Mitochondrial biogenesis mediated by melatonin in an APPswe/PS1dE9 transgenic mice model

ChaoYuan Song, MaoYu Li, LinLin Xu, Yang Shen, Hui Yang, Mao Ding, XiangTian Liu and ZhaoHong Xie

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disease, but the pathogenesis is unclear. Damaged mitochondrial biogenesis has been observed in AD. Increasing evidence suggests that mitochondrial biogenesis is involved in the pathogenesis of AD, but the exact mechanism is unclear. In this study, we used the amyloid precursor protein Swedish mutations K594N/M595L (APPswe)/presenilin 1 with the exon-9 deletion (PS1dE9) transgenic mouse model of AD, which was successfully established by the expression of amyloid β precursor protein and presenilin 1 (PS1). Then, we compared APPswe/PS1dE9 transgenic mice with and without melatonin (MT) in drinking water for 4 months (estimated 0.5 mg/day) and control C57BL/6J mice without MT for expression of mitochondrial biogenesis factors (mitochondrial transcription factor A, nuclear respiratory factor 1 and 2, peroxisome proliferator-activated receptor γ coactivator 1-α), mitochondrial structure, mitochondrial DNA to nuclear DNA ratio, behavioral changes, and amyloid β (Aβ) deposition and soluble Aβ levels in the cerebral cortex and hippocampus. Compared with controls, APPswe/ PS1dE9 mice with long-term MT intake showed increased levels of mitochondrial biogenesis factors, alleviated mitochondrial impairment, enhanced mitochondrial DNA copy number, improved spatial learning and memory deficits, and reduced Aβ deposition and soluble Aβ levels. Defective mitochondrial biogenesis may contribute toward the damaged mitochondrial structure and function in AD. MT may alleviate AD by promoting mitochondrial biogenesis. *NeuroReport* 29:1517–1524 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

Introduction

Alzheimer's disease (AD) is a chronic, progressive, irreversible neurodegenerative disorder pathologically characterized by amyloid β (Aβ) plaques, neurofibrillary tangles, and neuronal cell loss [1,2]. The most common early symptom is difficulty in remembering recent events (short-term memory loss) [3]. As the disease advances, symptoms can include problems with language, disorientation (including easily getting lost), mood swings, loss of motivation, and behavioral issues [4]. According to the Alzheimer's Disease International annual report, about 46.8 million individuals worldwide had dementia in 2015. In 2050, the estimated number of cases will be 131.5 million, 50–75% of them with AD.

Mitochondria are considered the energy provider in eukaryotic cell activity. Growing evidence indicates that mitochondrial dysfunction is the important cause of diseases such as neurodegenerative disease. Damage to mitochondrial

biogenesis plays an important role in mitochondrial dysfunction. Mitochondrial biogenesis is regulated by the peroxisome proliferator-activated receptor γ coactivator 1-α-nuclear respiratory factor 1 and 2-mitochondrial transcription factor A pathway (PGC-1α-NRF-TFAM). Recent research showed decreased expression of PGC-1α, NRF1, NRF2, and TFAM in the brains of AD patients and cells stably expressing amyloid β precursor protein (APP) [5]. In addition, the number of mitochondria was significantly reduced [6], which suggested that mitochondrial biogenesis was blocked in AD. An important cause of mitochondrial dysfunction in the process was impaired mitochondrial biogenesis [7].

Melatonin (MT) is a tryptophan metabolite synthesized mainly by the pineal gland and also synthesized by the mitochondria. It has many biological functions, such as regulating circadian rhythm, scavenging free radicals, improving immunity, and antioxidation [8]. MT was found to be significantly reduced in the brain of AD patients, and was found to be involved in sleep disorders with AD. In addition, MT plays an important role in the pathogenesis of AD [9]. Recent studies have shown that MT is synthesized in the pineal gland and also in other

cells. The concentration of MT is higher in the mito-chondria than in the plasma, cerebrospinal fluid, and peripheral blood [10]. MT can be transferred by peptide transporters 1 and 2. It is essential for maintaining the stability of mitochondrial morphology and function [10]. Finally, the pathological changes and cognitive function of APP/presenilin 1 (PS1) mice were found to be alleviated with oral MT treatment [11].

Increasing evidence suggests that mitochondrial biogenesis is involved in the pathogenesis of AD, but the exact mechanism is unclear. In this study, we treated APP/PS1 mice with MT to observe the expression of mitochondrial biogenesis factor, mitochondrial structure, mitochondrial DNA (mtDNA) to nuclear DNA (nDNA) ratio, behavioral changes, and Aβ deposition and soluble Aβ levels in the cerebral cortex and hippocampus. We aimed to analyze the role of mitochondrial biogenesis in the AD mouse model and the effect of MT on defective mitochondrial biogenesis and its underlying mechanisms.

