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MicroRNA-132 promotes neurons cell apoptosis and activates Tau phosphorylation by targeting GTDC-1 in Alzheimer's disease

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Abstract. – OBJECTIVE: Alzheimer's disease (AD) is a neurodegenerative disorder with limited success in the prognosis of patients worldwide. The microRNA (miRNA) technology shows an encouraging trend in the therapeutics of AD. The study aimed to investigate the role of miR-132 and its underlying mechanism involving neuronal apoptosis and Tau phosphorylation in the pathophysiology of AD.

MATERIALS AND MATERIALS: Frozen postmortem brain samples and neurons cells were used as the specimens. The expression of miR-132 was assessed in AD and mild-cognitive (MCI) group by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Then, miR-132 mimic, ASO-miR-132, corresponding controls were transfected into necrotic cells. Flow cytometry was used to detect apoptosis in necrotic cells. qRT-PCR, western blot, and immunoprecipitation were used to quantify and identify apoptosis-related proteins (Bax and Bcl-2), Tau phosphorylation, CDK-5, GTDC-1 levels in necrotic cells. Furthermore, Dual-Luciferase reporter assay was used to predict a direct target of miR-132.

RESULTS: The expression of miR-132 was significantly higher in patients with MCI and AD compared to the normal group. Overexpression of miR-132 induced neuronal apoptosis by increasing Bax and decreasing Bcl-2 and also dysregulated phosphorylation of Tau, Rb, Histone H1, and CDK-5 expressions. Besides, GTDC-1 was identified as a direct target gene of miR-132. However, GTDC-1 markedly reversed the effect of miR-132 on cell apoptosis and Tau phosphorylation.

CONCLUSIONS: MiR-132 plays an important role in the pathogenesis of AD *via* regulation of cell apoptosis and GTDC-1/CDK-5/Tau phosphorylation signaling mechanism. It may be a potential therapeutic target in patients with AD.

Key Words:
Alzheimer's disease, miR-132, Apoptosis, Tau protein, GTDC-1.

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease in the elderly people. It is characterized by neuropathological hallmarks that include amyloid plaques and neurofibrillary tangles, neuropil threads, and dystrophic neurites[1]. The abnormally hyperphosphorylated Tau protein gets aggregated into bundles of filaments and is seen as intraneuronal neurofibrillary tangles of paired helical filaments (PHF)[2]. Neurofibrillary degeneration of abnormally hyperphosphorylated Tau proteins is a significant component of AD pathogenesis[3]. Tau proteins are not limited only to AD, which is also considered as a vital component of various other neurodegenerative diseases, such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD), and argyrophilic grain disease[4].

Normally, the function of microtubule-associated protein (MAP) is to stabilize the microtubules for promoting neurite growth[5,6]. Tau isoforms with three (3R) or four (4R) microtubule binding repeats arise from exclusion or inclusion of exon 10 of the human MAPT gene on chromosome 17. The 3R Tau isoforms predominate in the developing brain contrary to those in the adult brain where both 3R and 4R Tau are expressed in equal amounts[7]. Any mutation in the MAPT gene causes frontotemporal dementia with parkinsonism and is characterized by intraneuronal aggregates of hyperphosphorylated Tau[8-10]. Fur-

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thermore, Tau kinases and phosphatases could cause Tau dysfunction and contribute to neurodegenerative disease pathophysiology[11].

MicroRNAs (miRNAs) are a class of small non-coding transcripts, which have been reported to regulate different biological processes in several neurodegenerative diseases[12]. Recent studies[13,14] have shown that the levels of some specific miRNAs such as miR-34a, miR-125b, and miR-146a were elevated during the disease progression. The up-regulation of these different miRNAs could lead to cell cycle entry (CCE) in cultured post-mitotic neurons. Absalon et al[15] revealed that the up-regulation of miR-26b in AD could activate CCE, Tau phosphorylation, and apoptosis in post-mitotic neurons. CCE suggests neuronal cell cycle regulatory failure leading to cell death is a significant component in AD pathogenesis[16]. Furthermore, miRNA profiling and qRT-PCR analysis have directly proven the correlation between miRNA and AD[17].

MiR-132 is located on chromosome 17 in human, which has been reported to exert anti-inflammatory and memory-promoting functions in the nervous system[18]. Wong et al[19] sugg[illegible] that miR-132 could control neuronal surv[illegible] directly modulate PTEN/AKT/FOXO3 sig[illegible]g axis in AD neurodegeneration. Additionally, i[illegible] 132 has been found consistently down-regula[illegible] in AD, and the levels correlate[illegible]he sever[illegible] ty of Tau pathology. Spillanti[illegible] a[illegible]owed *in vitro* that miR-132 could r[illegible]te Tau[illegible]ernative splicing by targeting po[illegible]midin[illegible] ing protein 2 (PTBP2)[illegible] mi[illegible]12[illegible]monstr[illegible]d *in vivo* that miR-12[illegible]as dire[illegible]mplicated by endogenous Tau [illegible]ssion, phosp[illegible]ation, and aggregation. H[illegible]w[illegible]e effects o[illegible]R-132 on the pathophys[illegible]logy i[illegible]nism of AD remain not fully i[illegible]stigated.

In th[illegible]esent study, we a[illegible]d to explore the role [illegible]iR-132[illegible]a potential biomarker in AD an[illegible]nder[illegible]d the possible underlying mechanism[illegible]d in mi[illegible]2 and Tau phosphory[illegible]n me[illegible]d ne[illegible]al apoptosis. The findings [illegible]provi[illegible]eas and strategies for clinical [illegible]tment o[illegible]D.

Materials and Methods

S[illegible]le Collection and Preparation

Frozen human *postmortem* brain specimens were collected from the Harvard Brain Tissue Resource Center. These specimens were divided into two patient groups of 10 mild cognitive impairment (MCI), 10 AD patients, and normal controls. The samples collection was performed in accordance with the poli[illegible]f Brigham and Women's Hospital in[illegible] review board.

