miR-149-5p inhibition reduces Alzheimer's disease β-amyloid generation in 293/APPsw cells by upregulating H4K16ac via KAT8

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Abstract. Alzheimer's disease (AD), the leading cause of age-related dementia, is characterized by abnormal β-amyloid accumulation. During learning, memory formation and consolidation, increased levels of histone H3 and H4 acetylation are observed. The present study reported significantly decreased level of H4K16ac in the plasma of patients with AD compared with healthy subjects via western blotting and reverse transcription-quantitative (RT-q)PCR. Lysine acetyltransferase 8 (KAT8) expression, the major lysine acetyltransferase responsible for the acetylation of H4K16, was significantly decreased in patients with AD compared with healthy subjects as determined via western blotting and RT-qPCR. The results indicated that aberrant expression patterns of H4K16ac and KAT8 might be associated with AD progression. Moreover, western blot analysis demonstrated that KAT8-overexpression cells displayed increased levels of H4K16ac, accompanied by higher levels of neuroprotective soluble amyloid precursor protein (sAPP) α and β-secretase (BACE)2, and decreased levels of sAPPβ and BACE1 compared with negative control and vector cells. In neurodegenerative disorders, microRNAs (miRNAs/miRs) are deregulated; however, the effect of miRNA dysregulation on histone acetylation is not completely understood. To the best of our knowledge, the present study identified a novel inhibitory interaction between miR-149-5p and KAT8 3'-UTR that contributed to the pathological alterations in an AD cell model for the first time, using bioinformatics and a dual-luciferase reporter assay. The western blotting results indicated that, compared with the inhibitor control group, miR-149-5p inhibitor markedly increased H4K16ac levels, which were significantly suppressed by co-transfection with KAT8 short hairpin (sh)RNA. KAT8 shRNA and miR-149-5p inhibitor co-transfection abolished the beneficial effects of miR-149-5p inhibitor. The results indicated that miR-149-5p regulated KAT8 and H4K16ac expression in an AD cell model, which may be associated with the pathological process of AD; therefore, miRNA may serve as a potential drug target for AD.

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Introduction

Alzheimer's disease (AD), the most common cause of dementia in elderly individuals worldwide, is characterized by progressive memory and cognitive impairments (1). The abnormal accumulation of extracellular β-amyloid (Aβ) plaques in the brain is an important molecular pathological feature of patients with AD (2). Aβ is produced by proteolytic cleavage of the amyloid precursor protein (APP) by β-secretases and γ-secretases (3,4), which generates soluble (s)APPβ, amyloid-β_{1-40} ($A\beta_{1-40}$) and $A\beta_{1-42}$ fragments. The alternative proteolytic pathway of APP involves the release of α-secretase, which prevents the formation of Aβ due to its cleavage site and produces sAPPα that serves as a neuroprotective player (5,6).

MicroRNAs (miRNAs/miRs) are a class of endogenous 22-nucleotide non-coding RNAs that base-pair with the 3'-untranslated region (UTR) of specific genes, thereby repressing gene expression. Certain miRNAs serve as crucial players of neuronal gene expression that contribute to neurogenesis, neuronal maturation, brain development and neuroplasticity (7,8). The involvement of miRNAs in the pathophysiology of AD has been demonstrated in a number of previous studies. For example, miR-15b inhibits Aβ accumulation by targeting NF-κB signaling and β-secretase (BACE)1 (9). microRNA-219 downregulation promotes neurodegeneration via post-transcriptional regulation of microtubule associated protein tau (10). Recently, miR-149-5p was reported to protect against high glucose-induced pancreatic β-cell apoptosis (11). Furthermore, miR-149-5p was also revealed to regulate pentraxin resistance in breast cancer (12). However,

the role of miR-149-5p in regulating APP and Aβ production is not completely understood. Epigenetic dysregulation serves a vital role in the onset and progression of AD (13-21), which include chromatin variation, DNA methylation and non-coding RNAs (22). Among the widespread epigenetic modifications, histone acetylation and deacetylation are chromatin variations that regulate gene transcription (23). Histone acetylation is downregulated at regulatory regions of memory genes in mouse models of AD (24). Nonselective histone deacetylase inhibitor treatments were reported to improve memory impairments by restoring normal histone acetylation levels (25,26). Among histone acetylation marks, H4K16ac facilitates gene activation and damaged DNA repair by influencing its chromatin structure (27). The largest meta-analytic genome-wide association study identified branched chain keto acid dehydrogenase kinase/KAT8 as a new AD-associated loci (28). KAT8 is the major lysine acetyl transferase responsible for the acetylation of H4K16 in flies and mammals (29). The present study compared serum histone acetylation and KAT8 in patients with AD and cognitively healthy individuals. In addition, the expression of miR-149-5p in an AD cell model *in vitro* was investigated, and the roles of miR-149-5p and its interaction with KAT8 in the AD cell model were also explored. Collectively, the results suggested that miR-149-5p levels were increased in patients with AD compared with healthy subjects, which regulated H4K16ac in 293/APPsw cells via targeting KAT8. Therefore, the present study indicated that targeting miR-149-5p may display neuroprotective effects in AD, suggesting that miR-149-5p may serve as a novel therapeutic target.

