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ORIGINAL ARTICLE

Intrahippocampal miR-342-3p inhibition reduces β-amyloid plaques and ameliorates learning and memory in Alzheimer's disease

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Abstract

Accumulation of extracellular amyloid-β (Aβ) in hippocampal subregions is a hallmark of Alzheimer's disease (AD), which promotes neuronal apoptosis, potentiates cognitive decline and play a causative role in AD pathogenesis. However, whether this process is controlled by distinct miRNAs at the posttranscriptional level remain fascinating but poorly understood. Using post mortem hippocampal samples from human AD patients and 3xTg-AD mouse, we demonstrate that miR-342-3p expression was significantly induced during the AD development. With the aid of intrahippocampal injection of miR-342-3p antagomir, we further show that in vivo miR-342-3p inhibition synergistically improved cognitive deficits in 3xTg-AD mice. The hippocampal Aβ-plaque burden in 3xTg-AD mice, as revealed by immunohistochemical analysis with 4G8 antibody, was attenuated also. Mechanistically, the upregulation of neuronal miR-342-3p is linked to an increase in the activation of the stress kinase c-Jun N-terminal kinase with the subsequent death of the neurons in Aβ-challenged HT22 hippocampal neuronal cells. These findings support the model that derangement of hippocampus signal transduction and subsequent neuronal apoptosis in AD arises as a consequence of increased Aβ burden and chronic activation of the JNK MAPK cascade in a miR-342-3p-dependent manner. Overall, we described for the first time the regulatory activity of miR-342-3p on relevant Aβ metabolism pathways in Alzheimer's disease.

Keywords Alzheimer's disease (AD) · Microribonucleic acids (miRNAs) · Amyloid-β · Apoptosis · JNK

Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder, affects more than 30 million people worldwide (Bonham et al. 2018). China is to believed to have more people suffering AD than any other country in the world, as assessed by the World Health Organization in 2016 (Shah et al. 2016). The most prominent pathological event in the development of AD is the accumulation of toxic amyloid-β (Aβ), a major component of extracellular plaques. Aβ induces oxidative stress and neuroinflammation, and thereby promotes neuronal apoptosis. Aβ aggregation can also impair neurotransmission and synaptic activity. Loss of both neurons and functional synapses eventually results in cognitive deficits including impaired learning, memory and motility (Branca et al. 2017). Although the pathophysiological process of AD has been well documented, the central molecular mechanisms governing the Aβ burden and potentiates cognitive decline remain largely unknown.

Microribonucleic acids (miRNAs), a class of small (~22 nt) non-protein-coding nucleotides, regulates gene expression at

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the posttranscriptional level by binding to the 3′ untranslated regions (3'UTR) of target mRNAs for degradation or translation repression. miRNAs are highly expressed in central nervous system, where they play criticle regulatory roles in neuron differentiation, apoptosis, and synaptogenesis. Deregulated expression of distinct miRNAs has been functionally linked to age-related cognitive changes [e.g. AD and Parkinson's disease (PD)](Ferrante and Conti 2017). For instance, miRNAs are reported to regulate Aβ processing, tau translating and neuronal apoptosis (Wen 2016). miR-132 provides neuroprotection for tauopathies via direct regulation of GSK3β kinase, RNA-binding protein Rbfox1 and Caspases 3/7(El Fatimy et al. 2018). miR-29a and miR-29b-1 are simultaneously upregulated in AD and repression of these two miRNAs results in Aβ overproduction (Lei et al. 2015). Furthermore, the various changes in miRNA expression are detectable not only in local tissues related to diseases, but frequently observed in the distant biological systems (e.g. serum and cerebrospinal fluid), which suggest miRNAs may serve as powerful circulating biomarkers in AD treatment (Wang et al. 2017).

Very recently, using miRCURY LNA array analysis in amyloid precursor protein (APP) and presenilin 1 (PS1) double transgenic AD mice, Wang LL et al. have shown that several miRNAs are differentially expressed during AD pathogenesis (Wang et al. 2017). Of particular interest, miR-342-3p expression was found to be conservatively upregulated in both human and murine AD samples (Tan et al. 2014). Mechanistically, target predicting analysis has demonstrated that miR-342-3p may have targets or down-stream pathways closely related to AD pathology (Wang et al. 2017). Nevertheless, the function of miR-342-3p (either as promoter or suppressor miRNA) and its corresponding mechanisms during the development and progression of AD remain unexplored.

Based on this rationale, we investigate here the expression profile of miR-342-3p in human AD tissues and 3xTg-AD mouse brains. With the aid of intrahippocampal injection, we further show that in vivo miR-342-3p inhibition synergistically attenuates Aβ deposition and ameliorates murine cognitive deficits. Overall, our study should pave the way for a better understanding of this unique miRNA in AD.

Materials and methods

Human samples and animal model

The use and handling of human samples was approved by the ethical committee of our hospital and the procedures were strictly conformed to the standards set by the 2008 Revised *Declaration of Helsinki*, with the acquisition of the family member's written permission. To be specific, post mortem hippocampal tissues of 8 AD patients and 5 age-matched controls were obtained from the Brain Tissue Resource Center in General Hospital of Heilongjiang Agricultural Reclamation Bureau. Donors' information is listed in Supplementary Table 1.