Human Cortical Neuron Cultu[illegible]

The human fatal cortical t[illegible]ues of [illegible]ional age of 16 weeks were prov[illegible] by Advan[illegible] science Resources. The[illegible]ues we[illegible]washed [illegible] Dulbecco's Modified [illegible]le's M[illegible]m (DMEM; Gibco, Grand Isla[illegible]d, [illegible], clean[illegible](meninges were re[illegible]d) a[illegible] into 2[illegible]^3 sizes using force[illegible]The tissue[illegible]t[illegible]nized in 0.25% tryp[illegible]o, Grand I[illegible] NY, USA) and was [illegible]bate[illegible]20 min at 37°C with occasional swirling. T[illegible] was washed off from the [illegible]Furthermo[illegible]ml of PR medium w[illegible]sed to wash the tissue twice (DMEM with [illegible] fetal bovin[illegible]erum, 2 mM L-glutamine, 1 [illegible] sodium pyr[illegible]te, and antibiotics). The tissu[illegible]rations w[illegible]performed using 5 × with a 10 [illegible]tic d[illegible]sable pipette. The cell suspension wa[illegible]d using a 70 µm followed by a 40 µm of cell strainer. The cells were centrifuged [illegible]0 rpm for 5 min, the supernatant was re[illegible]ov[illegible]nd the pellet was re-suspended in 1 ml of PR medium. The cells were plated in PR medium and the medium was replaced the next day with a fresh PR medium and with a neurobasal medium on day 5.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from frozen biopsy samples using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Equal portions of the white and grey matter were dissected from temporal lobe and homogenized. TaqMan microRNA Reverse transcription reagents and universal PCR master mix with microRNA Real Time-Polymerase Chain Reaction primers (Applied Biosystems, Foster City, CA, USA) were used for miRNA analysis using qRT-PCR. The mRNA expression analysis was performed using 1 µg of Reverse-Transcribed total RNA with TaqMan Reverse Transcription Reagents (Applied Biosystems N808-0234, Foster City, CA, USA) and gene expression was quantified using SYBR green (Applied Biosystems SYBR Green PCR master mix, Foster City, CA, USA).

3'UTR Luciferase Reporter Assay

3'UTR Luciferase reporter plasmids and miR-132 mimic and its controls were co-transfected in the cells. The cells were harvested at 48 h after transfection and the Luciferase activity was determined with Dual-Luciferase reporter assay system on a luminometer (Berthold, LB 9507, Bad Wildbad, Germany) following the manufacturer's information. The experiments were performed in triplicates. The relative ratio of Renilla Luciferase activity to Firefly Luciferase activity was calculated for each well.

Western Blot

Briefly, the cells were lysed in radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) and protease inhibitor cocktail (Roche, Basel, Switzerland) for 30 min on ice and were cleared by centrifugation. The lysates (20 µg) were boiled (at 100°C) for 5 min in 4X SDS loading buffer. The samples were separated by sodium dodecyl sulfate-polyacrylamid[illegible] electrophoresis (SDS-PAGE) and were[illegible] ferred to a polyvinylidene difluoride (P[illegible]F) membrane. The membranes were treated in[illegible] non-fat milk blocking buffer (Thermo Fi[illegible] er Scientific, Waltham, MA, U[illegible]hen incu[illegible] bated with primary antibod[illegible] at 4°C. Immunoblotting analysis [illegible]used t[illegible]tect the indicated antibodies usi[illegible]mobi[illegible] chemiluminescent H[illegible] s[illegible] (Millipore, Billerica, MA, US[illegible]he ban[illegible]nsities were quantified by de[illegible]metric analy[illegible]sing NIH Image J (Bio-R[illegible]les, CA, U[illegible]. All the experiments [illegible]re pe[illegible]ed in triplicates to represent [illegible]results.

Im[illegible]opr[illegible]itation Assay

Th[illegible]re har[illegible]d at day 7 after transion [illegible]40 h[illegible]uffer (50 mM Tris-HCl, p[illegible], 150[illegible]l, 1 mM EDTA, 0.1% NP-40) [illegible]d protea[illegible]nhibitors. The cell lysates were pr[illegible]as described above. Furthermore, the ly[illegible]00 µg) were incubated with 1 µg of [illegible]mmunoprecipitating antibody for 1 h at 4°C and [illegible] incubated overnight at 4°C with 20 µl of anti[illegible]it-IgG beads (eBioscience, San Diego, CA, USA). The immunoprecipitated complexes were washed (4 ×) with lysis buffer (centrifugation at 1000 g at 4°C for 5 min), recovered in 40 µl 2 × Laemmle buffer with 50 mM fresh dithiothreitol and boiled for 5 min. The samples were loaded onto SDS-PAGE gels and analyzed by immunoblotting.

Apoptosis Assay

Identification and quantificati[illegible] apoptotic cells were performed by flow cyt[illegible]y analysis using Annexin V-fluores[illegible]n isoth[illegible]nate/Propidium Iodide (FITC/P[illegible]poptosis d[illegible] kit (Biosea Biotechnol[illegible] Beijin[illegible] China). cells (100,000 cells/w[illegible]vere s[illegible]d in a 6 well-plate. The treated cell[illegible]e[illegible]shed tw[illegible] and re-suspended wi[illegible]old P[illegible]uffer. T[illegible] adherent and floati[illegible]ells were c[illegible]in[illegible]nd treated accordi[illegible] manufact[illegible] instruction and meas[illegible]d wi[illegible]low cytometer (Beckman Coulter, Fullerton, [illegible]USA) to differentiate apo[illegible]lls (Annexi[illegible]sitive and PI-negati[illegible]rom necrotic cells (A[illegible]nexin-V and PI-pos-i[illegible]).

