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Predicted Regulatory Pathways for Long Noncoding RNA-SNHG7 via

miR-34a and its Targets in Alzheimer's Disease

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Abstract

The long noncoding RNA (LncRNA) SNHG7 (small nuclear RNA host gene 7) is a new type of lncRNA, whose function as an oncogene has been studies. However, the role of SNHG7 in Alzheimer's disease (AD) remains to be revealed. In this study, the expression data of SNHG7 in AD brains (n=7) and normal brains (n = 5) were collected and calculated. The results indicated that low SNHG7 level in AD was correlated with high expressed microRNA-34a (miR-34a), and the decreased expression of Bcl2 (B-cell lymphoma 2), a target of miR-34a. Moreover, previous studies have shown that miR-34a and Bcl2 are involved into the development and progression of AD. Bioinformatic analysis predicted that SNHG7 has miR-34a binding sites. Therefore, it suggests that SNHG7 may regulate neuronal survive in AD brain through miR-34a/Bcl2 axis. In addition, miR-34a may regulate post-transcriptionally estimated hundred mRNAs. Using several bioinformatics tools, we can predict the regulatory pathways that SNHG7 participates in through miR-34a and its targets in AD. These findings indicate that the down-regulated lncRNA SNHG7 in AD may reduce the inhibition of miR-34s' function, then increase its function and decrease miR-34a target signals, thereby joining in the regulatory network of AD.

Key words: SNHG7, miR-34a, Alzheimer's Disease, Bcl2, apoptosis

Introduction

Alzheimer's disease (AD) is the most common degenerative disease of the central nervous system in the elderly, accounting for an estimated 60–80% of dementia cases [1]. The main clinical phenotypes are cognitive dysfunction, memory loss and personality changes. In fact, there are 50 million AD patients worldwide, and its incidence doubles every five years after the age of 65 years [2]. The principal pathologies seen in AD are amyloid beta (Aβ)-contained plaques and neurofibrillary tangles (NFTs) containing hyperphosphorylated tau protein. This may occur through a variety of mechanisms, including excitotoxicity, generation of reactive oxygen species (ROS), inflammatory responses, and apoptotic and cell death [3, 4]. It was recently found that microRNAs (miRNAs) are involved in cancer and neurodegenerative diseases including AD development and progression [5-7]. MiRNAs are 20-24 nucleotides in length and function to post-transcriptionally inhibit mRNA translation, each miRNA targeting 100 or more mRNAs. Several miRNAs including miR-34a [8] have been found to play critical roles in AD pathogenic pathways, including apoptosis, inflammation, and impaired neuronal function.

Currently, the authors have identified that at least 90% of the entire human genome is transcribed as noncoding RNAs(ncRNAs) with no protein-coding capacity. The ncRNA could be divided into several groups according to the transcript size, including miRNAs and long noncoding RNAs (lncRNAs, >200 nts) [9]. Accumulating evidence indicated that these ncRNAs have important roles in regulating gene expression in development, physiology, and pathology, including cancer [10, 11]. Some miRNAs and lncRNAs have been recognized as tumor

suppressor or oncogene during cancer development and progression. However, the study of lncRNA in AD just starts although the miRNAs studies have more reports in this field.

SNHG7 is a new lncRNA located on chromosome 9q34.3, which is 2,176 bp long [12]. LncRNA-SNHG7 (SNHG7) has been demonstrated that the expression of SNHG7 is significantly increased in tumors and cancer cells, including colorectal, breast, prostate and gastric origin [13]. SNHG7 was enhanced in tumor cells for tumor proliferation and survival [14]. According to previous studies, SNHG7 modulates tumorigenesis and cancer progression by acting as a competing endogenous RNA. Importantly, Deng Y. et al. reported that SNHG7 can sponge tumor suppressive miRNAs including miR-34a [15] and regulate its downstream signaling pathways [13]. However, the expression and function of SNHG7 in AD remains to be unexposed so far.

MiR-34a is involved in several neurological diseases, including AD, although previously, miR-34a was identified as a classic tumor-suppressive miRNA, which is related to tumor proliferation, cell cycle, and apoptosis [16]. In AD, the expression of miR-34a is upregulated [8], which is opposite to the expressions in some cancers [15]. The downstream pathways of miR-34a have been studied for a while in AD development, but the regulatory pathways for miR-34a itself is still unclear. In this study, the authors found that there may be an interaction between miR-34a and SNHG7 via the bioinformatic analysis. Therefore, they analyzed AD clinical significance of SNHG7; investigated the expression of SNHG7, miR-34a, and its target Bcl2; and predict the network of SNHG7 through miR-34a and its targets in AD.