Adult female 3xTg-AD mice, the widely-used murine model resembling pathological or behavioral abnormalities of AD (Ren et al. 2008), were obtained from Jackson Lab (Bar Harbor, ME, USA). Animals were housed 4–5 per cage under a 12-h light/dark cycle, with ad libitum access to food and water. To inhibit the miR-342-3p expression at the in vivo level, mice were injected intrahippocampally with miR-342-3p micrOFF™-antagomir (50 nM, RiboBio, Guangzhou, China) or negative control (NC) in 4-μL volume at the age of 8 months (n = 10/group, coordinates:±3.2 mm medial/lateral, −2.7 mm anterior/posterior, −2.7 mm dorsal/ventral from the bregma). 2 months later, animal behavioral analysis was performed, followed by animal sacrifice and tissue harvest (SFig. 1). All animal experiments were performed in accordance with the Principles of Laboratory Animal Care (NIH, Publication 85–23) and approved by the IACUC of our hospital (HARB #3142-6742I).

Behavioral analyses

Two months after the intrahippocampal injection, we subjected mice to behavioral tests (Branca et al. 2017). The open field test, as described previously, was used to qualitatively and quantitatively evaluate the general locomotor activity and compliance in mouse. The radial arm water maze (RAWM) test was employed to measure the hippocampal-dependent spatial reference and working memory in mouse. Two experimenters, who were blind to mouse genotype and treatment, carried out these two tests according to published procedures and scored the entries into arms (Branca et al. 2017). The dependent measures were set as incorrect arm entries (reference memory errors) and reentries (working memory errors).

Cell treatment

The HT22 mouse hippocampal neuronal cells (ThermoFisherScientific, Shanghai, China) were cultured in DMEM-HAMS F12, containing 10% fetal calf serum, L-glutamine (100 mM) and 1% antibiotics (penicillin, streptomycin) in a 5% CO_2 and 95% air humidified atmosphere. Cells were seeded onto a 96-well plate precoated with 50 mg/ml poly D-lysine (Sigma-Aldrich, Shanghai, China) and cultured as described elsewhere (Ren et al. 2008). Following a 72-h cell culture, cells were challenged for 24 h either with 50 μM of Aβ (1–40) peptide (APExBIO, Boston, MA, USA) or with no Aβ treatment (Muthaiyah et al. 2011; Ren et al. 2008). To study the signal transduction pathways involved, HT22 cells were challenged with Aβ, in the

presence of pretreatment with different pathway inhibitors (U0128, an ERK inhibitor, 10 μM; SP600125, a JNK inhibitor, 20 μM; SB203580, a p38 MAPK inhibitor, 20 μM. All inhibitors were from Selleck, Shanghai, China), for 2 h. Following Aβ treatment, cells were harvested and subjected to measurement of neuronal survival using Vybrant® MTT Assay Kit (ThermoFisherScientific). To study the effects of miR-342-3p overexpression on neuronal apoptosis, HT22 cells were transfected with miR-342-3p mimics or NC (ThermoFisherScientific) using HiPerFect Transfection Reagent (Qiagen, Shanghai, China). 48 h later, cells were subjected to other assays.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from human brain tissues and HT22 cells was isolated using the mirPremier microRNA Isolation Kit (Sigma-Aldrich, Shanghai, China). Subsequent reverse transcription was performed using TaqMan™ MicroRNA RT Kit (Applied Biosystems, Foster City, CA, USA). The cDNA samples were then PCR amplified with the aid of TaqMan™ MicroRNA Assays System (Applied Biosystems), with human U6 snRNA or mouse snoRNA202 serving as internal controls. Relative expression levels of miR-342-3p were determined by $2^{-\Delta\Delta Ct}$ method (Dong et al. 2016).

In situ hybridization

Localization of miR-342-3p in mouse hippocampal tissues was determined using in situ hybridization. Briefly, the a 3'and 5′-digitoxin-labeled locked nucleic acid (LNA) probe for miR-342-3p was purchased from Exiqon (Woburn, MA, USA). In situ hybridization was then performed on 4% paraformaldehyde-fixed hippocampal frozen sections. The slides were incubated with hybridization buffer containing 0.25% CHAPS, 0.1% Tween 20 and 20 nM of LNA digitoxin-labeled probe at 60 °C overnight. After a thorough rinse, sections were incubated with an anti-DIG-alkaline phosphatase antibody (1:1000, Sigma-Aldrich) at 4 °C overnight. Final positive signals were developed by immersing slides in B3 buffer supplemented with 340 μg/mL nitroblue tetrazolium (NBT), 175 μg/mL 5-bromo-4-chloro-3-indolyl phosphate (BCIP), 2.4 mM levamisole, and 0.05% Tween 20 (Sigma-Aldrich), for 2 h.

Western blot and immunohistochemistry

Protein samples were extracted using ReadyPrep™ Protein Extraction Kit (Bio-Rad, Shanghai, China), separated on SDS-PAGE and transferred to the PVDF membrane (ThermoFisherScientific). Subsequent Western blot was carried out as described in detail in previous report (Zhang et al. 2014).