S[illegible]tical An[illegible]is

I[illegible]tud[illegible] statistical analysis of the data is perfo[illegible]ing SPSS 16.0 (SPSS Inc., Chicago, IL, USA). All the data are expressed as mean [illegible]ard deviation (SD). One-way analysis of [illegible] (ANOVA)/ Student's t-test followed by Duncan's multiple-range test were used to assess the statistically significant differences. A p-value of < 0.05 was considered to be significant.

Results

MiR-132 Was Highly Expressed in AD

The human postmortem brain specimens were divided into three groups of normal, mild cognitive impairment (MCI) and AD, respectively. The expression of miR-132 was assessed using qRT-PCR. As shown in Figure 1, the expression of miR-132 was significantly higher in patients with MCI ($p < 0.05$) and AD ($p < 0.05$) compared to the normal group. Moreover, the expression of miR-132 was markedly higher in patients with AD than those with MCI ($p < 0.001$). These data indicated that high expression of miR-132 existed in AD brain.

MiR-132 Promoted Primary Neuronal Cell Apoptosis

The influence of miR-132 on cell apoptosis was analyzed and the results were shown in Figure 2A-2B. Cell apoptosis was significant-

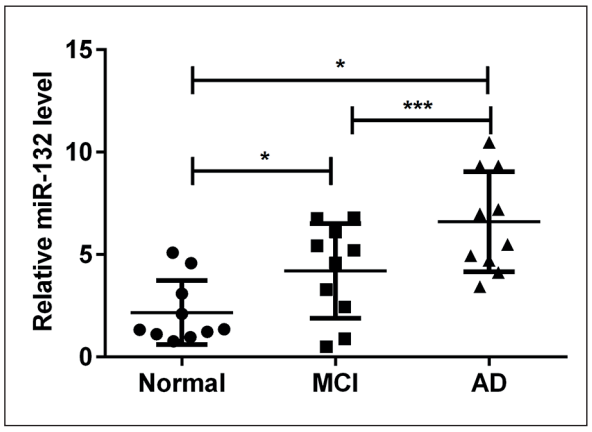


Figure 1. MiR-132 was highly expressed in AD. Thirty human postmortem brain specimens were divided into three groups of normal, MCI and AD, respectively. Then, the relative expression of miR-132 in these three groups was analyzed by qRT-PCR. MiR: microRNA; AD: Alzheimer's disease; MCI: mild cognitive impairment; qRT-PCR: quantitative Real Time-Polymerase Chain Reaction. *$p < 0.05$ and ***$p < 0.001$.

ly increased by the overexpression of miR-132 compared to the control groups ($p < 0.01$). [illegible]trarily, the suppression of miR-132 rem[illegible] decreased cell apoptosis ($p < 0.05$). Fu[illegible]r-more, apoptosis-related factors (Bax and B[illegible] were detected by qRT-PCR and Western b[illegible] respectively. The results show[illegible] the ove[illegible] expression of miR-132 mar[illegible]y p[illegible]oted the expression of Bax ($p < 0.0$[illegible] and in[illegible]ited the expression of Bcl-2 ($p <$ [illegible]). The[illegible] of miR-132 showed t[illegible] opp[illegible]lt of t[illegible]e two factors expres[illegible]s (Figu[illegible]-2D). These data revealed tha[illegible]R-132 may [illegible] primary neuron apopto[illegible]by affecting the [illegible] apoptosis-related fac[illegible]s exp[illegible]ns.

MiR[illegible] Acti[illegible]ed Tau Phosphorylation in[illegible]ary [illegible]rons

Th[illegible] of mi[illegible]2 on phosphorylation [illegible] wer[illegible]lored [illegible] Western blot. The results [illegible]d tha[illegible]ere 3 increasing phosphorylation sites b[illegible]e overexpression of miR-132 (Fi[illegible]2A). Cyclic-dependent kinase-5 (CDK-[illegible] a[illegible]ortant kinase that is involved in [illegible]osphorylation of Tau protein. Therefore, the [illegible]ession of CDK5 was measured by Immunop[illegible]tation (anti-p35/25) and Western blot. The results showed that the overexpression of miR-132 promoted the expression of CDK5 and the accumulation of p35/p25 (Figure 3B). Immunoprecipitation studies also showed that miR-132 promoted the expression of CDK5 and phosphorylation sites (Figure 3C). These results revealed that miR-132 could activate Tau phosphor[illegible]tion in primary neurons.

Glycosyl Transferase Like Do[illegible] Containing -1 (GTDC-1) [illegible]s a Direct Target of MiR-1[illegible]

The HEK-293 cells w[illegible]ased as[illegible] model [illegible] vestigate the target ge[illegible] miR-[illegible] The relationship between 3'UTR [illegible]T[illegible]1 and [illegible]-132 was correlated [illegible] Luc[illegible]e repo[illegible] assay. The results s[illegible]ed that fl[illegible]e intensity of GTDC-1[illegible] reased by [illegible]pression of miR-132 [illegible] incre[illegible]y suppression of miR-132 ($p < 0.01$; Figure 4A-[illegible] However, no significant diff[illegible] were found [illegible]DC-1 mut. In additi[illegible]qRT-PCR and Weste[illegible] blot analysis showed [illegible] expression o[illegible]GTDC-1 was down-regulated [illegible]iR-132 ove[illegible]ression, but up-regulated by m[illegible]32 suppre[illegible]n ($p < 0.01$; Figure 4D and 4E). [illegible] d[illegible]onfirmed that GTDC-1 was a direct t[illegible]iR-132 and was negatively regu[illegible]ted by miR-132.

GTDC-1 Inhibited Neuronal Cell Apoptosis and Tau Phosphorylation

To further explore the effect of GTDC-1 on cell apoptosis and Tan phosphorylation, flow cytometry and Western blot assays were performed. The results showed that GTDC-1 markedly inhibited neuronal cells apoptosis compared to the control group ($p < 0.01$; Figure 5A). Additionally, GTDC-1 decreased the expression of Bax and increased the expression of Bcl-2 (Figure 5B). Furthermore, as shown in Figure 5C, GTDC-1 remarkably inhibited phosphorylation of Tau, Rb, and Histone H1 levels. These results indicated that GTDC-1 could inhibit neuronal cell apoptosis and Tau phosphorylation.