Databases and Methods

Databases

A small RNA-seq dataset from six Alzheimer disease and seven control brains were downloaded from EMBL-EBI website (https://www.ebi.ac.uk/arrayexpress/, dataset EGEOD-63501). In this dataset, total RNA was isolated from human post-mortem autopsy whole brain, and deep sequencing of small RNAs was performed as described by Hafner et al.[17]. Here, counts of two major sequences for miR-34a TGGCAGTGTCTTAGCTGGTTGT and TGGCAGTGTCTTAGCTGGTTGTT, differing only in the latter containing one extra final T residue, were combined.

The profiles of mRNA transcript from whole brain tissues of seven AD patients and five controls were downloaded from EMBL-EBI website (https://www.ebi.ac.uk/arrayexpress/, dataset E-MEXP-2280). The expression levels of SNHG7, Bcl2, and SHNK3 were obtained from this dataset. The gene expression in the heatmap was also obtained from this dataset.

Methods

To perform gene annotation and pathway analysis, we have used the gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Database for Annotation, Visualization and Integrated Discovery (DAVID, v6.8), and g:Profiler (http://biit.cs.ut.ee/gprofiler/gost).

The results are analyzed by the Statistical Package for the SigmaPlot 14 (Systat Software Inc.). Paired t-tests or unpaired t-tests tests were used to compare the two groups $p < 0.05$ was considered statistically significant. After calculating the Z-score of associated genes, the heatmap is generated by MeV (4.9.0) bioinformatic software.

Results

The expression of lnc-RNA-SNHG7 and miR-34a in AD patients

To discover the clinical significance of SNHG7 in AD patients, the authors collected the microarray data from AD brain tissues and normal brain tissues (n = 7 and 5, respectively). The expressions of SNHG7 indicated that the SNHG7 level was decreased significantly in AD brains when compared with the normal brains (Fig. 1A).

The expression of miR-34a in AD was examined using a small RNA-seq dataset from six AD brains and seven control brains were downloaded from EMBL-EBI. In this dataset, total RNA was isolated from human post-mortem autopsy whole brain, and deep sequencing of small RNAs was performed as described by Hafner et al. [17]. We found that miR-34a expression was increased ~three folds in human AD brains compared to controls. (Fig. 1B). Here, we combined two major sequences of miR-34a as its counts. This result is consistent to the previous report although testing methods are different [18].

Bioinformatic analysis (http:// starbase.sysu.edu.cn/index.php) revealed putative complementary sequences for miR-34a in human SNHG7, and predicted miR-34a binding sites were found by Deng Y. et al.(Fig. 1C) [15]. Because the expression of SNHG7 was found to be decreased in AD tissues, they suspected that SNHG7 upregulated the function of miR-34a in AD. These data implicated that down regulated SNHG7 and upregulated miR-34a might predict the poor clinical outcome of AD patients. As an inhibitor of gene expression, reduced SNHG7 might release the inhibition of the expression or function of miR-34a in AD; while in cancer, miR-34a is a classic tumor suppressor, which is often down regulated in cancer cells correlated with SNHG7 up-regulation.
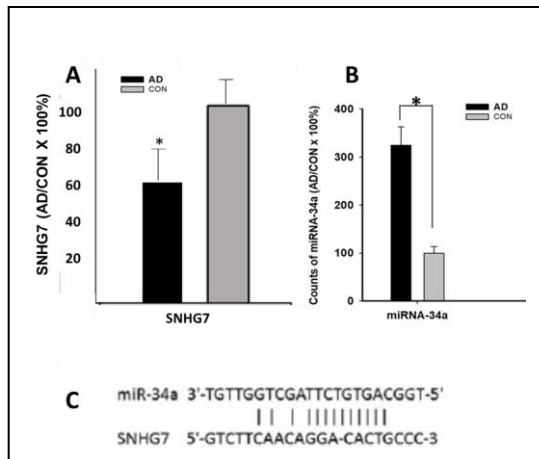

Fig. 1. The expression of SNHG7 and miR-34a in AD brains.

A) The expression of SNHG7 was down regulated in human AD brains (n=7) compared to normal brain (n=5), *p=0.029 (AD/COM x 100%).