Immunohistochemistry was performed on 4% paraformaldehyde-fixed hippocampal frozen sections using VECTASTAIN® ELITE® ABC Kits (Vector Lab, Burlingame, CA, USA).

Details regarding the antibodies used for Western blot and immunohistochemistry were listed in Supplementary Table 2.

Aβ ELISA

Aβ1–40 and Aβ1–42 levels were assayed in mouse hippocampal tissues using a commercial ELISA kit from ThermoFisherScientific, as per the manufacturer's instructions.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed with the assistance of SPSS 19.0 software. For in vitro experiments, data were analyzed by an unpaired *Student's t* test. For in vivo experiments, a 2-way repeated measures ANOVA was employed. $P < 0.05$ or $P < 0.01$ were considered statistically significant or very significant, respectively.

Results

Confirmation of miR-342-3p upregulation in AD

Because has-miR-342-3p and mmu-miR-342-3p share the same DNA sequence (www.mirbase.org), so we firstly used human AD samples to verify the expression profile of miR-342-3p. qRT-PCR assay confirmed a consistent up-regulation of miR-342-3p in hippocampal tissues of AD patients as compared with the age-matched non-AD controls (3.77 ± 1.18 v.s. 0.75 ± 0.86, Fig. 1a). Consistently, qRT-PCR analysis using hippocampal tissues from 3xTg-AD mouse also underscored a ~2.7 fold of increase in the miR-342-3p levels of the AD brain vs. control (3.69 ± 0.37 v.s. 1.00 ± 0.08, degree of freedom/df = 5, Fig. 1b). Furthermore, this increase correlated well to the positive staining of miR-342-3p in hippocampal regions, as shown by digitoxin-labeled LNA in situ hybridization (Fig. 1c). Thus, miR-342-3p expression is significantly induced during AD pathogenesis.

miR-342-3p inhibition improves learning and memory in 3xTg-AD mouse

As further exploration of the functional meaning of miR-342-3p up-regulation, we treated 8-month-old female 3xTg-AD mice with miR-342-3p micrOFF™-antagomir or NC by intrahippocampal injection. Two months later, we determined

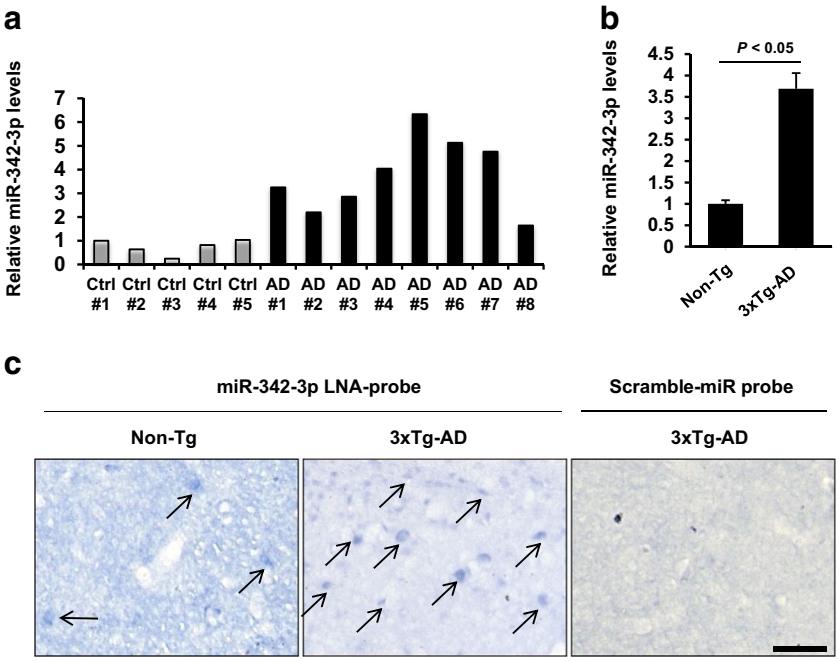

Fig. 1 Induction of miR-342-3p expression in AD. (**a**) qPCR analyses of miR-342-3p expression in post mortem hippocampal tissues of 8 AD patients and 5 age-matched controls. (**b**) qPCR analyses of miR-342-3p expression in hippocampal tissues from 3xTg-AD and wild-type mice. Date are shown as mean ± SEM, $n = 5$/group. (**c**) Localization of miR-342-3p in murine hippocampal tissues was determined using in situ hybridization. Final positive signals (arrows) were enlightened by staining with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP)

the effects of chronic miR-342-3p inhibition on behavioral changes. This time-point was chosen because 3xTg-AD mice are supposed to display AD symptoms at 10 months old (Coronas-Samano et al. 2014). Following the confirmation of hippocampal miR-342-3p inhibition by qRT-PCR analysis (reduced by ~58.7% in the miR-342-3p micrOFF™-antagomir--treated mice, df = 9, Fig. 2a), we subjected mice to the open-field activity test to measure general motor function. Apparently, the spontaneous activity and general motor function were not different among different experimental groups (Fig. 2b, c). Similarly, open-field thigmotaxis tests showed that the time spent in the periphery and the center was similar among experimental groups (Fig. 2d, e), indicating that miR-342-3p inhibition has no effects on general anxiety and stress. Together, these results suggest mice are well tolerated to the intrahippocampal injection with antagomir or NC.