Discussion

AD is a neurodegenerative disease caused by various factors and there is still no effective treatment option for the management of AD[22-23]. The mechanisms involved in the pathogenesis of AD, especially those which lead to neuronal degeneration and neuronal loss, are incomplete and speculative. Therefore, further investigation of

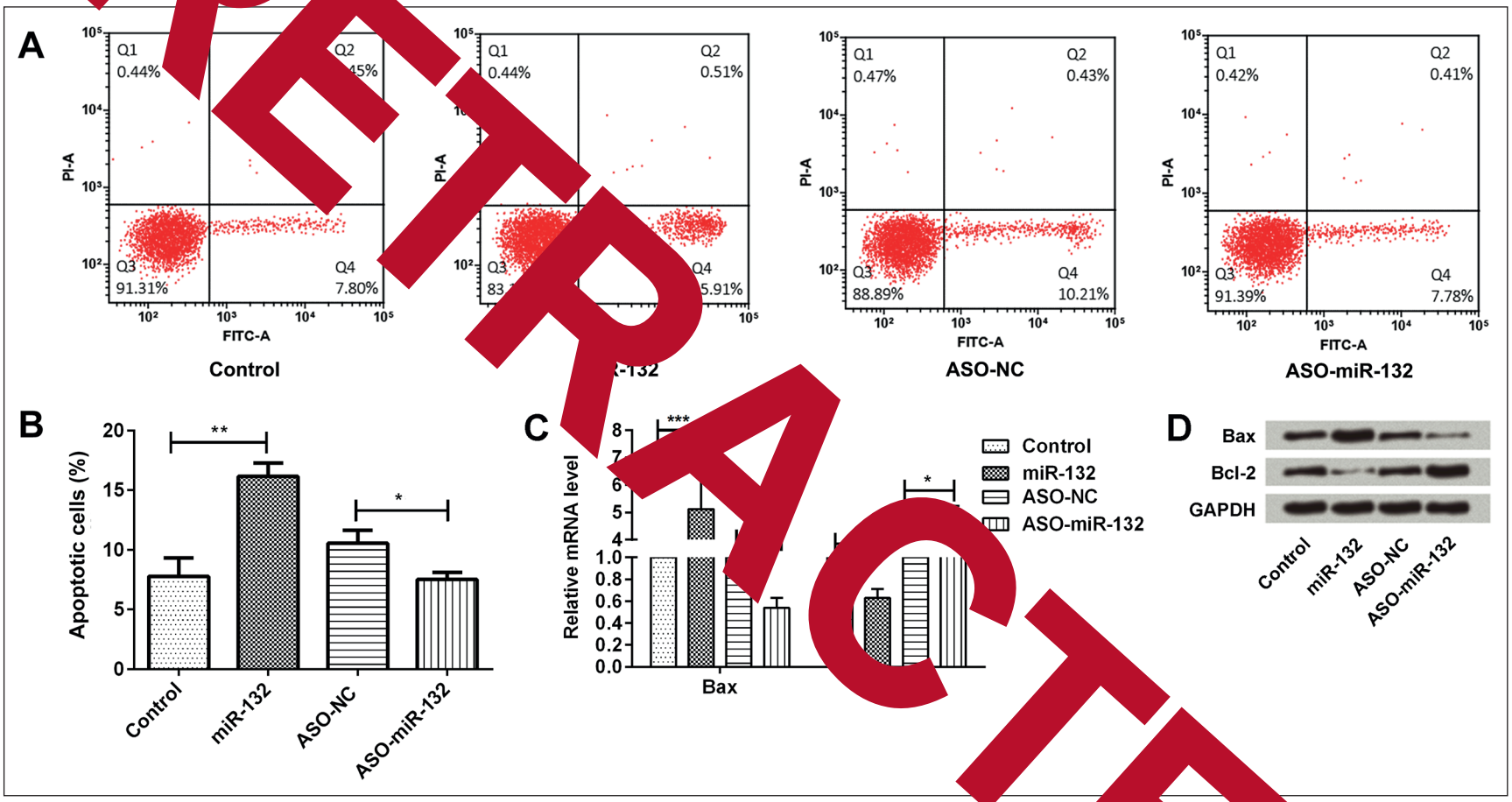


Figure 2. MiR-132 promoted primary neuronal cell apoptosis. Primary neuronal cells were transfected with miR-132 mi[illegible]-miR-13[illegible] to overexpress or suppress miR-132 expression. **A, B,** The percentages of apoptotic neurons in each group of transfected with different miR-132 vect[illegible]re de[illegible] by flow cy[illegible]etry. **C, D,** Relative mRNA and protein expressions of apoptosis-associated factors were examined by qRT-PCR and Western blot. MiR: mi[illegible]A; AD: [illegible]eimer's [illegible] ASO: antisense oligonucleotides; qRT-PCR: quantitative Real Time-Polymerase Chain Reaction. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