Materials and methods

Plasma analyses. Blood samples were obtained from patients with AD (n=30) and healthy volunteers (n=30) with typical cognitive performance. Patients with AD were confirmed by screening cognitive impairment by conducting a Mini-Mental State Examination (patients with a score ≤24 were included in the present study) and a neuropsychological test via Clinical Dementia Rating (an assay that can identify very mild dementia; dementia scale: 0, none; 0.5, very mild; 1, mild; 2, moderate; and 3, severe) (30). All patients with AD had an overall CDR of ≥0.5. Patients with AD were diagnosed according to NINCDS-ADRDA criteria (31). To further diagnose AD based on imaging-based techniques, the magnetic resonance image (MRI) relaxation time constant was examined in the brains of patients with AD according to a previous report (32). Neuropsychological impairment was measured according to the Global Deficit Score for classifying Neuropsychological impairment (33). All healthy subjects had no brain diseases, as assessed by MRI and computed tomography. In addition, healthy subjects did not have a history or symptoms of ischemic or hemorrhagic stroke. Exclusion criteria were as follows: i) history of any vascular or systemic disease; ii) any other neurodegenerative disease; iii) any psychiatric disease; iv) epilepsy; and v) substance or drug abuse. The present study was approved by the Institutional Ethics Committee of First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (approval no. KY-E-2017-12-30). Written informed consent was obtained from all patients and healthy subjects. Blood samples were collected in EDTA-containing tubes and processed within 2 h. After collection, blood samples were centrifuged at 1,000 x g for 10 min at 4˚C. Plasma was isolated and stored 1 ml aliquots at -80˚C until further analysis.

Cell Culture. Human embryonic kidney (293) cells carrying the Swedish mutation of APP (293/APPsw), which were kindly provided by the Centre for Translational Medicine, Tianjin University of Traditional Chinese Medicine, were used in the present study. APPsw is an established AD cell model. Cells were cultured in DMEM/F-12 supplemented with 10% FBS and 500 μg/ml G418 (all from Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in a humidified atmosphere of 5% CO_2.

Transfection. 293/APPsw cells were plated at $5x10^4$ cells per well in a 24-well plate and transfected with 100 nM miR-149-5p inhibitor, inhibitor negative control (inhibitor NC), short hairpin RNA (sh)-Scramble (sh-NC) orsh-KAT8, which were all purchased from GenePharma. Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used to transfect cells according to the manufacturer's protocol. Subsequent *in vitro* assays were performed 3 days post-transfection. Sequences of the oligonucleotides were as follows: miR-149-5p inhibitor: 5'-GGGAGUGAAGACACGGAGCCAGA-3' and inhibitor NC: 5'-CGAACGUGUCACGUTT-3. For stable KAT8 overexpression, at 50% confluence, 293/APPsw cells were infected with purified lentiviral particles expressing KAT-8 (LV-KAT-8-puromycin; Shanghai GeneChem Co., Ltd.) or NC lentiviral particles (LV-puromycin; Shanghai GeneChem Co., Ltd.) at MOI=20. Subsequently, KAT8 overexpression cells were selected using puromycin for 72 h at 37˚C in a humidified atmosphere of 5% CO_2. Reverse transcription-quantitative PCR and western blotting were performed to assess transfection efficiency.

ELISA assay. $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in the cell medium were determined using a specific sandwich ELISA kit ($A\beta_{1-40}$, cat. no. MBS263658; $A\beta_{1-42}$, cat. no. MBS703888; both MyBioSource, Inc.) according to the manufacturer's protocol.

Western blotting. Total protein was extracted from cells using RIPA buffer (Beyotime Institute of Biotechnology). Protein concentration was determined using the Bicinchoninic Acid Protein Assay (Pierce; Thermo Fisher Scientific, Inc.). Equal amounts of protein (20 μg/lane) were separated via 8-12% SDS-PAGE and transferred onto PVDF membranes (EMD Millipore). After blocking with 5% skim milk in TBS for 2 h at room temperature, the membranes were incubated with the following primary antibodies at 4˚C overnight: Anti-H3K4ac (Abcam; 1:500; cat. no. ab232931), anti-H3K27ac (Abcam; 1:500; cat. no. ab4729), anti-H4K12ac (EMD Millipore; 1:3,000, 04-119), anti-H4K16ac (EMD Millipore; 1:3,000; cat. no. 07-329), anti-APP (EMD Millipore; 1:1,500; cat. no. AB5300), anti-sAPPα (EMD Millipore; 1:1,000; cat. no. JP11088), anti-sAPPβ (EMD Millipore; 1:1,000; cat. no. MABN640), anti-BACE1 (Epitomics; 1:2,000; cat. no. ABCA0158537), anti-BACE2 (Epitomics; 1:2,000; cat. no. ABCA0157823) and anti-GAPDH (Santa Cruz Biotechnology, Inc.; 1:1,000; cat. no. sc-47724). After washing with TBST (0.1% Tween 20), the membranes were incubated