Materials and methods
Animals and tissues
Male mice were used in this study to avoid the possible influence of sex on Aβ plaque formation in AD [12]. All mice were purchased from Beijing HFK Bio-Technology Co., Beijing, China. Mice were housed at $25 \pm 2°C$ with a relative humidity of $70 \pm 5°C$ in controlled rooms on a 12/12 h light, dark cycle. Treatment of mice followed the guidelines of the ethical committee for animal experiments of Shandong University (Jinan, China).

The APP/PS1 mouse is an AD mice model harboring mutant human transgenes of APP and presenilin 1 (PS1) [13]. Aβ deposition appears in this mouse model early in life. Some research has shown that Aβ plaques were present at 4 months of age and increased with age [14]. We used C57 mice as a control. Mice were divided into three groups ($n = 15$/group): C57 mice, APP/PS1 mice, and APP/PS1 mice that received MT in 4% PBS (100 mg/l) from 4 to 8 months of age, when they were killed. Mice drink an average of 5 ml water/day; thus, their daily MT intake was estimated at 0.5 mg. Controls received PBS in water. Finally, there was 4% PBS in the drinking water.

Morris water maze
Behavior of mice was detected by the Morris water maze (MWM) after a 4-month treatment with MT. The aim of the MWM test was to test the spatial learning and memory ability of mice [15]. The maze consisted of a circular pool filled with water. Within the pool, there were four quadrants. A platform was placed in the center of a quadrant and submerged 1 cm below water. A camera was placed on the top of the MWM to track the swimming path of mice. In the place navigation test, mice had to find the platform within 60 s after they were released into water and the time until they found the platform was recorded. If they could not find the platform within 60 s,

escape latency was recorded as 60 s and mice were guided to the platform. Mice underwent four trials per day. The place navigation test was performed for 5 consecutive days. At 24 h after the end of testing, the hidden platform was removed and the spatial probe test was performed. Mice were placed in the water for 60 s to find the platform. The number of times the platform was crossed and the time spent in the target quadrant were recorded. All of the results were analyzed using the software of SPSS (SPSS Inc., Chicago, Illinois, USA).

Western blot analysis
After the MWM test, mice were deeply euthanized by 10% chloral hydrate, followed by cervical dislocation. The cerebral cortex and hippocampus from one hemisphere were isolated from three mice of each group. Tissues were homogenized in ice-cold RIPA lysing buffer and then centrifuged at $12\,000g$ for 15 min at 4°C. The protein level of samples was tested using the bicinchoninic acid method. Protein samples were electrophoresed by SDS-PAGE (140 V) with a 10% separation gel and a 6% stacking gel, and transferred by constant current (180 mA, 1.5 h) to a polyvinylidene difluoride membrane that was blocked with milk powder at room temperature for 1 h and incubated overnight at 4°C with the following primary antibodies: rabbit anti-APP (1 : 1000), rabbit anti-PS1 (1 : 1000), rabbit anti-TFAM (1 : 5000; Abcam, Cambridge, Massachusetts, USA), rabbit anti-NRF1 (1 : 3000; Abcam), rabbit anti-NRF2 (1 : 3000), rabbit anti-PGC-1α (1 : 1000; Abcam); or mouse β-actin (1 : 1000; Golden Bridge International, Beijing, China). After washing with TBST three times, membranes were incubated at room temperature for 1.5 h with secondary antibodies: goat anti-rabbit IgG (1 : 5000) and goat anti-mouse IgG (1 : 5000; Golden Bridge International). Immunoreactive bands were visualized using electrochemiluminescence. The density of bands was analyzed by Image J (National Institutes of Health, Bethesda, Maryland, USA).

Transmission electron microscopy
The mice were deeply euthanized by 10% chloral hydrate, followed by cervical dislocation after the MWM test. We used three mice of each group for TEM. According to the conventional TEM sample preparation method [16], the brains were rapidly removed after mice were killed and cut into 1-mm-thick blocks, placed in glutaraldehyde, and embedded using Epon 812 (Sigma-Aldrich, St. Louis, Missouri, USA). Tissues were collected on formvar-coated, single-slot grids, and viewed by transmission electron microscopy.