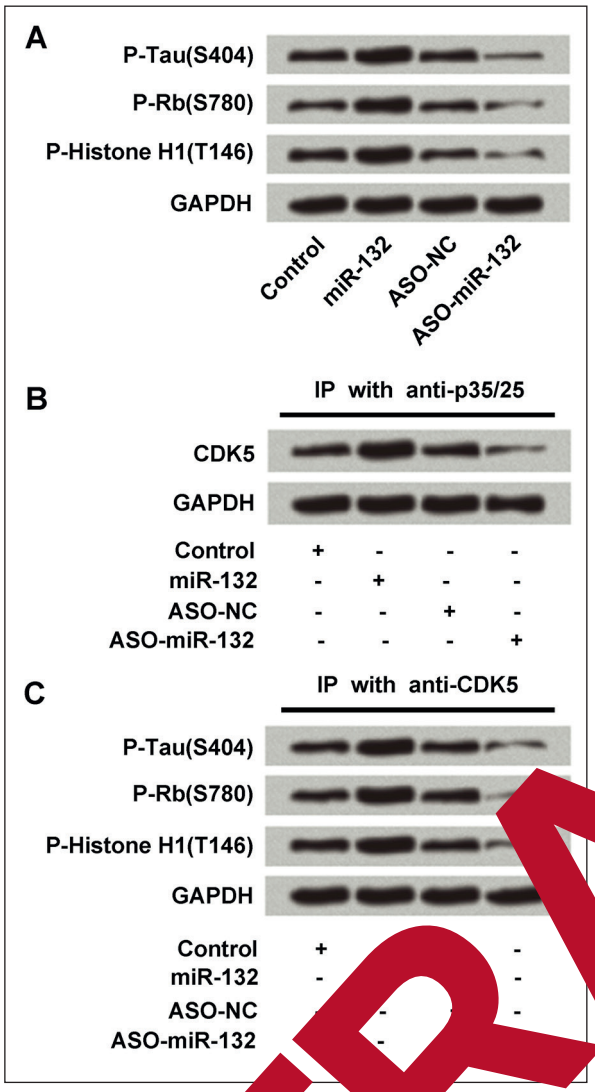
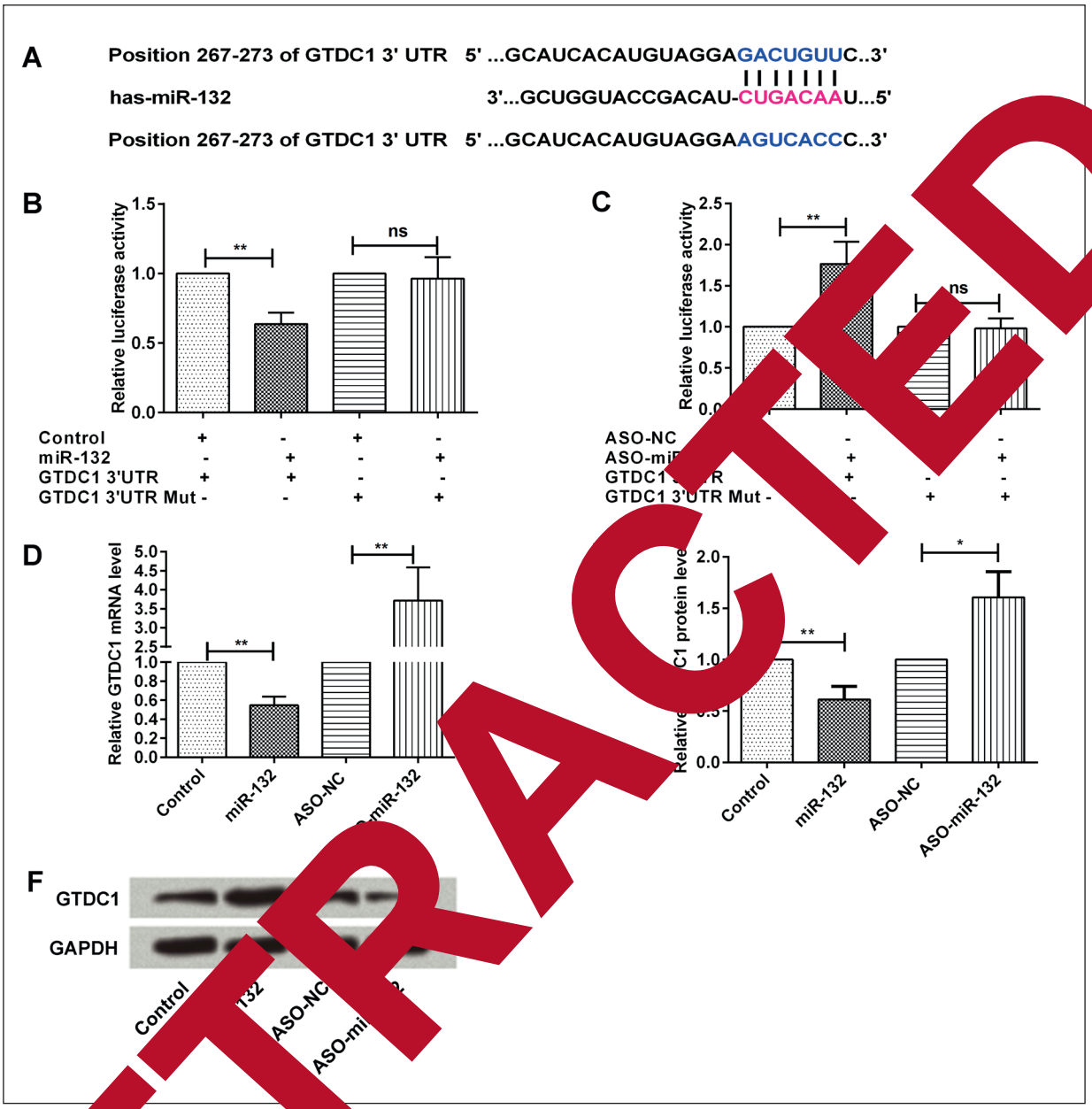


Figure 3. MiR-132 ... ated Tau pho... lation in primary neurons. Prim... nal cells were ... fected with miR-132 mimic ... AS... R-132. **A,** Pro... levels of phosphorylation ... Tau, Rb ... istone H1 were tested by Western blo... er immunopr... tion with anti-p35/25 and anti-... 5, protein levels o... DK-5 and **C,** phosphoryl... of Tau, ...b and Histone ... were measured by West... not again... R: microRNA; ASO: antisense oligonucl... CD... cyclin-d... endent kinase-5.