with horseradish peroxidase-conjugated corresponding secondary antibodies (Santa Cruz Biotechnology, Inc.; 1:5,000; cat. no. sc-2357 for anti-rabbit IgG-HRP, and cat. no. sc-2005 for anti-mouse IgG-HRP) at 37˚C for 1 h. Protein bands were visualized using ECL substrate (Beyotime Institue of Biotechnology). Protein expression levels were semi-quantified using ImageJ software (v1.8.0, National Institutes of Health) with GAPDH (KAT8, APP, sAPPα, sAPPβ, BACE1 and BACE2) or H3 and H4 (H3K4ac, H3K27ac, H4K12ac and H4K16ac) as the loading controls (30,31).

RT-qPCR. Total RNA was extracted from transfected 293/APPsw cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit (Takara Biomedical Technology (Beijing) Co., Ltd.), and the reverse transcription conditions were 37˚C, 15 min; 85˚C, 5 sec. qPCR was performed using SYBR Premix Ex Taq kit (Takara Biomedical Technology Co., Ltd.) according to the manufacturer's instructions. The thermocycling conditions were 95˚C for 10 min (initial denaturation) followed by 40 cycles of 95˚C, 15 sec (denaturation); 60˚C, 1 min (annealing). The final stage was 95˚C, 5 min; 60˚C, 1 min; 95˚C, 15 sec for 1 cycle to obtain a dissolution curve. The following primers were used for qPCR: miR-149-5p forward, 5'-GGCTCTGGCTCCGTGTCTT-3' and reverse, 5'-CAGTGCAGGGTCCGAGGTATT-3'; KAT8 forward, 5'-GTCACGGTGGAGATCGGAGA-3' and reverse, 5'-CCCTCCTGGTCGTTCACTC-3'; U6 forward, 5'-GCTTCG GCAGCACATATACTAAAAT-3' and reverse, 5'-CGCTTC ACGAATTTGCGTGTCAT-3'; and GAPDH forward, 5'-GCC TTCCGTGTCCCCACTGC-3' and reverse, 5'-CAATGCCAG CCCCAGCGTCA-3'. miRNA and mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (34) and normalized to the internal reference genes U6 and GAPDH, respectively.

Luciferase reporter assay. 293/APPsw cells ($2x10^4$ cells/well) were incubated in 24-well plates for 24 h. Subsequently, cells were co-transfected with 50 ng pGL3 luciferase vector carrying wild-type (WT) or mutated (MUT) 3'-UTR of KAT8 (Promega Corporation) and 50 nM miR-149-5p-mimic or mimic-NC using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). At 48 h post-transfection, luciferase activities were detected using a Dual-Luciferase Reporter Assay system (Guangzhou RiboBio Co., Ltd.) according to the manufacturer's instructions. Relative luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. All experiments were repeated at least three times independently. Data are presented as the mean ± SEM. Statistical analyses were conducted using SPSS software (version 20.0; IBM Corp.). The figures and graphs were prepared using GraphPad Prism 7 (GraphPad Software, Inc.). The unpaired Student's t-test was used to compare two groups, and comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. Pearson's correlation coefficient analysis was used to analyze the correlation between miR-149-5p and KAT8. $P<0.05$ was considered to indicate a statistically significant difference.

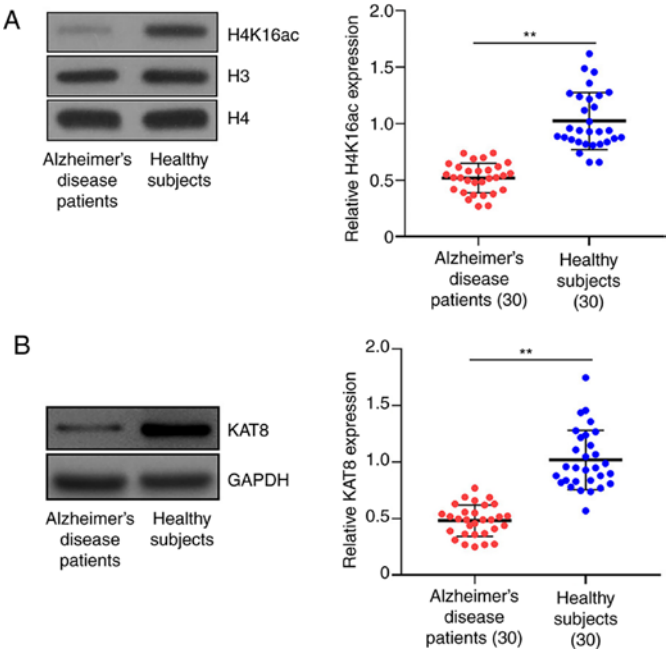

Figure 1. Aberrant expression of H4K16ac and KAT8 in the plasma of patients with AD. (A) H4K16ac and (B) KAT8 expression in the plasma of patients with AD (n=30) and healthy subjects (n-30). **P<0.01. KAT8, lysine acetyltransferase 8; AD, Alzheimer's disease.