B) The expression of miR-34a in human AD brains is shown as the counts of miR-34a (AD/CON x100%) (n=6, *P<0.05 vs control).

C) Bioinformatic analysis predicted that SNHG7 harbored miR-34a binding sites [15].

The predicted targets of SNHG7 via miR-34a in AD

MiR-34a may regulate neuronal death through Bcl2-related pathways. Bioinformatics methods examining the Bcl2 3'- UTR predict that Bcl2 may be a direct target of miR-34a [19, 20]. Bcl2 is an essential component of the intrinsic apoptotic death pathways, acting as an inhibitor of cytochrome C release and mitigating other effects on mitochondria promoted by pro-apoptotic Bcl family members such as Bax [21-24]. Decreased expression of Bcl2 increases susceptibility to apoptotic death, and increased Bcl2 expression increases cell survival following exposure to various injurious stimuli.

To explore cell death mechanisms in AD, we examined the expression of Bcl2 and SHANK3. The mRNA transcript profiles from whole brain tissues of seven AD patients and five

controls were downloaded from EMBL-EBI website (https://www.ebi.ac.uk/arrayexpress/, dataset is E-MEXP-2280), and the expression of Bcl-2 and SHANK3 expression were examined in the same dataset of SNHG7. Bcl2 expression was found to be decreased significantly in human AD brains compared to control brains (Fig. 2A), and it might cause the increase of neuron apoptosis in AD brain. However, there was no significant change in the expression of SHANK3.
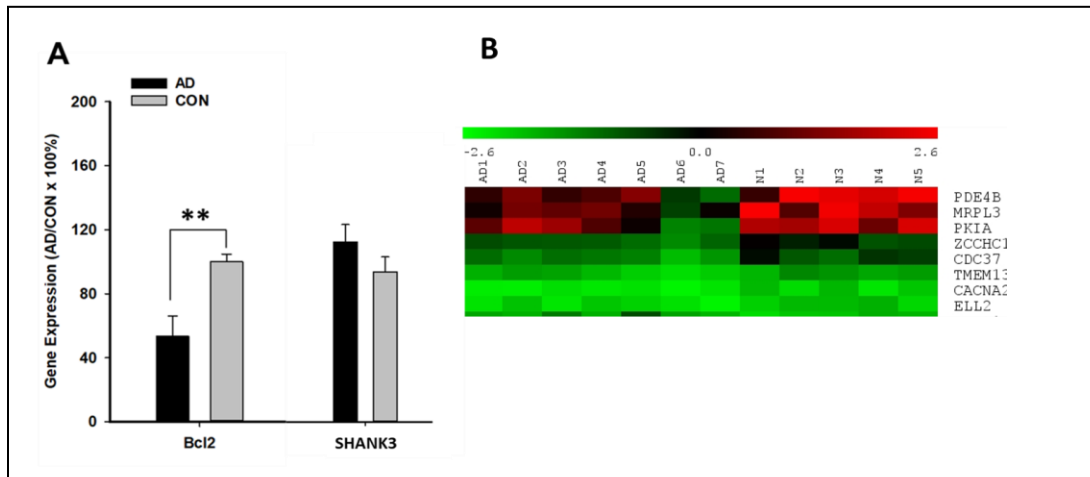

Fig. 2. The expression of Bcl2 and other miR-34A target genes.

A) Bcl2 expression was decreased in human AD brains compared to control brains (n=7, p<0.01 vs controls). There was no significant change in the expression of SHANK3 in human AD brains vs control brains.

B) The heat map shows the expression levels of SNHG7/miR-34a AD associated genes, including PDE4B, MRPL3, PKIA, ZCCHC, CDC37, THEM13, CACNA2D1, and ELL2. In the upper part of the picture, from -2.6 to 2.6 of the Z-score of gene expression, the heatmap is shown from green to red.

Using the TargetScan7.2 program, we found there are 178 predicted targets for miR-34a. Then, using profiling data from AD patients and normal controls, statistic calculating, and clustering, we scanned all these genes and selected eight genes as SNHG7 regulatory targets via miR-34a or as the SNHG7/miR-34a associated AD genes, because those eight genes were significantly decreased in AD brains compared to normal controls. Those eight gene IDs are PDE4B, MRPL3, PKIA, ZCCHC, CDC37, THEM13, CACNA2D1, and ELL2. Their expression heat map was shown in Fig 2B.

