a were not significant. miR-9 expression in CB region was significantly reduced.

Although genetics of AD and familial AD (FAD) are different, converging evidences from PIB-PET (Bateman et al., 2012), MRI (Reiman et al., 2012) and the resting state functional connectivity MRI (Thomas et al., 2014) imaging studies suggest that late onset AD and familial AD have similar disease processes. In order to further characterize the similarities between the two AD types, we profiled miRNA expression in brain regions from familial AD model of transgenic AD mice (3xTg AD). Expression of miR-34a was significantly increased in TC of 3xTg AD mice by 12-month of age (Fig. 1D) but not in the FC and CB (Fig. 1D) as we have seen in human AD cases. miR-34a expression was also increased in the hippocampus of these mice (Fig. 1D) as reported in human AD (Zovoilis et al., 2011). Expression levels of the other four miRNAs in FC, TC, and CB, and in the hippocampus were not significantly altered compare to the age matched control (Table 3).

### 2.2. Functional analysis of the repression of target proteins in increased miR-34a AD brain regions

It has been reported that miR-34c, a family member of miR-34, targets the SIRT1 gene, and elevated level of miR-34c correlates with memory impairment in AD mice (Agostini et al., 2011a). Also, it has been shown that ectopic expression of miR-34a in primary cortical neurons affects dendritic spine morphology and the reduction of amplitude/frequency of miniature excitatory synaptic current (mEPSC) (Agostini et al., 2011b). Scanning all genes targeted by both miR-34 family and miR-146a, we selected miR-34a as a novel candidate based on its potential to target the maximal number of genes involved in brain energy metabolism, synaptic plasticity, and synchronous activity in RSN (Table 4). To assess expression of miR-34a targeted proteins in AD brains, we measured protein levels of selected synaptic plasticity related genes, VAMP2, SYT1, HCN1, NR2A and GLUR1; oxidative phosphorylation related genes, NDUFC2, SDHC, UQCRB, UQCRQ, and COX10; and genes in the glycolytic pathway, H6PD, PFK1, and PFK2 in the TC and CB of the same AD and AMC samples used in our microRNA

**Table 1**
Human subject list with pathological scores.

| ID | Age | Sex | APOE | PMD | Diagnoses | B&B stage | Atherosclerosis[a] | Amyloid angiopathy[a] | Other pathology |
|---|---|---|---|---|---|---|---|---|---|
| 591 | 78 | F | 33 | 5.5 | Normal CERAD 1A | 1 | | | |
| 673 | 80 | F | 33 | 1.2 | Normal CERAD 1A | 1 | Mild | | |
| 981 | 74 | F | 24 | 4.7 | Normal CERAD 1B | 3 | | | Metastatic Carcinoma |
| 1035 | 72 | F | 33 | 30 | Normal CERAD 1B | 2 | | | |
| 196 | 75 | M | 33 | 18.9 | Normal CERAD 1B | 1 | | | |
| 381 | 78 | M | n/a | 9.8 | Normal CERAD 1A | 1 | | | |
| 837 | 75 | M | 22 | 29 | Normal CERAD 1A | 1 | | | Lacunes |
| 543 | 72 | F | 34 | 3 | Normal CERAD 1A | 1 | | | |
| 707 | 80 | M | 33 | 4.3 | Normal CERAD 1B | 1 | | | |
| 795 | 81 | M | 34 | 7.2 | Normal CERAD 1B | 1 | | | Lacunes |
| 357 | 75 | M | 33 | 3 | AD | 3 | | Mild | |
| 347 | 77 | M | 33 | 16.8 | AD | 3 | | | |
| 601 | 76 | F | 33 | 10.5 | AD | 3 | | | |
| 602 | 78 | M | 34 | 0.8 | AD | 3 | Mild | | |
| 731 | 76 | F | 44 | 5.5 | AD | 3 | Mild | Mild | |
| 1054 | 75 | F | 34 | 20.1 | AD | 3 | Severe | Mild | |
| 1052 | 71 | M | 33 | 25.2 | AD | 6 | | Mild | |
| 1068 | 77 | M | 44 | 25.4 | AD | 6 | Mild | Mild | |
| 1560 | 74 | F | 34 | 20.5 | AD | 6 | | | |
| 360 | 75 | F | 33 | 13.3 | AD | 6 | Mild | | |
| 1664 | 79 | F | n/a | 20.8 | AD | 6 | | Moderate | Infarcts |
| 49 | 75 | F | 34 | 24 | AD | 6 | Severe | | |
| 254 | 75 | M | 34 | 2.6 | AD | 6 | Moderate | | |

[a] Pathological scores were analyzed by a pathologist for atherosclerosis and amyloid angiopathy using CERAD guidelines and NIA-Reagan criteria.