Results

H4K16ac and KAT8 were downregulated in the plasma of patients with AD. First, primary acetylated histone protein (H3K4ac, H3K27ac, H4K12ac and H4K16ac) levels in the plasma of healthy subjects and patients with AD were analyzed via western blotting. Table I summarizes the characteristics of patients with AD and cognitively healthy volunteers. Although no difference was identified in H3K4ac and H3K27ac levels between healthy subjects and patients with AD, the H4K12ac level in patients with AD was notably downregulated compared with healthy subjects (Fig. S1). H4K16ac protein levels were notably decreased in patients with AD compared with healthy controls (Fig. 1A), which was further indicated at the mRNA level via RT-qPCR (Fig. 1A). The expression profile of KAT8, the major lysine acetyltransferase responsible for the acetylation of H4K16, was also evaluated. The RT-qPCR results demonstrated a reduction in KAT8 mRNA and protein expression in patients with AD compared with healthy subjects (Fig. 1B). Collectively, the results demonstrated that aberrant expression patterns of H4K16ac and KAT8 could be related to the progression of AD.

KAT8 overexpression increases H4K16ac expression, reduces APP processing and decreases the $A\beta_{1-40}/A\beta1_{-42}$ ratio in 293/APPsw cells. Histone-associated heterochromatin structural alterations are involved in the formation of long-term memory (35). Certain studies reported that increased levels of H3 and H4 acetylation are closely associated with memory consolidation (36,37). In the present study, KAT8-overexpression 293/APPsw cells were constructed to explore the effect of KAT8 on the acetylation status of core histones *in vitro*. KAT8 mRNA and protein expression

Table I. Characteristics of patients with AD and healthy subjects.

Variable	Patients with AD (n=30)	Healthy subjects (n=30)	Statistical value	P-value
Sex (male/female)	17/13	14/16	0.601[a]	0.438
Age (years)	63.4±18.0	64.1±16	0.341[b]	0.899
BMI (kg/m^2)	24.5±3.4	24.7±4.4	0.413[b]	0.931
Diabetes/non-diabetes	8/22	5/25	0.884[a]	0.347
High blood pressure/normal blood pressure	10/20	7/23	0.739[a]	0.390
Coronary heart disease/non-coronary heart disease	6/24	3/27	1.176[a]	0.278
Smoker/non-smoker	11/19	10/20	0.073[a]	0.787
Drinker/non-drinker	15/15	16/14	0.067[a]	0.796
Educational years	11.3±3.4	11.7±3.7	0.968[b]	0.867
Left handed/right handed	3/27	5/25	0.577[a]	0.448
Duration of illness (years)	8.5±4.7	-	-	-
Pathological MRI (%)	75 (60%)	-	-	-
Neuropsychological impairment (2 T-scores 35; %) (51,52)	90 (64%)	-	-	-
MMSE scores	18.9±3.6	25.4±2.8	5.65[b]	0.001
CDR scale				
CDR 0	0	30	-	-
CDR 0.5	7	-	-	-
CDR 1	11	-	-	-
CDR 2+	12	-	-	-

[a]χ^2 test; [b]Student's t-test. AD, Alzheimer's disease; MRI, magnetic resonance imaging; MMSE, Mini-Mental State Examination; CDR, Clinical Dementia Rating; -, not applicable.

profiles were assessed via RT-qPCR and western blotting (Fig. 2A and B). Cellular H4K16ac was markedly higher in KAT8-overexpression 293/APPsw cells compared with NC and vector cells (Fig. 2C). Furthermore, cellular H4K12ac was slightly increased in KAT8-overexpression cells compared with NC and vector cells; however, there was no notable difference in H3K4ac and H3K27ac levels between KAT8-overexpression and control cells (Fig. S2). The results indicated that KAT8 overexpression in 293/APPsw cells induced H416K acetylation. Subsequently, the effects of KAT8 on APP processing in 293/APPsw cells were assessed. KAT8-overexpression 293/APPsw cells displayed markedly increased neuroprotective sAPPα expression levels, and decreased expression levels of sAPPβ and APP expression compared with NC and vector 293/APPsw cells (Fig. 2D). The $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio was significantly increased in KAT8-overexpression 293/APPsw cells compared with vector cells (Fig. 2E). The expression level of enzymes involved in proteolytic cleavage of APP, BACE1 (a key enzyme in sAPPβ generation) and BACE2 (a homolog of BACE1, which functions as an antagonist of BACE1 and blocks Aβ production), were assessed (38,39). The western blotting results indicated that BACE2 expression was markedly upregulated in KAT8-overexpression cells compared with NC and vector cells. Likewise, BACE1 expression was downregulated in KAT8-overexpression cells compared with NC and vector cells (Fig. 2D), which suggested that KAT8 was associated with the proteolytic processing of APP protein.