...hogen... D and search for the specific and ...nsitive ... arkers will be helpful for the ea... osis of the disease[24]. In this study, we ...lore ...nctions of miR-132 in AD. Highly ...pressed miR-132 was observed in patients with ...I and AD. Moreover, miR-132 overexpression in... d neuronal apoptosis and also increased phosphorylation of Tau, Rb, Histone H1 and CDK-5 expressions. Besides, whether GTDC-1 was a direct target gene of miR-132 has been clarified. The promoting effect of miR-132 on cell apoptosis and Tau phosphorylation was reversed by GTDC-1.

The role of miRNAs in the pathogen... of neurodegenerative disorders is still i... liminary stages and offers many ...rtunities for further understanding the me... sm of neuronal death[25]. Recently, altered ... ions of miRNAs have been implica... in sev... uro-degenerative disorders[22]. ... prominen... of miRNAs on variou... ular ...nts su... cellular proliferation ...ptosis ...A repair a...d gene expression at the ...t... cription ...level in a variety of si... ing p... ys is w... n mentioning in this ...text[26]. Mi... ...dentified as a specifi... which wa... egulated in AD patie... t is ...nown that miR-132 could regulate some targe... TDC-1 and Tau hyperpho... ation) there... using programmed c... eath signaling netw...rks[17]. Therefore, the ...ent work aim... o understand the role of miR-... in the patho... siology of AD and extrapola... function... ignificance in early diagnosis ... ...atm... f the disease. Our study first detecte... pression level of miR-132 in the ...atients with MCI and AD. The results revealed ...R-132 was highly expressed in patients ...h ...I and AD. Similar with Xie et al[26], we demonstrated that the levels of miR-206 and miR-132 in MCI patients' serum were markedly increased compared to normal controls. These data indicated that miR-132 might be involved in regulation of the pathophysiology of AD.

The previous work has established the neuromodulatory function of miR-132 in neurodegenerative cell model[27]. A large number of neuronal losses are observed during AD mainly due to apoptosis that is induced by the expression of apoptosis-related genes[28]. Therefore, the level of neuronal apoptosis might reflect the situation of AD to some extent. Wong et al[19] has shown that the downregulation of miR-132 could induce expression of pro-apoptotic proteins (Bima activated caspase-3 signaling) leading to neuronal apoptosis at both basal and stress conditions. Transient activation of apoptotic pathways results in conditions such as local synapse elimination and dendritic pruning without leading to cell death[29]. Low levels of miR-132 in neuronal cells are likely to be associated with dendritic decay and neuronal cell death in the AD patients and lead to both synaptic and neuronal loss with the progression of the disease[30]. In the present work, we observed that miR-132 could promote



Figur GTDC-1 as a direct targ t of miR-132. Primary neuronal cells were transfected with miR-132 mimic and ASO-miR **A,** The icted GTDC-1 binding site on miR-132 was analyzed by TargetScan and microRNA database. **B, C,** GTD as ted as a get of miR-132 using Luciferase reporter assay. **D, E, F,** Relative mRNA and protein levels of GTDC essed b PCR and Western blot. GTDC-1: glycosyl transferase like domain containing-1; miR: microR- ASO nse ol cleotides; qRT-PCR: quantitative Real Time-Polymerase Chain Reaction. *$p < 0.05$, **$p < 0.01$ repres icant difference.

ells apoptosis by the up-regulation Bax and down-regulation of Bcl-2, suggest- promoting effect of miR-132 on neuronal c optosis. Currently, it is generally accepted that changes in the regulation of Tau expression and metabolism are involved in the development of AD and Tau protein seems to be a predicted target for miR-132[31]. Hyperphosphorylation of Tau proteins has been targeted in the past to improve the abilities of memory and learning[32]. Therefore, the therapeutic target of miR-132, which modulates Tau phosphorylation, plays an essential role in AD treatment. Truncation of Tau protein makes it a favorable target for hy-

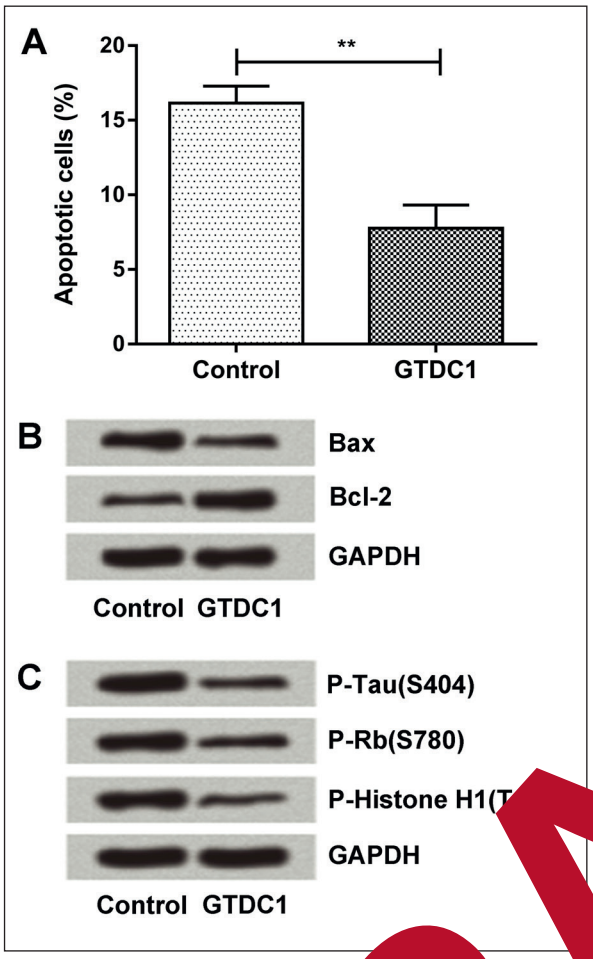


Figure 5. GTDC-1 inhibited neuronal cell apoptosis and Tau phosphorylation. **A,** Neuronal cell apoptosis was detected by flow cytometry. Protein levels of **B,** apoptosis-related factors and **(C)** phosphorylation of Tau, Rb and Histone H1 were determined by Western blot. GTDC-1: glycosyl tran domain containing -1. $^{**}p < 0.01$.