We next evaluated the effects of miR-342-3p inhibition on cognitive function using RAWM test. The overall reference errors between Day 1 and Day 2 of training were all significantly decreased among four groups (df = 9, Fig. 2f), suggesting that all experimental subjects had learned the training. On Day 2 of training, however, 3xTg-AD mice made more errors than WT mice (43.6 ± 3.78 in WT mice v.s. 18.4 ± 2.05 in 3xTg-AD mice, df = 9), and these cognitive deficits in 3xTg-AD mice were completely reversed by antagomir treatment (22.5 ± 1.74 in the miR-342-3p micrOFF™-antagomir-treated 3xTg-AD mice v.s. 41.2 ± 3.55 in the NC-treated 3xTg-AD mice, df = 9, Fig. 2g). A similar rescuing effects by miR-342-3p inhibition were also observed upon measurement of working memory errors (df = 9, Fig. 2h, i). Thus, deregulated miR-342-3p expression is a critical contributor to cognitive deficits in 3xTg-AD mice.

miR-342-3p inhibition ameliorates hippocampal Aβ-plaque burden in 3xTg-AD mice

Extracellulary Aβ plaques are closely associated with neurodegeneration in 3xTg-AD mice. To clarify whether the therapeutic effects of antagomir treatment on cognitive deficits is mediated through altering the Aβ-plaque burden, we determined Aβ expression in 3xTg-AD brain sections. Immunohistochemical staining with 4G8 antibody in subicula region revealed that the plaque burden was decreased by ~53.3% ($P < 0.01$) in 3xTg-AD mice treated with miR-342-3p micrOFF™-antagomir compared to NC-treated 3xTg-AD mice (df = 9, Fig. 3a, b). Moreover, by using Aβ ELISA analysis, we showed that the amounts of soluble (Aβ1–40) and insoluble Aβ species (Aβ1–42) in 3XTg-AD brain extracts were reduced by ~32.7% (df = 9, Fig. 3c) and ~41.8% (df = 9, Fig. 3d) upon antagomir treatment, respectively. To sum up, targeting neuronal miR-342-3p bears therapeutic effects against Aβ-plaque load. To ask which factors may contribute to the reduction of Aβ in Antagomir-treated 3xTg-AD mice, we evaluated the changes in APP processing and Aβ clearance-related factors using hippocampal regions from WT and 3xTg-AD mice. As expected, 3xTg-AD mice had higher expression levels of full-length APP, p-APP (Thr668) and β-site APP-cleaving enzyme 1 (BACE1, an Aβ producing enzyme) but lower levels of neprilysin (NEP, an Aβ clearing enzyme) compared to WT mice. Interestingly, treatment with Antagomir effectively reduced the expression levels of full-length APP and p-APP (Thr668), but exerted no effects on expression levels of BACE1 and NEP (df = 9, Fig. 3e). These data suggest that miR-342-3p inhibition may reduce