Real-time PCR to detect mitochondrial DNA copy number
Mice were deeply euthanized by 10% chloral hydrate, followed by cervical dislocation after the MWM test. Brains were rapidly removed after mice were killed and cut into 1-mm-thick blocks for TEM. The rest of the

brains were used for Real-time PCR (three mice per group). MtDNA copy number was measured by real-time PCR using the Step one plus real-time PCR System (Applied Biosystems, Carlsbad, California, USA) with the SYBR Green detection method. Total intracellular DNA in the cortex and hippocampus CA1 neuronal cells was extracted using the QIAamp DNA mini kit (Qiagen, Germantown, Maryland, USA) following the manufacturer's instruction. Each real-time PCR reaction (20 μl total volume) contained 3 μl template DNA (50 lg), 10 μl of $2\times$ SYBR Green Real-time PCR Master (Applied Biosystems), 1 μl each forward and reverse primers, and 5 μl ultrapure water. The primers for a subunit of the human electron transport chain used for mtDNA amplification were as follows: forward, 5′-CAAAC-CTACGCCAAAATCCA-3′ and reverse, 5′-GAAATGA-ATGAGCCTACAGA-3′ [17]. The mtDNA primers (human complex II, fragment length 164 bp) were designed to minimize amplification of the mtDNA pseudogenes embedded in the nuclear DNA. Primers for human nuclear 18 s, as an internal control, were as follows: forward, 5′-ACGGACCAGAGCGAAAGCA-3′ and reverse, 5′-GACATCTAAGGGCATCACAGAC-3′. Relative amounts of mtDNA and nDNA copy numbers were compared. The $\Delta\Delta C_t$ (mtDNA to 18 s) represents the mtDNA copy number in a cell [18].

Thioflavin S
After the MWM test, the mice were anesthetized with chloral hydrate, and then immediately cardiac perfused with a 0.9% saline solution, followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). After the perfusion, the brains were excised and postfixed overnight at 4°C. The brain tissue was then incubated in 30% sucrose at 4°C until equilibration (six mice per group). Brain sections were removed and cut in a sagittal plane at 30 μm thickness on a freezing microtome and mounted on slides. Slides were stained with 1% thioflavin-S for 5 min, and rinsed with 70% ethanol and distilled water. Images of plaques were captured with a camera connected to a fluorescence microscopy (1 × 71S1F; Olympus Tokyo, Japan). Plaque burden was calculated as percentage of the thioflavin-S staining area to the total area. Image Pro Plus 6 Media Cybernetics (Silver Spring, Maryland, USA) was used to analyze images.

ELISA
Mice were deeply euthanized by 10% chloral hydrate, followed by cervical dislocation after the MWM test. Brain samples were isolated from the mice (three mice per group). Hemibrains were flash frozen and stored at −80°C until homogenization. The secretion of $A\beta_{1-40}$ and $A\beta_{1-42}$ in brain tissue was assessed by ELISA. Brain tissues were lysed in guanidine-Tris buffer and centrifuged at 12 000 rpm/min for 30 min; 50 μl sample was added to each well of plates, then incubated with antibodies for $A\beta_{1-40}$ and $A\beta_{1-42}$, and quantified by ELISA

kits (Invitrogen, Carlsbad, California, USA) following the manufacturer's instruction. The wavelength of absorbance was set at 450 nm.

Statistical analysis
All data were expressed as mean ± standard error. Student's *t*-test was used to compare two groups. In two-variable experiments, two-way repeated-measures analysis of variance was used to evaluate the significance of differences between group means. *P* value less than 0.05 was considered statistically significant. All experiments were repeated independently at least three times. Data were analyzed using SPSS 17.0 software (SPSS Inc.).

Results
Effect of melatonin on protein expression of transcription factor A pathway, nuclear respiratory factor 1, nuclear respiratory factor 2, and proliferator-activated receptor γ coactivator 1-α
We verified the AD mouse model by measuring the protein level of APP and PS1. The expression of APP and PS1 was greater in APPswe/PS1dE9 transgenic (APP/PS1) than C57 mice (Fig. 1a). The protein expression of TFAM, NRF1, NRF2, and PGC-1α in the cortex and the hippocampus was lower in APP/PS1 than C57 mice ($P < 0.05$); MT restores the expression of TFAM, NRF1, NRF2, and PGC-1α in APP/PS1 mice (Fig. 1b and c). Thus, MT promotes mitochondrial biogenesis in AD in mice.

Effect of melatonin on the mitochondrial structure in Alzheimer's disease mice
The mitochondrial structure was severely damaged in cortex and hippocampus CA1 neuronal cells of APP/PS1 mice compared with C57 mice (Fig. 2). The structures of mitochondrial membrane and mitochondrial crista were obviously destroyed. Arrows showed altered structures: (a) mitochondria split rings formed; (b) mitochondria swelling with broken cristae; And (c) mitochondria split rings significantly increased. MT could reverse the damaged mitochondrial structure.