perphosphorylation. Exp ents i rats expressing huma Tau Tau 151-391) show eurofil y degeneration in the cells icating the ficance of Tau hyperpho n in a prog sive AD disease mode Base these previous studies, we f that miR-1 uld activate Tau phosph ation in primary rons. Through thes dings, ight be suggested that miR-13 fun as a odulator of Tau protein expre Tau m ted neuronal functions n AD dition number of previous studned t the target gene of miR-132 to apolate function in an indication[19,34]. So es such as p250GAP[35], methyl-CpG ein 2 (MeCP2)[36], PTBP2[37] and 00[38] were reported to be directly interacting the miRNAs. In neuronal apathies, PTBP-2 a 00 have been established to be directly interacted with the infamous Tau proteins, which form the significant core[39]. MiR-132 mediated dysregulation of PTBP-2 expression is known to contribute in the abnormal splicing of Tau protein during progressive supranuclear palsy in patients[38]. Moreover, P300 is also an established target for miR-132, which is involved in miR-132 mediated neuronal death and Tau acetylations in various taupathies[39]. However, whether GTDC-1 also participated in miR-132 regulated neuronal apoptosis and Tan phosphorylation has not been explored. In the present work, we provided evidence that GTDC-1 was a direct target of miR-132 and it was negatively regulated by miR-132. GTDC-1 has shown to inhibit neuronal apoptosis and Tau phosphorylation, indicating that miR-132 is an ideal biomarker in therapeutic interventional studies for AD.

Conclusions

These results demonstrate that miR-132 could induce neurons cell apoptosis and activate Tau phosphorylation by targeting GTDC-1, indicating

the promoting effect of miR-132 on the development of AD. The current study further suggests that miR-132 is an essential molecule contributing in the maintenance of normal neuronal cell physiology and miR-132 may be an important candidate for therapeutic intervention of AD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Biol 2011; 3: a006189.

2) Shimura M, Matsushita Y, Ikezaki K, Ichiyanagi K, Miyasaka T, Hiroshi S, Ihara Y, Sasaki YC. Anomalous stiffness changes of Tau protein in X-ray single molecule observations. Biophys J 2015; 108: 62a-62a.

3) Iqbal K, Liu F, Gong CX, Grundkeiqbal I. Tau in Alzheimer disease and related tauopathies. Curr Alzhei-mer Res 2010; 7: 656-664.

4) Goedert M. Tau protein and neurodegeneration. Semin Cell Dev Biol 2004; 15: 45-49.

5) Hernández F, Borrell J, Eacute, G[illegible] C, Avila J, [illegible] cas JJ. Spatial learning deficits enic mice that conditionally over-express GS[illegible]3 in the brain but do not form tau[illegible]ments. J [illegible]urochem 2002; 83: 1529-1533.

6) Lee G, Rook SL. [illegible]kes [illegible] protein non-neuronal cell [illegible]icrotubule binding and stabilization. J Cell [illegible] 1992; 102 ([illegible]227-237.

7) Rodríguez-M[illegible]ler AM, Lau[illegible], Mórotz GM, De V[illegible], Gil[illegible] Coleman MP, Hanger DP. Reduced number of a[illegible] mitochondria and tau hyperphosphorylation in [illegible] P301L tau knock-in mice. Neurobiol Dis 2[illegible]85: 1-10.

8) [illegible]on M, [illegible]n CL, Rizzu P, Baker M, Froelich S, [illegible]den J[illegible]kering-Brown S, Chakraverty S, Isaacs A[illegible], Hackett [illegible]damson J, Lincoln S, Dickson [illegible]ies P, [illegible]en RC, Stevens M, de Graaff [illegible] Wau[illegible]ren J, Hillebrand M, Joosse M, [illegible]on JM, [illegible]y P, Che LK, Norton J, Morris JC, [illegible]ed LA, Tro[illegible]owski J, Basun H, Lannfelt L, Neystat [illegible] Dark F, Tannenberg T, Dodd PR, Hayward [illegible], Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998; 393: 702-705.

9) Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neu-rol 1998; 43: 815-825.

10) Rodriguez-Martin T, Anthony K, Garcia-Blanco MA, Mansfield SG, Anderton BH, Gall[illegible] rection of tau mis-splicing caused [illegible] MAPT mutations by spliceosome[illegible]iated RNA trans-splicing. Hum Mol Genet[illegible] 18: 3266-3273.

11) Smith PY, Hernandez-Rapp J[illegible]ivette [illegible]urs C, Bisht K, Goupil C, Dorval [illegible]rsi S, Morin[illegible] E, Bennett DA, Fernan[illegible]Gomez EJ, Serge[illegible] Buée L, Tremblay M[illegible]alon F[illegible]ert SS. [illegible] 132/212 deficiency impairs tau metabolism and promotes pathological aggregation in vivo. Hum Mol Genet 2015; 24: 67[illegible]

12) Zhang B, C[illegible]F, Wang AH[illegible]OR[illegible]-16 regulates cell[illegible] Alzheimer's disease by targeting am[illegible] pr[illegible]or protein. Eur Rev Med Pharmacol Sci 2015; [illegible]20-4027.

13) [illegible] Liu P, Zhu [illegible]Y, Ma C, Dai X, Huang [illegible], Zhang L, Qin [illegible]miR-34a, a microRNA up-regulated in a double transgenic mouse model of Alzheimer's disease, inhibits bcl2 translation. Brain Res Bull 2009; 80: 268-273.

14) [illegible]kiw WJ, Al[illegible]drov PN. Regulation of complement factor H (CFH) by multiple miRNAs in Alzheimer's disease (AD) brain. Mol Neurobiol 2012; 46: 11-19.

15) Absalon S, Kochanek DM, Raghavan V, Krichevsky AM. MiR-26b, upregulated in Alzheimer's disease, activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons. J Neurosci 2013; 33: 14645.