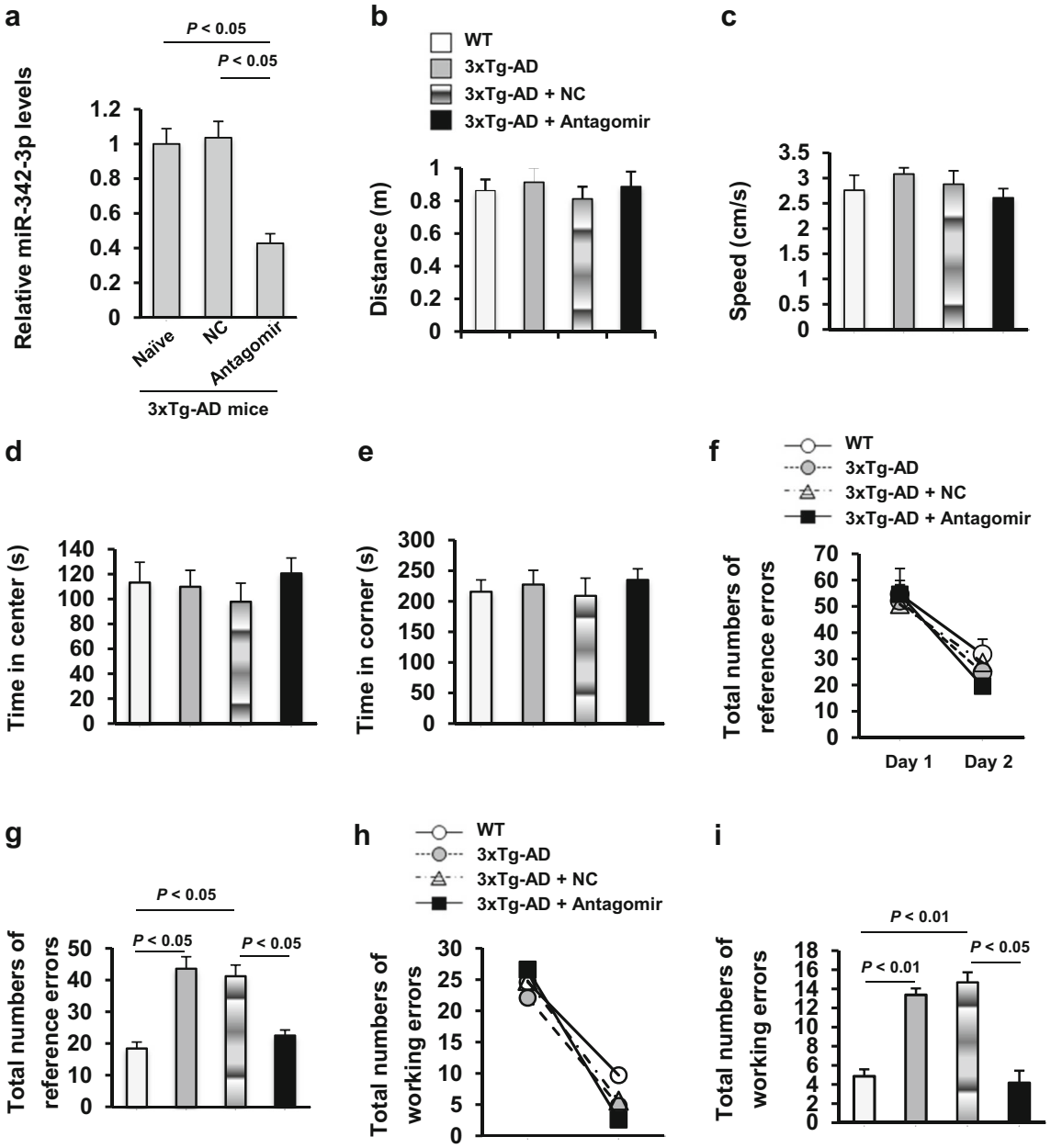

Fig. 2 Intrahippocampal inhibition of miR-342-3p ameliorates cognitive deficits in 3xTg-AD mice. (**a**) Validation of miR-342-3p inhibition by intrahippocampal inhibition of antagomir was achieved using qPCR analyses 2 months after injection. Date are shown as mean ± SEM. (**b**-**c**) Total distance traveled and speed during the open-field test. (**d**-**e**) Time spent in the center and periphery of the arena during open-field testing. (**f**) All groups show a decrease in total spatial errors at day 2 of training. (**g**) Total number of spatial errors at day 2 of training. (**h**) All the groups learned the task as reflected by working memory errors. (**i**) Working memory errors were different in experimental groups at day 2. Data from behavioral test were analyzed using 2-way repeated measures ANOVA

Aβ production via regulation of APP processing, but not by Aβ clearance.

miR-342-3p inhibition ameliorates hippocampal Aβ-plaque burden in HT22 cells

To begin understanding the mechanisms underlying the improvement of Aβ-impaired cognitive function by miR-342-3p inhibition, we treated HT22 cells with Aβ, in the presence of pretreatment with different MAPK pathway inhibitors including U0128 (an ERK inhibitor), SP600125 (a JNK inhibitor) and SB203580 (a p38 MAPK inhibitor). Interestingly, stimulation with Aβ alone significantly induced miR-342-3p expression in all experimental settings, but only pretreatment with SP600125 substantially reversed Aβ-elicited miR-342-3p upregulation in HT22 cells. By contrast, pretreatment with U0128 or with SB203580 both failed to reverse Aβ-elicited miR-342-3p upregulation in HT22 cells (df = 4, Fig. 4a-c),