The predicted regulatory network of SNHG7 via miR-34a

Given a set of down-regulated genes in AD in the above studies, we performed GO analysis and KEGG analysis, or as a non-enrichment analysis, to find out which GO terms and pathways are under-represented using annotations for that gene set. Using the g:Profile analysis tool and setting the significant threshold to 0.01 (combined GO and KEGG analysis), we found a KEGG pathway (KEGG:00230) and several GO terms (data not shown); however, these terms seem

not specific to the principal pathologies of AD. Using DAVID (v6.8) analysis, we found one disease cluster, one KEGG pathway, and several functional annotations (not shown).

A generalized anxiety disease (GAD) cluster was found in the DAVID analysis by using the SNHG7/miR-34a associated down-regulated genes in AD. The GAD cluster showed in Table 1. Epidemiological-, genetic-, and neuropathological-based evidence continue to suggest that AD is a particularly heterogeneous disorder [8]. Previous studies have shown that the frequency of GAD or generic symptoms of anxiety in AD to range 5% to 70% [25, 26]. Recently, it has found that increasing symptoms of anxiety and depression may be linked to an increase in beta-amyloid proteins, a hallmark characteristic of Alzheimer's disease [27]. Therefore, SNHG7/miR-34a network may be associated with AD development, but it seems not a direct link to the AD principal pathologies including Aβ-contained plaques and NFTs containing p-tau pathologies.

Table 1. A cluster associated with SNHG7/miR-34A in AD

Functional Annotation Clustering

Help and Manual

Current Gene List: List_1
Current Background: Homo sapiens
11 DAVID IDs
⊞ Options   **Classification Stringency** Medium ▾
[Rerun using options] [Create Sublist]

1 Cluster(s)   🖫 Download File

Annotation Cluster 1	Enrichment Score: 1.55	G		Count	P_Value	Benjamini
☐ GAD_DISEASE	Echocardiography	RT	▬▬▬	3	1.7E-2	5.2E-1
☐ GAD_DISEASE	Blood Pressure	RT	▬▬▬	3	2.4E-2	5.2E-1
☐ GAD_DISEASE	Tobacco Use Disorder	RT	▬▬▬▬	5	5.7E-2	8.3E-1

[12 terms] were not clustered.

Discussion

By bioinformatic analysis and profiling from AD patients, we predicted that down regulated SNHG7 in AD decreased the inhibition of miR-34a, so it increased the expression or the function of miR-34a. Through the targets of miR-34a, down-regulated SNHG7 may increase neuron apoptosis through miR-34a/Bcl2 axis in AD. Using several bioinformatics tools, we can predict the regulatory pathways that SNHG7 are involved via miR-34a in AD. This manuscript is a bioinformatic study, so the function of SNHG7 on AD needs to be approved by experiments.

According the studies in cancer, the function of SNHG7 was suspected that it negatively regulates the expression of miR-34a, as a gene expressive inhibitor. In several cancers, the miR-34a expression were decreased; while the expression of SNHG7 showed the converse results [28]. Second, knockdown of SNHG7 in two osteosarcoma (OS) cell lines MG63 and SaOS2 restored the miR-34a expression levels. Third, the inhibiting function of SNHG7 was inhibited when using the miR-34A mimic with mutant miR-34a binding sites [15]. Therefore, we think the function of SNHG7 in AD is similar to the function in cancer, which is the inhibition of miR-34a expression. The reduced SNHG7 expression might release the inhibition of miR-34a expression and function, causing the increased miR-34a expression and function and down-regulation of miR-34a target genes including Bcl2. This is just a bioinformatic prediction for SNHG7, so everything needs to be proven, including the binding site to miR-34a and the regulation of miR-34a in neuronal cells.

There should be some unexposed mechanisms of the SNHG7/miR-34a regulatory network in AD. For example, miR-34a may also regulate neuronal function and apoptosis through SHANK3, which is also its target gene. The SHANK family of scaffolding proteins contain multiple domains which support extensive protein-protein interactions, including ankyrin (ANK) repeats, and are key players for both synapse formation and the modulation of synaptic transmission and synaptic plasticity [29]. Extra-neural levels of Aβ peptide oligomers have been shown to strongly correlate with the loss of synaptic SHANK3, disruption of synaptic function, and with the severity of cognitive impairment [30, 31]. However, we didn't find any significant changes in the expression of SHANK3 in these AD samples compared to normal controls, as shown in Fig. 2A.