KAT8 is a direct target of miR-149-5p. Since miRNAs inhibit transcription of specific mRNAs by binding to their 3'-UTRs (40), starBase (version 2.0, http://starbase.sysu.edu.cn/starbase2/) computational analyses were conducted to predict potential miRNA interactions in the 3'-UTR of KAT8. A putative miR-149-5p target site in the 3'-UTR of KAT8 was identified (Fig. 3A). To validate the interaction between miR-149-5p and KAT8, a luciferase reporter assay was conducted. The luciferase activity of 293/APPsw cells co-transfected with miR-149-5p mimic and WT 3'-UTR was significantly reduced compared with cells co-transfected with miR-NC and WT 3'UTR (Fig. 3B); however, the luciferase activity was not significantly different between cells co-transfected with miR-149-5p mimic and MUT 3'-UTR, and cells co-transfected with miR-NC and MUT 3'-UTR (Fig. 3B). The results suggested that KAT8 may serve as a potential target of miR-149-5p. Since miRNAs can circulate in the blood and cerebrospinal fluid, they have been identified as biomarkers of various diseases, including AD (41). Subsequently, the expression of miR-149-5p in the plasma of patients with AD and healthy controls was assessed. miR-149-5p expression levels were significantly upregulated in the plasma of patients with AD compared with healthy subjects (Fig. 3C). Pearson's correlation coefficient analysis revealed a negative linear correlation between the expression level of miR-149-5p and KAT8 (Fig. 3D), which further indicated that KAT8 may serve as a potential target of miR-149-5p. To further clarify the negative relationship between miR-149-5p and KAT8, miR-149-5p

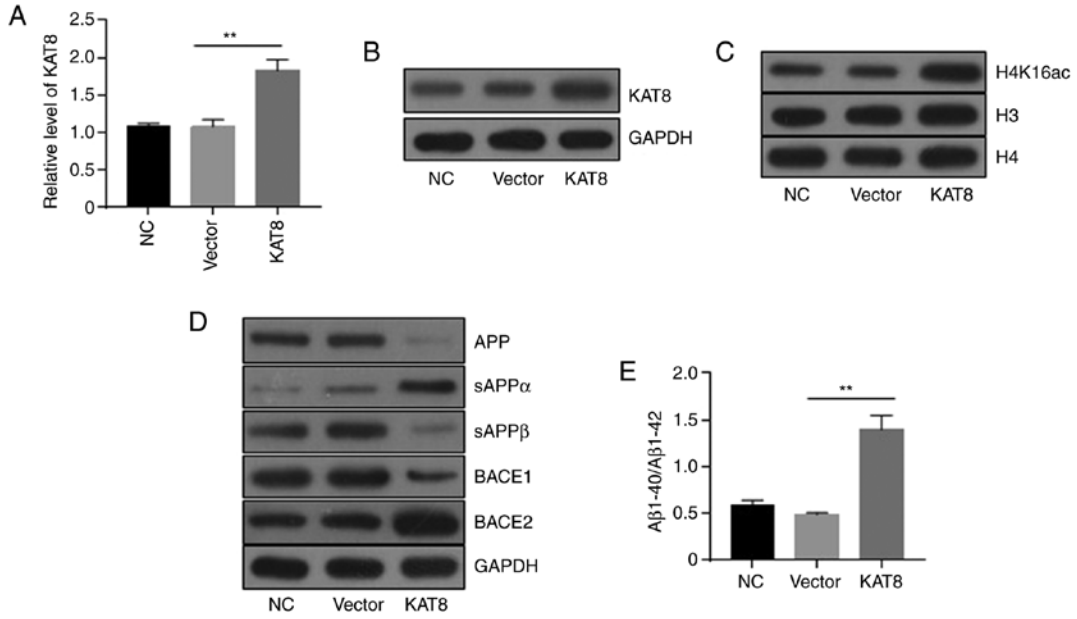

Figure 2. KAT8 overexpression increases H4K16 acetylation, inhibits amyloidogenic APP processing and reduces the $A\beta_{1-40}/A\beta_{1-42}$ ratio. KAT8 (A) mRNA and (B) protein expression levels in KAT8-overexpression 293/APPsw cells. (C) H4K16ac expression in KAT8-overexpression 293/APPsw cells. (D) KAT8 overexpression reduces protein expression levels of amyloidogenic APP, sAPPβ and BACE1, and increases neuroprotective sAPPα and BACE2 expression levels. (E) KAT8 overexpression reduces the $A\beta_{1-40}/A\beta_{1-42}$ ratio. **P<0.01. KAT8, lysine acetyltransferase 8; APP, amyloid precursor protein; sAPP, soluble APP; Aβ, β-amyloid; BACE, β-secretase; NC, negative control.