16) Lee HG, Casadesus G, Zhu X, Castellani RJ, McShea A, Perry G, Petersen RB, Bajic V, Smith MA.. Cell cycle re-entry mediated neurodegeneration and its treatment role in the pathogenesis of Alzheimer's disease. Neurochem Int 2009; 54: 84-88.

17) Piscopo P, Albani D, Castellano AE, Forloni G, Confaloni A. Frontotemporal lobar degeneration and mi-croRNAs. Front Aging Neurosci 2016; 8: 17.

18) Salta E, Sierksma A, Vanden EE, De SB. MiR-132 loss de-represses ITPKB and aggravates amyloid and TAU pathology in Alzheimer's brain. EMBO Mol Med 2016; 8: 1005.

19) Wong HK, Veremeyko T, Patel N, Lemere CA, Walsh DM, Esau C, Vanderburg C, Krichevsky AM. De-repression of FOXO3a death axis by microRNA-132 and -212 causes neuronal apoptosis in Alzheimer's dis-ease. Hum Mol Genet 2013; 22: 3077.

20) Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in famili-al multiple system tauopathy with presenile dementia. Proc Natl Acad Sci U S A 1998; 95: 7737-7741.

21) Smith PY, Hernandezrapp J, Jolivette F, Lecours C, Bisht K, Goupil C, Dorval V, Parsi S, Morin F, Planel E. miR-132/212 deficiency impairs tau metabolism and promotes pathological aggregation in vivo. Hum Mol Genet 2015; 24: 6721.

22) Viswambharan V, Thanseem I, Vasu MM, Poovathinal SA, Anitha A. miRNAs as biomarkers of neuro-degenerative disorders. Biomark Med 2017; 11: 151.

23) Singh S, Kushwah AS, Singh R, Farswan M, Kaur R. Current therapeutic strategy in Alzheimer's disease. Eur Rev Med Pharmacol Sci 2012; 16: 1651.

24) Grasso M, Piscopo P, Confaloni A, Denti MA. Circulating miRNAs as biomarkers for neurodegenerative disorders. Molecules 2014; 19: 6891-6910.

25) Saito Y, Saito H. MicroRNAs in cancers and neurodegenerative disorders. Front Genet 2012; 3: 194.

26) Xie B, Zhou H, Zhang R, Song M, Yu L, Wang L, Liu Z, Zhang Q, Cui D, Wang X, Xu S. Serum miR-206 and miR-132 as potential circulating biomarkers for mild cognitive impairment. J Alzheimers Dis 2015; 45: 721-731.

27) Lungu G, Stoica G, Ambrus A. MicroRNA profiling and the role of microRNA-132 in neurodegeneration using a rat model. Neurosci Lett 2013; 553: 153.

28) Jembrek MJ, Hof PR, Šimić G. Ceramides in Alzheimer's disease: key mediators of neuronal apoptosis induced by oxidative stress and Aβ accumulation. Oxid Med Cell Longev 2015; 2015: 346783.

29) Chen FQ, Hill K, Guan YJ, Schacht J, Sha SH. Activation of apoptotic pathways in the abse[illegible] cell death in an inner-ear immortomouse [illegible]. Hear Res 2012; 284: 33.

30) Wang Y, Veremeyko T, Wong AH, El FR, Wei Z[illegible] W, Krichevsky AM. Downregulation of miR-132/[illegible] impairs S-nitrosylation balance and induces t[illegible] phosphorylation in Alzheimer[illegible] Neurobiol Aging 2017; 51: 156.

31) Smith P, Hebert S. Regu[illegible]n of tau [illegible]pression by miR-132. Alzheime[illegible]ment [illegible]: P461-P461.

32) Bai J, Zhang SL, Yang NN. Effect of pioglitazone on the learning memory and glycogen synthase kinase 3 and hyperphosphorylated tau protein in model of AD rates. National Medical Frontiers of China 2010.

33) Zilka N, Filipcik P, Koson P, Fialova L[illegible], Zilkova M, Rolkova G, Kontsekova E[illegible] M. Truncated tau from sporadic Alzheim[illegible]disease suffices to drive neurofibrillary dege[illegible]n in vivo. FEBS Lett 2006; 580: 3582-3588.

34) Rahimian P, He JJ. HIV-1 T[illegible]ortened ne[illegible] growth through regula[illegible] of micRNA-1[illegible] its target gene exp[illegible]on. J N[illegible]mflamma[illegible] 2016; 13: 247.

35) Vo N, Klein ME, [illegible]RLA[illegible]KELLER DM, [illegible]MAMOTO T, Goodm[illegible]H, Impey [illegible]AMP-[illegible]onse element bi[illegible]protein-indu[illegible]NA regulates n[illegible]orphogenes[illegible]c Natl Acad Sci U[illegible]200[illegible]: 16426-1643[illegible].

36) Liu DY, Shen XM, [illegible]F, Guo OY, Zhong Y, Chen [illegible]ZQ, Wu J. Th[illegible]iology of BDNF and its [illegible]ationship with ADHD[illegible]ol Neurobiol 2015; 52: 1467-1476.

[illegible] Smith PY, Dela[illegible]Girard J, Papon MA, Planel E, Sergeant N, Buée[illegible]Hébert SS. MicroRNA-132 loss is [illegible]sociated w[illegible]au exon 10 inclusion in progres-[illegible]ear palsy. Hum Mol Genet 2011; 20[illegible].

[illegible] Lagos D, Pollara G, Henderson S, Gratrix F, Faba-[illegible]M, Milne RS, Gotch F, Boshoff C. miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. Nat Cell Biol 2010; 12: 513-519.

39) Min SW, Cho SH, Zhou Y, Schroeder S, Haroutunian V, Seeley WW, Huang EJ, Shen Y, Masliah E, Mukherjee C. Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron 2010; 67: 953-966.