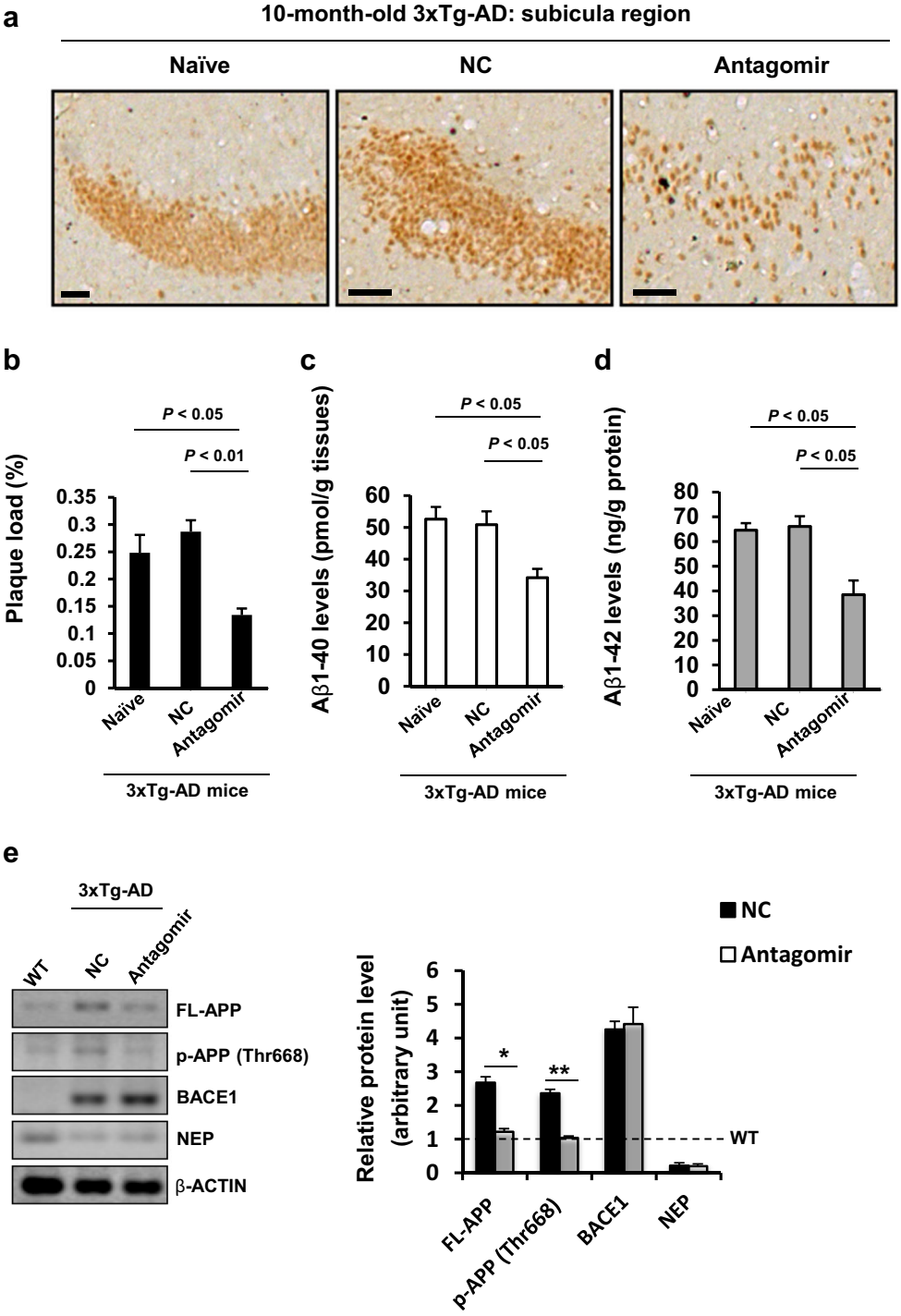

Fig. 3 Intrahippocampal miR-342-3p inhibition attenuates Aβ-plaque burden in 3xTg-AD mice. (**a**) Immunohitochemical labeling of Aβ with 4G8 antibody in subicula regions was assayed two months after intrahippocampal injection. (**b**) Quantification of relative 4G8 intensity in Penal A using Image J (n = 5/group). Aβ1–40 (**c**) and Aβ1–42 (**d**) levels from the mouse hippocampal tissues were measured by sandwich ELISA (n = 5/group). (**e**) Expression levels of full-length amyloid precursor protein (FL-APP), p-APP, β-site APP-cleaving enzyme 1 (BACE1) and neprilysin (NEP) were assayed in hippocampal tissues from different experimental groups. Densitometric scanning of immunoblots was performed in which the level of a target protein was normalized against the values in WT mice, which was arbitrarily set at 1 (A right panel, $*P < 0.05$ and $** P < 0.01$)

suggesting that Aβ may stimulate miR-342-3p expression via modulating specific activation of the JNK pathway. To further elucidate whether miR-342-3p and JNK pathway can regulate Aβ-challenged HT22 cells in a coordinated manner, we transfected HT22 cells with miR-342-3p mimics or NC (6.35 ± 0.54 in the miR-342-3p mimics-treated HT22 cells v.s. 1.05 ± 0.11 in the mimics NC-treated HT22 cells, df = 4, Fig. 4d), followed by Aβ stimulation. The relative viability of HT22 cells was reduced by ~42.4% upon Aβ treatment alone (0.58 ± 0.06 in the Aβ-treated HT22 cells v.s. 1.00 ± 0.05 in the untreated HT22 cells, df = 4), and this reduction was further enhanced by transfection with miR-342-3p mimics (0.28 ± 0.04 in the miR-342-3p mimics plus Aβ-treated HT22 cells v.s. 0.53 ± 0.07 in the mimics NC plus Aβ-treated HT22 cells, df = 4, Fig. 4e). In line with the cell viability changes, miR-342-3p overexpression significantly augmented Aβ-induced JNK activation, evidenced by Western blot analysis of p-JNK and p-c-Jun expression (df = 4, Fig. 4f). To be noted,