Effect of melatonin on mitochondrial DNA copy number
The number of mitochondria was significantly reduced in neurons of mice with AD (Fig. 3). The mtDNA/nDNA ratio in the cortex and hippocampus was lower in APP/PS1 than C57 mice. However, with MT treatment for 4 months, the mtDNA/nDNA ratio was increased significantly in APP/PS1 mice.

### Effect of melatonin treatment on Morris water maze behavior
The escape latency was longer for APP/PS1 than C57 mice, and MT treatment could ameliorate the latency in APP/PS1 mice (Fig. 4a). The effect of MT was similar for the number of platform crosses and the time spent in the target quadrant (Fig. 4b and c). The swimming route to

Fig. 1
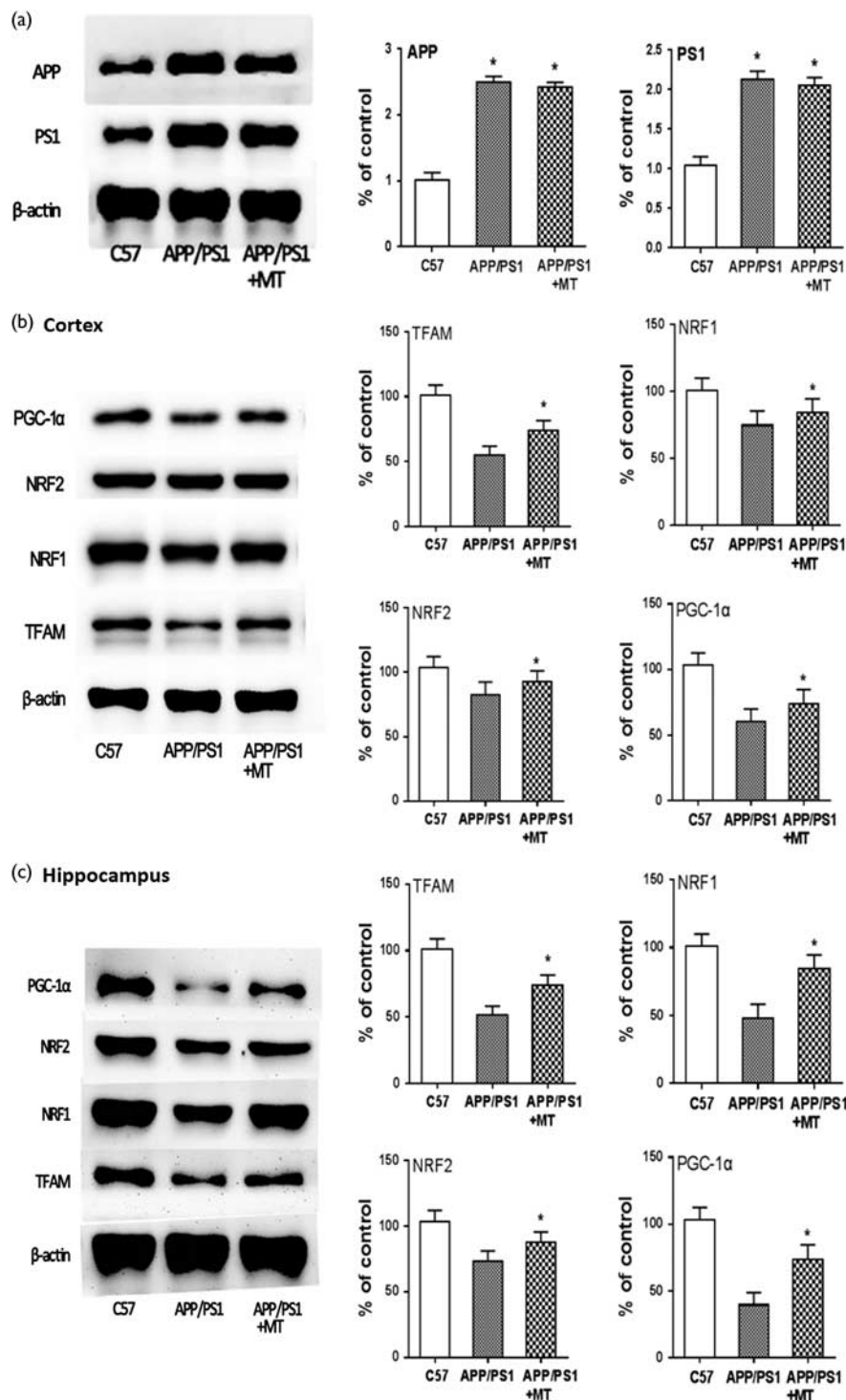
Verification of AD mouse model and expression of mitochondrial biogenesis factors. Western blot analysis of protein levels of APP and PS1 (a) and TFAM, NRF1, NRF2, and PGC-1α in the cortex (b) and hippocampus (c) of APP/PS1 and C57 mice. The scanned image of western blot was analyzed using the software Image J. Data are mean ± SE; *$P < 0.05$ versus APP/PS1 alone. AD, Alzheimer's disease; APP, amyloid precursor protein; MT, melatonin; NRF, nuclear respiratory factor 1; PGC-1α, proliferator-activated receptor γ coactivator 1-α; PS1, presenilin 1; TFAM, transcription factor A pathway.