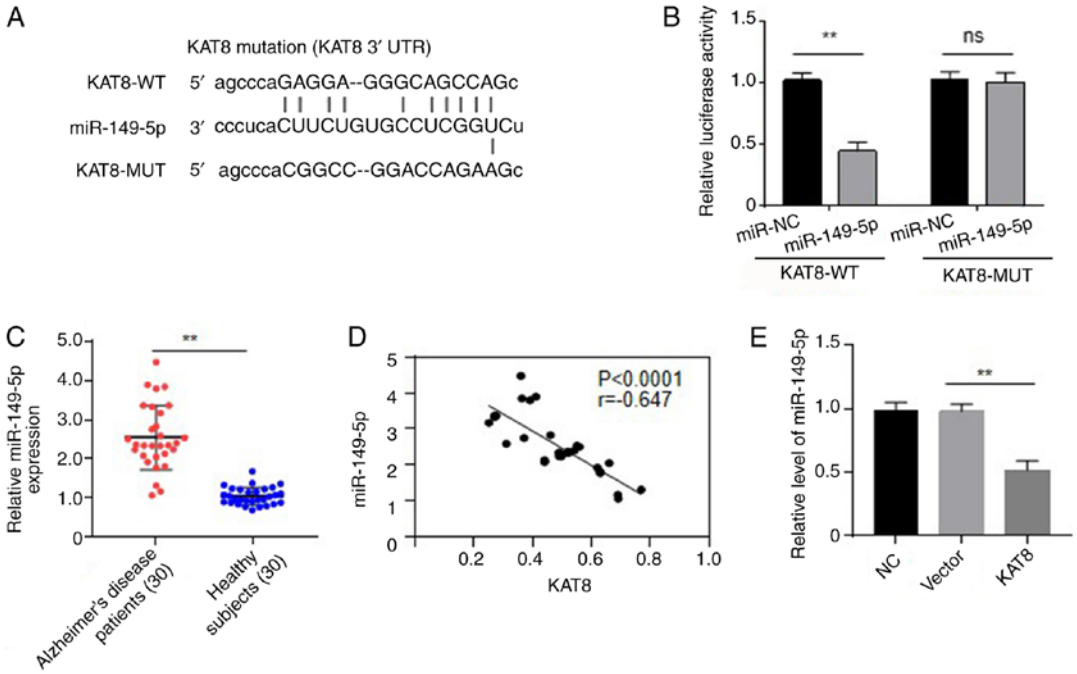

Figure 3. KAT8 is a direct target of miR-149-5p. (A) The binding site between KAT8 and miR-149-5p. (B) Luciferase activity in 293/APPsw cells co-transfected with KAT8-WT or KAT8-MUT and miR-NC or miR-149-5p mimic, **P<0.01 compared with miR-NC group. (C) miR-149-5p expression level in the plasma of patients with AD (n=30) compared with healthy subjects (n=30). (D) Pearson's correlation coefficient analysis identified a negative correlation between serum miR-149-5p and KAT8 in patients with AD. (E) Effect of KAT8 overexpression on miR-149-5p expression. **P<0.01. KAT8, lysine acetyltransferase 8; miR, microRNA; WT, wild-type; MUT, mutated; NC, negative control; AD, Alzheimer's disease; UTR, untranslated region; ns, not significant.

expression was assessed in KAT8-overexpression 293/APPsw cells. The RT-qPCR results suggested that miR-149-5p expression was significantly decreased in KAT8-overexpression cells compared with vector control cells (Fig. 3E). Collectively, the results indicated that KAT8 may serve as a potential target of miR-149-5p.

miR-149-5p negatively regulates H4K16ac and promotes amyloid pathology by targeting KAT8 in 293/APPsw cells. To explore the effect of miR-149-5p on the pathological alterations associated with KAT8 in patients with AD, the levels of H416Kac, sAPPα, sAPPβ, BACE1 and BACE2 were assessed following KAT8 knockdown and co-transfection with