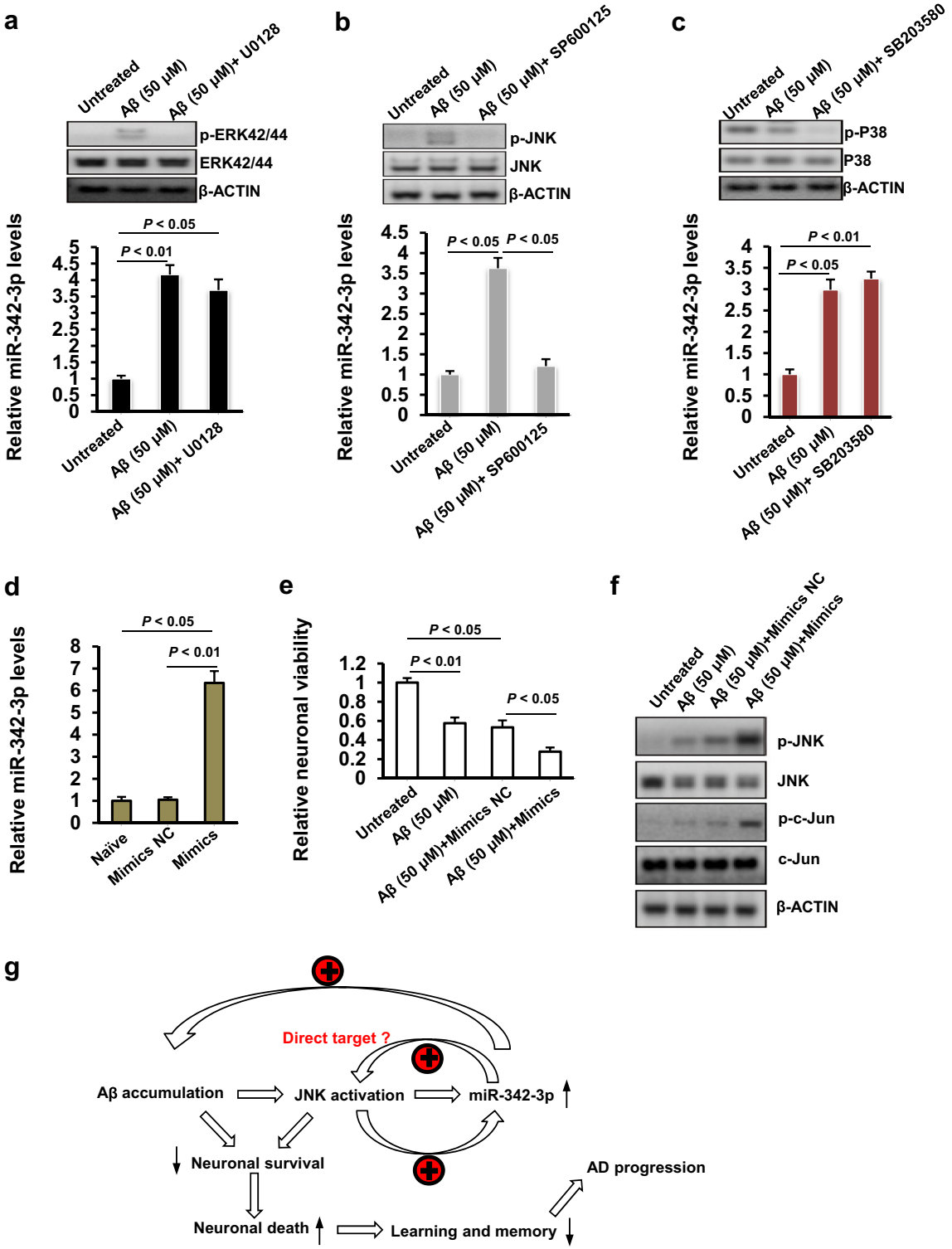

Fig. 4 miR-342-3p acts both as a target and as a modulator of the Aβ circuitry, in the regulation of neuronal apoptosis. (**a**-**c**) The HT22 mouse hippocampal neuronal cells were challenged with 50 μM Aβ peptide, in the presence of pretreatment with different pathway inhibitors (U0128, an ERK inhibitor, 10 μM; SP600125, a JNK inhibitor, 20 μM; SB203580, a p38 MAPK inhibitor, 20 μM), for 2 h. Following Aβ treatment, cells were harvested and miR-342-3p expression was assessed using qPCR. Date are shown as mean ± SEM (*Student's t* test). (**d**) HT22 cells were transfected with miR-342-3p mimics or NC for 48 h, followed by qPCR analysis. (**e**) 48 h after transfection with miR-342-3p mimics or NC, HT22 cells challenged with 50 μM Aβ peptide for another 2 h, followed by measurement of neuronal survival using Vybrant® MTT Assay Kit. (**f**) 48 h after transfection with miR-342-3p mimics or NC, HT22 cells challenged with 50 μM Aβ peptide for another 2 h, followed by Western blot analysis of JNK, p-JNK, c-Jun and p-c-Jun expression levels. (**g**) Summary of identified reciprocal events from the miR-342-3p-Aβ interaction during AD progression

transfection with miR-342-3p mimics or mimics-NC alone exhibited no effects on JNK activation (SFig. 2). Taken together, it would appear that miR-342-3p and JNK pathway may regulate Aβ-induced neuronal damage in a reciprocal manner.