Fig. 2
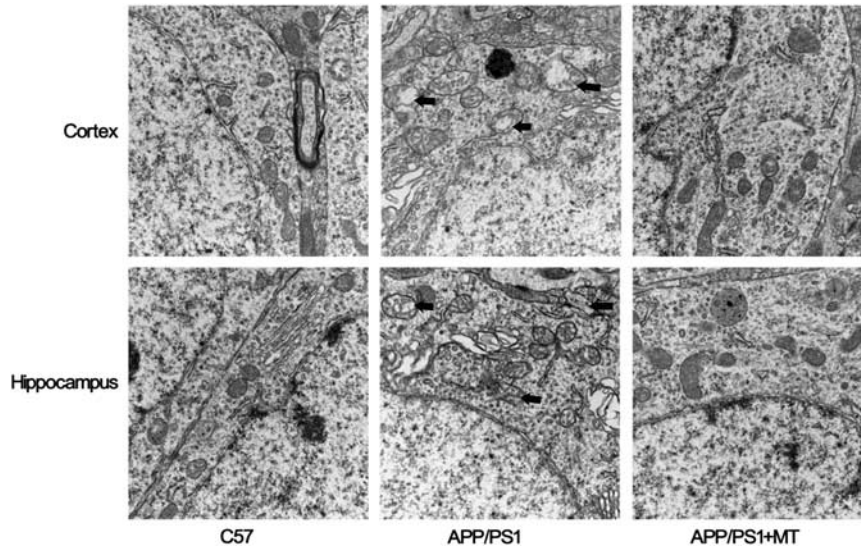
Melatonin alleviated the damaged mitochondrial structure in the AD mouse model. Arrows show the altered structure of mitochondria. Transmission electron microscopy of the mitochondrial structure of cortex and hippocampus CA1 neuronal cells in APP/PS1 mice with and without melatonin and in C57 mice. AD, Alzheimer's disease; APP, amyloid precursor protein; MT, melatonin; PS1, presenilin 1.

Fig. 3
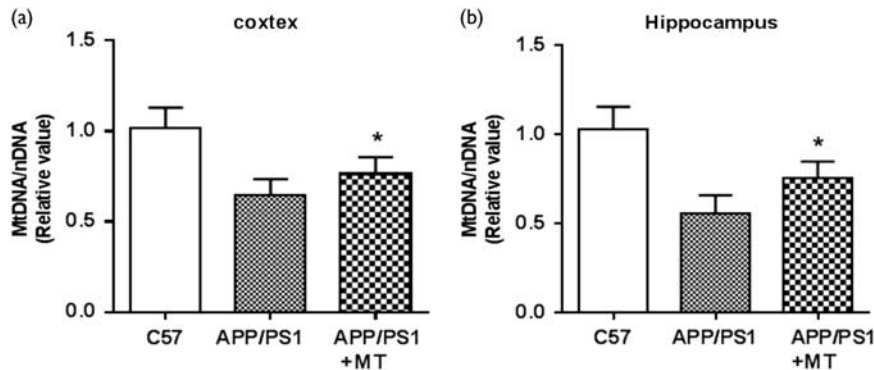
Effect of melatonin on the mtDNA/nDNA ratio in cortex and hippocampus CA1 neuronal cells of mice with AD. Relative amounts of mtDNA and nDNA copy numbers were measured by real-time PCR. Data are mean $\pm$ SE; *$P < 0.05$ versus APP/PS1 alone. AD, Alzheimer's disease; APP, amyloid precursor protein; mtDNA, mitochondrial DNA; MT, melatonin; nDNA, nuclear DNA; PS1, presenilin 1.

the previous goal location was longer for APP/PS1 than C57 mice (Fig. 4e) and was shorter with MT treatment. However, the swimming speed of the three groups did not differ (Fig. 4d). Hence, MT could alleviate the spatial learning and memory deficits in APP/PS1 mice and similar results have been shown previously by O'Neal-Moffitt *et al.* [11].