SNHG7/miR-34a axis may regulate other neurological disorders except AD. This article is the first report that the SNHG7/miR-34a axis may also regulate GAD disease. In Table 1, blood pressure and tobacco use disorder as associated phenotypes of GAD are involved. Importantly, these phenotypes are also deeply relevant with AD development [32, 33]. In addition, we found that miR-34a was involved in traumatic brain injury and ischemic stroke (data not shown), so there is possible that SNHG7/miR-34a axis might be also involved in the brain injury. Therefore, SNHG7/miR-34a axis could be a novel therapeutic target for AD and other neurological disorders.

Reference

1. Alzheimer's, A., *2016 Alzheimer's disease facts and figures.* Alzheimers Dement, 2016. **12**(4): p. 459-509.
2. Fan, L., C. Mao, X. Hu, S. Zhang, Z. Yang, Z. Hu, et al., *New Insights Into the Pathogenesis of Alzheimer's Disease.* Front Neurol, 2019. **10**: p. 1312.
3. Shi, J., *Traumatic Brain Injury May Lead to Alzheimer's Disease and Related Dementia.* On J Neur & Br Disord, 2020. **4**(3): p. 363-365.
4. Butterfield, D.A. and D. Boyd-Kimball, *Oxidative Stress, Amyloid-beta Peptide, and Altered Key Molecular Pathways in the Pathogenesis and Progression of Alzheimer's Disease.* J Alzheimers Dis, 2018. **62**(3): p. 1345-1367.
5. Shi, J., Huang, S., *Predicting and Identifying Human Glioblastoma MiRNA Targets Using RRSM and qPCR Methods.* The Grant Medical Journals, 2017. **02**(02): p. 7-12.
6. Shi, J., *Regulatory networks between neurotrophins and miRNAs in brain diseases and cancers.* Acta Pharmacol Sin, 2015. **36**(2): p. 149-57.
7. Amakiri, N., A. Kubosumi, J. Tran, and P.H. Reddy, *Amyloid Beta and MicroRNAs in Alzheimer's Disease.* Front Neurosci, 2019. **13**: p. 430.
8. Jaber, V.R., Y. Zhao, N.M. Sharfman, W. Li, and W.J. Lukiw, *Addressing Alzheimer's Disease (AD) Neuropathology Using Anti-microRNA (AM) Strategies.* Mol Neurobiol, 2019. **56**(12): p. 8101-8108.
9. Jandura, A. and H.M. Krause, *The New RNA World: Growing Evidence for Long Noncoding RNA Functionality.* Trends Genet, 2017. **33**(10): p. 665-676.
10. Yoon, J.H., K. Abdelmohsen, and M. Gorospe, *Functional interactions among microRNAs and long noncoding RNAs.* Semin Cell Dev Biol, 2014. **34**: p. 9-14.
11. Kallen, A.N., X.B. Zhou, J. Xu, C. Qiao, J. Ma, L. Yan, et al., *The imprinted H19 lncRNA antagonizes let-7 microRNAs.* Mol Cell, 2013. **52**(1): p. 101-12.

12.   Imanishi, T., T. Itoh, Y. Suzuki, C. O'Donovan, S. Fukuchi, K.O. Koyanagi, et al., *Integrative annotation of 21,037 human genes validated by full-length cDNA clones.* PLoS Biol, 2004. **2**(6): p. e162.

13.   Bian, Z., W. Ji, B. Xu, W. Huang, J. Jiao, J. Shao, et al., *The role of long noncoding RNA SNHG7 in human cancers (Review).* Mol Clin Oncol, 2020. **13**(5): p. 45.

14.   She, K., H. Yan, J. Huang, H. Zhou, and J. He, *miR-193b availability is antagonized by LncRNA-SNHG7 for FAIM2-induced tumour progression in non-small cell lung cancer.* Cell Prolif, 2018. **51**(1).

15.   Deng, Y., F. Zhao, Z. Zhang, F. Sun, and M. Wang, *Long Noncoding RNA SNHG7 Promotes the Tumor Growth and Epithelial-to-Mesenchymal Transition via Regulation of miR-34a Signals in Osteosarcoma.* Cancer Biother Radiopharm, 2018. **33**(9): p. 365-372.

16.   Slabakova, E., Z. Culig, J. Remsik, and K. Soucek, *Alternative mechanisms of miR-34a regulation in cancer.* Cell Death Dis, 2017. **8**(10): p. e3100.