Discussion

miRNAs are emerging as critical regulators of the development and progression of AD. In the current proof-of-concept study, we showed that miR-342-3p expression was significantly upregulated in brain samples from AD patients and in hippocampal regions from 3xTg-AD mice. These results are in line with previous studies demonstrating that miR-342-3p expression is noticeably increased in human serum samples (Tan et al. 2014) and whole-brain samples from murine AD models (Wang et al. 2017). Collectively, miR-342-3p is critically involved in the pathogenesis of AD and may therefore represent a signature miRNA and a potential therapeutic target for AD treatment.

The discovery that miR-342-3p expression is enriched in hippocampal fractions from both postmortem human AD patients and 3xTg-AD mice is a novel observation. Given that hippocampal accumulation of tau pathology is a hallmark of AD, these data suggest a possible contribution of miR-342-3p to Aβ deposition. Emerging data evidence an essential involvement of lipometabolism in AD pathogenesis. Central obesity, frequently occurring in human middle age, is closely associated with an increased risk of dementia. Virtually the vast majority (>70%) of patients with dementia will eventually show Aβ accumulation and develop late onset Alzheimer's disease (LOAD). A very recent study using a large-scale longitudinal population-based cohort even shows that measurement of central obesity can predict LOAD better than BMI body mass index (BMI) in the elderly people (Murakami et al. 2013). Consistently in mice, supplement with high-fat diet significantly compromises spatial and contextual associative memory, and this cognitive deficits are rapidly reversed by switching mice from a high-fat diet back to a low-fat diet (McLean et al. 2018). Thus, disruption of lipometabolism plays an important role in the development of cognitive decline. Interestingly, independent groups have shown that miR-342-3p may represent a functional link between posttranscriptional regulation and lipometabolism. miR-342-3p expression is significantly induced during the development of murine obesity and miR-342-3p is later found to be enriched in the adipose tissue of obese mice (Chartoumpekis et al. 2012). Functionally, the obesity-associated miR-342-3p promotes adipogenesis of mesenchymal stem cells via suppression of CtBP2 signaling (Wang et al. 2015). These data thus suggest that the link between miR-342-3p deregulation and AD pathogensis is solid. Importantly, using in vivo antagomir treatment, we further showed that miR-342-3p inhibition diminished Aβ deposition and improved cognitive deficits in 3xTg-AD mice (Figs. 2 and 3). miR-342-3p inhibition may reduce Aβ production via regulation of APP processing (Fig. 3). Taken together, it is plausible that miR-342-3p upregulation may play a causative role in the development and progression of AD.

As a"multivalent" regulator, one miRNA is able to target multiple genes, which makes it more advantageous to underlie the cause of AD if the upstream regulatory factors controlling miRNA expression could be further identified (Wang et al. 2017). Stress-responsive mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase/stress activated protein kinase (JNK/SAPK) and p38 pathways, are central mediators regulating many fundamental cellular processes. Compelling data suggest MAPK pathways are all activated in AD (Zhu et al. 2001). Treatment with soluble Aβ1–42 in mouse hippocampal slices resulted in ERK activation, whereas JNK activation was observed in both cortical neurons and differentiated PC12 cells upon Aβ1–42 exposure. Similarly, stimulation by Aβ also induces increased p38 activity in microglia (Savage et al. 2002). In our study, however, treatment with 50 μM Aβ could only elicit ERK and JNK activation, with no effects on p38 activity (Fig. 4a-c). Two reasons may account for this discrepancy: i) The chronological relationship between activated ERK, JNK and p38 during AD progression is differential. All these kinases are found to be activated in mild and severe AD cases (Braak stages III–VI), but only ERK and JNK activation were detected in non-demented cases with limited pathology (Braak stages I and II) (Zhu et al. 2001). To this end, treatment with 50 μM Aβ in HT22 cells may only represent a non-demented AD cases. ii) Compared to p38, ERK and JNK kinases appear to play more important roles in the regulation of neuronal death upon noxious stimulus (Bhowmick et al. 2018). Of particular interest, JNK pathway has been confirmed to regulate critically the apoptosis and survival signaling in neurodegenerative diseases (e.g. PD and AD). Dopamine (DA), a neurotransmitter and also a neurotoxin, acts as a major pathological factor contributing to the selective loss of dopaminergic neurons in PD. DA-induced neuronal death has been functionally linked to the JNK activation. Conversely, repression of SEK1, an upstream kinase of JNK, significantly attenuated Aβ-elicited apoptosis (Peng and Andersen 2003). Taken together, the sole regulation of miR-342-3p by JNK pathway strongly suggest that this miRNA may play a unique role in the pathogenesis of Aβ burden. miR-342-3p acts both as a target and as a modulator of the Aβ circuitry, in the regulation of neuronal responses to Aβ stimulation (Fig. 4g).

In summary, we report that miR-342-3p expression is noticeably induced along AD pathogenesis, and in vivo miR-342-3p inhibition synergistically attenuated Aβ deposition

and improved cognitive deficits in 3xTg-AD mice. This is noteworthy as there is growing appreciation that miRNAs serve as valuable biomarkers and therapeutic targets for neurodegenerative diseases. In this context, the identification of central down-stream targets of neuronal miR-342-3p signaling, which is currently in progress in our lab, represents a step forward in generating novel therapeutic strategy for AD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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