**Effect of melatonin on amyloid β plaques and levels of Aβ$_{1-40}$ and Aβ$_{1-42}$**

We used thioflavin-S staining to examine Aβ plaques. The number of plaques in the cortex and hippocampus was significantly greater in APP/PS1 than C57 mice (Fig. 5a and b). MT reduced the number of plaques in

APP/PS1 mice. We used ELISA to examine the levels of Aβ$_{1-40}$ and Aβ$_{1-42}$. The levels of Aβ$_{1-40}$ and Aβ$_{1-42}$ were higher in APP/PS1 than C57 mice ($P < 0.05$); MT could reverse the levels in APP/PS1 mice ($P < 0.05$; Fig. 5c and d). Similar results have been reported previously by O'Neal-Moffitt *et al.* [11].

Discussion

In this study, we observed the effect of MT on the regulators of mitochondrial biogenesis in the model. We also studied the relation between mitochondrial biogenesis and the AD model. Compared with the controls, APP/PS1 mice with long-term oral MT intake showed increased levels of mitochondrial biogenesis factors, alleviated mitochondrial

Fig. 4
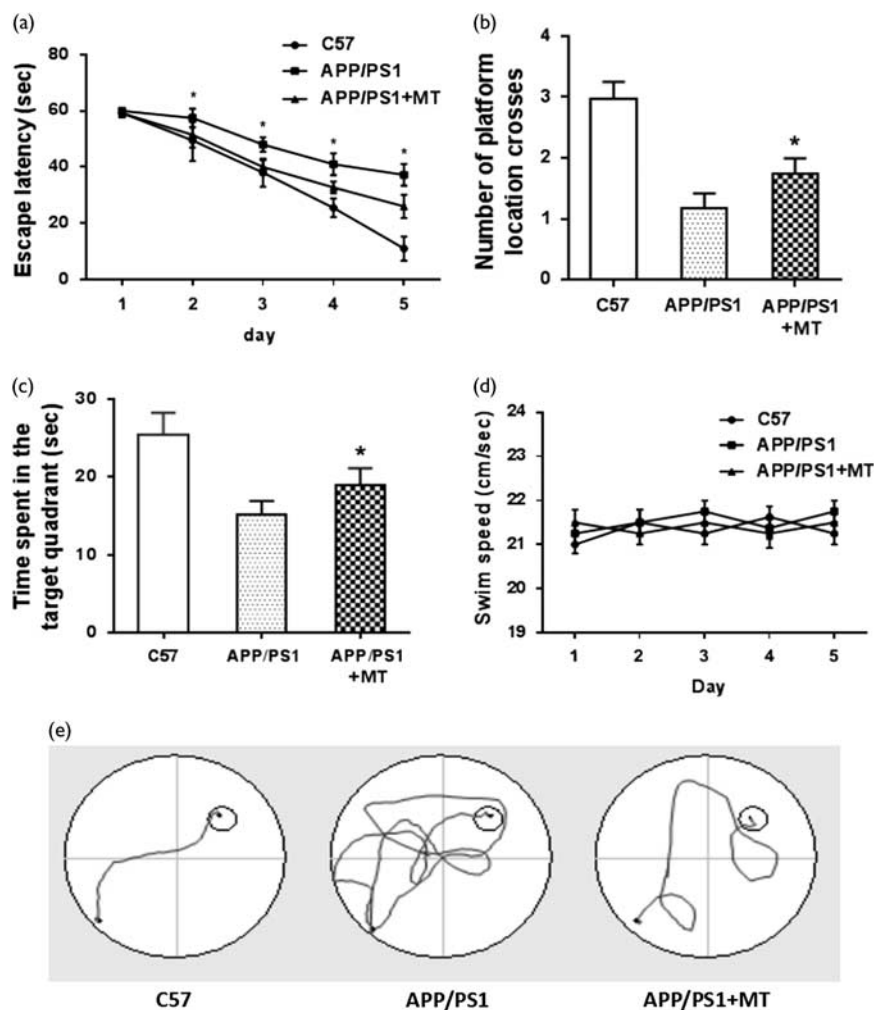
Melatonin improved spatial learning and alleviated memory impairments in mice with AD by the Morris water maze test. (a) Spatial learning measured as escape latency per day (*n* = 15 mice/group).(b, c). Spatial memory evaluated by the number of platform location crossings. (b)Time spent in the target quadrant (c). (d) Swimming speed. (e) Representative swimming route to previous goal location after 5 days of training. Data are mean ± SE; *P < 0.05 versus APP/PS1 alone. AD, Alzheimer's disease; APP, amyloid precursor protein; MT, melatonin; PS1, presenilin 1.

impairment, enhanced mtDNA copy number, improved spatial learning and memory deficits, and reduced Aβ plaque deposition and soluble Aβ levels.