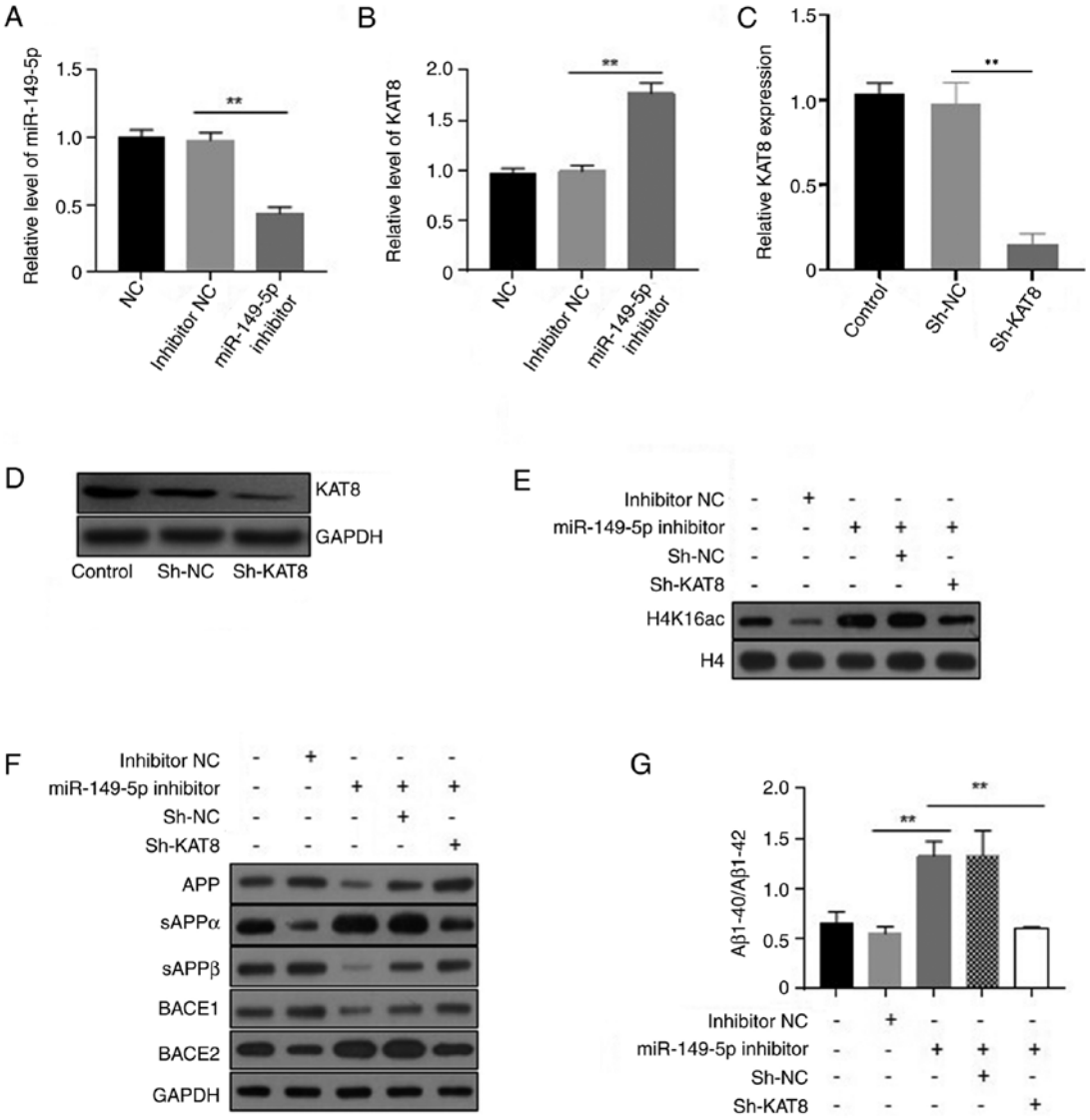

Figure 4. miR-149-5p inhibition exhibits neuroprotective properties in 293/APPsw cells, which are blocked by KAT8 knockdown. (A) Transfection efficiency of miR-149-5p inhibitor. (B) Effect of miR-149-5p inhibitor on KAT8 expression. Transfection efficiency of sh-KAT8 as determined by (C) reverse transcription-quantitative PCR and (D) western blotting. (E) Effect of miR-149-5p inhibitor and sh-KAT8 on H4K16ac, (F) APP, sAPPα, sAPPβ, BACE1, BACE2 and (G) the ratio of Aβ1-40/Aβ1-42. **P<0.01. miR, microRNA; KAT8, lysine acetyltransferase 8; sh, short hairpin RNA; APP, amyloid precursor protein; sAPP, soluble APP; BACE, β-secretase; Aβ, β-amyloid; NC, negative control.