17.   Hafner, M., N. Renwick, M. Brown, A. Mihailovic, D. Holoch, C. Lin, et al., *RNA-ligase-dependent biases in miRNA representation in deep-sequenced small RNA cDNA libraries.* RNA, 2011. **17**(9): p. 1697-712.

18.   Zhao, Y., V.R. Jaber, A. LeBeauf, N.M. Sharfman, and W.J. Lukiw, *microRNA-34a (miRNA-34a) Mediated Down-Regulation of the Post-synaptic Cytoskeletal Element SHANK3 in Sporadic Alzheimer's Disease (AD).* Front Neurol, 2019. **10**: p. 28.

19.   Misso, G., M.T. Di Martino, G. De Rosa, A.A. Farooqi, A. Lombardi, V. Campani, et al., *Mir-34: a new weapon against cancer?* Mol Ther Nucleic Acids, 2014. **3**: p. e194.

20.   Li, L., L. Yuan, J. Luo, J. Gao, J. Guo, and X. Xie, *MiR-34a inhibits proliferation and migration of breast cancer through down-regulation of Bcl-2 and SIRT1.* Clin Exp Med, 2013. **13**(2): p. 109-17.

21.   Mrschtik, M. and K.M. Ryan, *Lysosomal proteins in cell death and autophagy.* FEBS J, 2015. **282**(10): p. 1858-70.

22.   Strasser, A. and D.L. Vaux, *Viewing BCL2 and cell death control from an evolutionary perspective.* Cell Death Differ, 2018. **25**(1): p. 13-20.

23.   Obulesu, M. and M.J. Lakshmi, *Apoptosis in Alzheimer's disease: an understanding of the physiology, pathology and therapeutic avenues.* Neurochem Res, 2014. **39**(12): p. 2301-12.

24.   Shi, J., L.F. Parada, and S.G. Kernie, *Bax limits adult neural stem cell persistence through caspase and IP3 receptor activation.* Cell Death Differ, 2005. **12**(12): p. 1601-12.

25.   Chemerinski, E., G. Petracca, F. Manes, R. Leiguarda, and S.E. Starkstein, *Prevalence and correlates of anxiety in Alzheimer's disease.* Depress Anxiety, 1998. **7**(4): p. 166-70.

26.   Starkstein, S.E., R. Jorge, G. Petracca, and R.G. Robinson, *The construct of generalized anxiety disorder in Alzheimer disease.* Am J Geriatr Psychiatry, 2007. **15**(1): p. 42-9.

27.   Metti, A.L., J.A. Cauley, A.B. Newman, H.N. Ayonayon, L.C. Barry, L.M. Kuller, et al., *Plasma beta amyloid level and depression in older adults.* J Gerontol A Biol Sci Med Sci, 2013. **68**(1): p. 74-9.

28.   Sun, X., T. Huang, Z. Liu, M. Sun, and S. Luo, *LncRNA SNHG7 contributes to tumorigenesis and progression in breast cancer by interacting with miR-34a through EMT initiation and the Notch-1 pathway.* Eur J Pharmacol, 2019. **856**: p. 172407.

29.   Lee, Y., H. Kang, B. Lee, Y. Zhang, Y. Kim, S. Kim, et al., *Integrative Analysis of Brain Region-specific Shank3 Interactomes for Understanding the Heterogeneity of Neuronal Pathophysiology Related to SHANK3 Mutations.* Front Mol Neurosci, 2017. **10**: p. 110.

30.   Rajendran, L. and R.C. Paolicelli, *Microglia-Mediated Synapse Loss in Alzheimer's Disease.* J Neurosci, 2018. **38**(12): p. 2911-2919.

31.   Pham, E., L. Crews, K. Ubhi, L. Hansen, A. Adame, A. Cartier, et al., *Progressive accumulation of amyloid-beta oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice*

is accompanied by selective alterations in synaptic scaffold proteins. FEBS J, 2010. **277**(14): p. 3051-67.

32. Arvanitakis, Z., A.W. Capuano, M. Lamar, R.C. Shah, L.L. Barnes, D.A. Bennett, et al., *Late-life blood pressure association with cerebrovascular and Alzheimer disease pathology.* Neurology, 2018. **91**(6): p. e517-e525.

33. Vnukova, M., R. Ptacek, J. Raboch, and G.B. Stefano, *Decreased Central Nervous System Grey Matter Volume (GMV) in Smokers Affects Cognitive Abilities: A Systematic Review.* Med Sci Monit, 2017. **23**: p. 1907-1915.