NRF1 and NRF2, which control the nuclear genes to encode mitochondrial protein, and TFAM, which drives transcription and replication of mtDNA, were significantly increased in APP/PS1 mice with long-term oral MT compared with controls as shown in Fig. 1. We believed that MT alleviated mitochondrial impairment, and enhanced mtDNA copy number through improving the expression levels of TFAM, NRF1, and NRF2. The expressions of NRF1, NRF2, and TFAM are regulated by PGC-1α [19]. Therefore, we speculate that MT can improve the structure and function of the mitochondria by the PGC-1α-NRF-TFAM pathway.

In Fig. 5, MT can reduce Aβ plaque deposition and soluble Aβ levels. In addition, MT can improve spatial

learning and memory of APP/PS1 mice as shown in Fig. 4. Similar results have been reported previously by O'Neal-Moffitt *et al.* [11]. Their findings showed that prophylactic MT significantly reduced AD neuropathology and associated cognitive deficits in a manner that was independent of antioxidant pathways. However, they did not reveal the exact mechanism of MT's action. According to Sheng *et al.* [7], they reported that damaged mitochondrial biogenesis was associated closely with mitochondrial dysfunction and played an important role in the pathogenesis of AD. In our study, MT can increase the expression levels of TFAM, NRF1, NRF2, and PGC-1α. Therefore, we speculate that the PGC-1α-NRF-TFAM pathway plays an important role in the pathogenesis of AD and the increase in spatial learning and memory and the decline in Aβ plaque deposition and soluble Aβ levels may have a potential connection with

Fig. 5
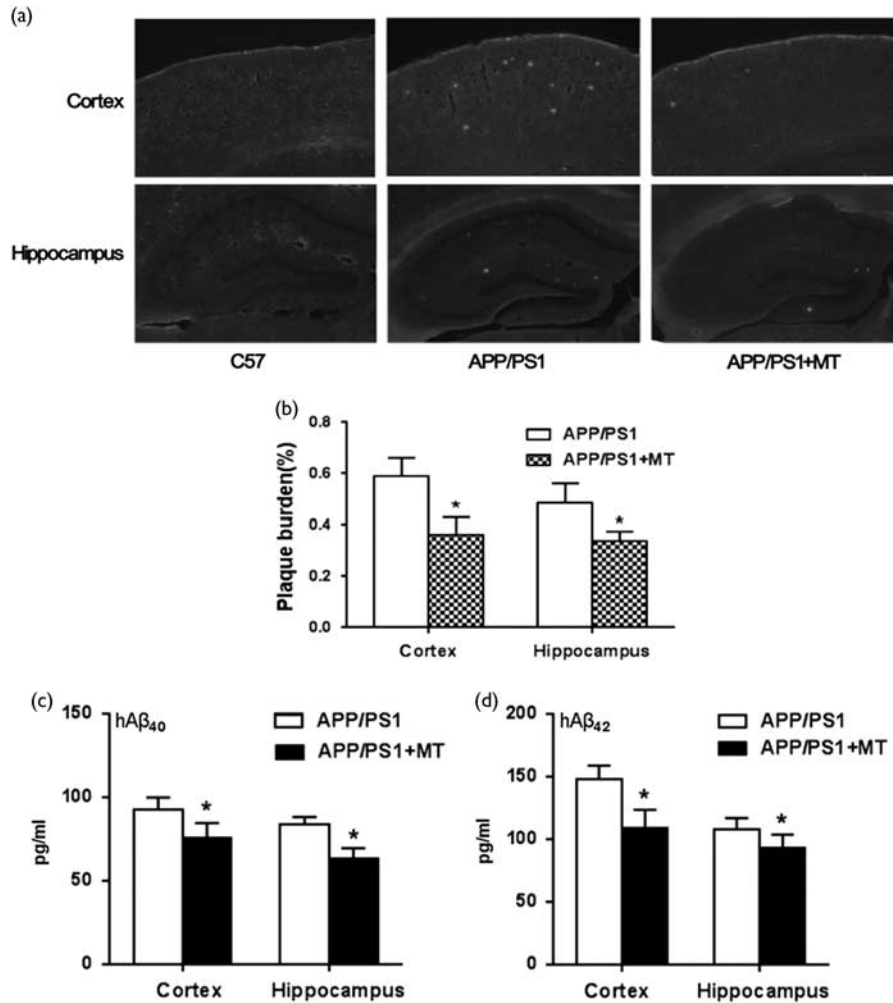
Melatonin reduced Aβ deposition. (a) Thioflavin-S staining for senile plaques of the cerebral cortex and hippocampus in mice. Scale bar, 200 μm. (b) Quantification of thioflavin-S staining ($n = 6$ mice/group). The plaque burden was calculated as the percentage of thioflavin-S staining area over the total area ($n = 6$ mice/group). Image Pro Plus 6 (Media Cybernetics) was used to analyze the images. Melatonin reduced soluble Aβ levels in AD mice. ELISA was used to detect $Aβ_{1-40}$ (c) and $Aβ_{1-42}$ (d) levels in the cortex and hippocampus of mice. Data are mean ± SE; *$P < 0.05$ versus APP/PS1 alone. MT, melatonin. Aβ, amyloid β; AD, Alzheimer's disease; APP, amyloid precursor protein; PS1, presenilin 1.