miR-149-5p inhibitor in 293/APPsw cells. miR-149-5p inhibitor significantly decreased miR-149-5p expression and increased KAT8 mRNA expression in 293/APPsw cells compared with the inhibitor NC group (Fig. 4A and B). The results indicated that exogenous miR-149-5p may inhibit KAT8 expression via mRNA destabilization. Based on the finding that KAT8 promoted H4K16 acetylation, whether KAT8 knockdown and co-transfection with miR-149-5p inhibitor altered H4K16ac levels was investigated. The knockdown efficacy of sh-KAT8 was assessed (Fig. 4C and D). Compared with the inhibitor NC group, the western blotting results indicated that miR-149-5p inhibitor markedly increased H4K16ac levels, which were notably suppressed by co-transfection with KAT8 shRNA. miR-149-5p inhibitor markedly reduced sAPPβ and BACE1 expression levels, increased sAPPα and BACE2 expression levels, and increased the $A\beta_{1-40}/A\beta_{1-42}$ ratio compared with the inhibitor NC group. Moreover, co-transfection of sh-KAT8 and miR-149-5p inhibitor reversed miR-149-5p inhibitor-mediated effects, resulting in increased levels of sAPPβ and BACE1, and a decreased $A\beta_{1-40}/A\beta_{1-42}$ ratio, but reduced APPα and BACE2 production (Fig. 4F and G). In summary, the results indicated that inhibiting miR-149-5p delivery could promote amyloidogenic APP processing and decrease the $A\beta_{1-40}/A\beta_{1-42}$ ratio by upregulating KAT8 and H4K16ac expression.

Discussion

Several reports indicated that histone acetylation, one of the main epigenetic modifications, could contribute to AD onset and progression (17,19,42). The present study compared acetylation of several core histones in the plasma of patients with AD and cognitively healthy individuals. H4K16ac was significantly downregulated in the plasma of patients with AD compared with healthy subjects. In addition, KAT8 expression levels, the major lysine acetyltransferase responsible for the acetylation of H4K16 in flies and mammals (29), were

decreased in patients with AD compared with healthy subjects, which implied that aberrant expression patterns of H4K16ac and KAT8 could be related to the progression of AD. AD is a multifactorial neurodegenerative disease, and the abnormal deposition of Aβ is a critical feature of the disease (43). The present study indicated that KAT8 overexpression increased the level of H4K16ac in 293/APPsw cells compared with the NC and vector groups. Moreover, compared with the NC and vector groups, KAT8 overexpression also increased expression levels of neuroprotective sAPPα and BACE2, and significantly decreased the levels of neurotoxic sAPPβ and BACE1, which suggested that KAT8 may serve as a critical player in APP processing via regulating acetylation of core histones.

Specific miRNAs have been reported to participate during the initiation and progression of AD (9,44,45); however, the effect of miRNAs on histone acetylation and deacetylation during AD development is not completely understood. To the best of our knowledge, for the first time, the present study identified a novel inhibitory interaction between miR-149-5p and KAT8 3'-UTR that participated in the pathological alterations of an AD cell model via regulation of H416Kac levels. Numerous dysregulated miRNAs such as miR-223 (46), miR-455-3p (47), and miR-16 (48) were reported among patients with AD, animal AD models and AD model cell lines, which served an essential role in amyloidogenic APP processing, synaptic dysfunction and other pathophysiological processes of AD. Although previous studies revealed that miR-149-5p is involved in cell migration, proliferation, apoptosis and major signaling pathways in various cell carcinomas (49,50), the expression profile of miR-149-5p in AD has not been previously reported. The present study indicated that miR-149-5p was significantly increased in the plasma of patients with AD and the AD cell model compared with healthy subjects, and NC and vector cells, respectively. Additionally, the dual-luciferase reporter assay indicated a negative relationship between miR-149-5p and KAT8 in the plasma of patients with AD, which might promote AD pathogenesis via regulating KAT8 expression levels. Furthermore, miR-149-5p displayed neurotoxic effects on 293/APPsw cells, as indicated by miR-149-5p inhibitor reducing APP and Aβ levels in 293/APPsw cells via regulating KAT8 expression and H4K16 acetylation.

The present study suggested that miR-149-5p could negatively regulate KAT8 and H4K16ac *in vitro*. Since histone acetylation serves a critical role in learning and memory consolidation (37), the present study revealed that KAT8 overexpression increased H4K16 acetylation compared with the NC and vector groups. KAT8-induced increases in H4K16 acetylation altered histone-associated heterochromatin structures, which could regulate a variety of AD-related gene transcriptions and suppress neurotoxic APP processing. The effect of miR-149-5p knockdown on KAT8 expression and Aβ formation requires further investigation. In conclusion, the present study suggested a potential novel approach to attenuate the pathological progression of AD by reducing miR-149-5p expression.

The present study had a key limitation; 293 cells carrying the Swedish mutation of APP (293/APPsw) were used as an established AD cell model. However, 293 cells lacking the Swedish mutation of APP were not used as a control group to evaluate whether the inhibitory effect of miR-149-5p on amyloid-β generation was due to the APPsw mutation. Therefore, future studies should use 293 cells lacking the Swedish mutation of APP as a control group to verify the results of the present study.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FC contributed to designing the study, and collecting, analyzing and interpreting the data. FC, HC, YJ, HL and QT contributed to collecting the data, drafting the manuscript and performing the literature search. XZ contributed to designing the study and drafting the manuscript. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of First Teaching Hospital of Tianjin University of Traditional Chinese Medicine. Written informed consent was obtained from all patients and healthy subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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