the PGC-1α-NRF-TFAM pathway. We infer that MT improves mitochondrial dysfunction by promoting mitochondrial biogenesis, resulting in an increase in spatial learning and memory and a decline in Aβ plaque deposition and soluble Aβ levels.

Overall, this study showed reduced mitochondrial biogenesis signaling in the AD mouse model. Defective mitochondrial biogenesis may contribute to mitochondrial dysfunction in AD, and the use of MT, which can enhance mitochondrial biogenesis, may be a potential approach for the treatment of AD.

Conflicts of interest
There are no conflicts of interest.

References
1 Katzman R, Saitoh T. Advances in Alzheimer's disease. *FASEB J* 1991; **5**:278–286.
2 Mattson MP. Pathways towards and away from Alzheimer's disease. *Nature* 2004; **430**:631–639.
3 Burns A, Iliffe S. Alzheimer's disease. *BMJ* 2009; **338**:b158.

4   Tokuchi R, Hishikawa N, Sato K, Hatanaka N, Fukui Y, Takemoto M, *et al.* Differences between the behavioral and psychological symptoms of Alzheimer's disease and Parkinson's disease. *J Neurol Sci* 2016; **369**:278–282.

5   Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci USA* 1994; **91**:1309–1313.

6   Hirai K, Aliev G, Nunomura A. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci* 2001; **21**:3017–3023.

7   Sheng B, Wang X, Su B, Lee HG, Casadesus G, Perry G, Zhu X. Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. *J Neurochem* 2012; **120**:419–429.

8   Miller E, Morel A, Saso L, Saluk J. Melatonin redox activity. Its potential clinical applications in neurodegenerative disorders. *Curr Top Med Chem* 2015; **15**:163–169.

9   Urrestarazu E, Iriarte J. Clinical management of sleep disturbances in Alzheimer's disease: current and emerging strategies. *Nat Sci Sleep* 2016; **8**:21–33.

10 Tan DX, Manchester LC, Qin L, Reiter RJ. Melatonin: a mitochondrial targeting molecule involving mitochondrial protection and dynamics. *Int J Mol Sci* 2016; **17**:2124.

11 O'Neal-Moffitt G, Delic V, Bradshaw PC, Olcese J. Prophylactic melatonin significantly reduces Alzheimer's neuropathology and associated cognitive deficits independent of antioxidant pathways in AβPP(swe)/PS1 mice. *Mol Neurodegener* 2015; **10**:27.

12 Casadesus G, Garrett MR, Webber KM, Hartzler AW, Atwood CS, Perry G, *et al.* The estrogen myth: potential use of gonadotropin-releasing hormone

agonists for the treatment of Alzheimer's disease. *Drugs R D* 2006; **7**:187–193.

13 Garcia-Alloza M, Robbins EM, Zhang-Nunes SX, Purcell SM, Betensky RA, Raju S, *et al.* Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. *Neurobiol Dis* 2006; **24**:516–524.

14 Ruan L, Kang Z, Pei G, Le Y. Amyloid deposition and inflammation in APPswe/PS1dE9 mouse model of Alzheimer's disease. *Curr Alzheimer Res* 2009; **6**:531–540.

15 Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* 2006; **1**:848–858.

16 Yan J, Liu XH, Han MZ, Wang YM, Sun XL, Yu N, *et al.* Blockage of GSK3β-mediated Drp1 phosphorylation provides neuroprotection in neuronal and mouse models of Alzheimer's disease. *Neurobiol Aging* 2015; **36**:211–227.

17 Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, Yano M, *et al.* Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes* 2006; **55**:120–127.

18 Schefe JH, Lehmann KE, Buschmann IR, Unger T, Funke-Kaiser H. Quantitative real-time RT-PCR data analysis: current concepts and the novel 'gene expression's CT difference' formula. *J Mol Med* 2006; **84**:901–910.

19 Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, *et al.* Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999; **98**:115–124.