Behavioural Brain Research 495 (2025) 115805

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research article

Downregulation of Nrf2 deteriorates cognitive impairment in APP/PS1 mice by inhibiting mitochondrial biogenesis through the PPARγ/PGC1α signaling pathway

Weigang Luo [a,b,1], Wei Bu [c,1], Guisong Zhang [a], Yujuan Dong [a], Yuling Wang [a], Jinyang Wang [a], Cuicui Liu [a], Xiaokai Hu [a], Yanan Jia [a], Huiling Ren [a,b,*]

[a] Department of neurology, Hebei Medical University Third Hospital, Shijiazhuang, Hebei 050000, China
[b] Hebei Key Laboratory of Neurodegenerative Disease Mechanism, Shijiazhuang, Hebei 050000, China
[c] Neurosurgery, Hebei Medical University Third Hospital, Shijiazhuang, Hebei 050000, China

ARTICLE INFO

Keywords:
Alzheimer's disease
Nrf2
PGC1α
PPARγ
Mitochondrial biogenesis
Cognitive impairment

ABSTRACT

Background: Mitochondrial dysfunction is considered to be an important pathogenesis of cognitive impairment in Alzheimer's disease(AD). Activation of Nrf2 can improve cognitive impairment in AD mice, but the underlying mechanism remains to be elucidated. This research aims to investigate the intrinsic molecular mechanism of Nrf2 in mitochondrial biogenesis related to cognitive impairment of AD mice.

Methods: APP/PS1 mice were used as AD model mice, and Nrf2 down-regulated mouse model was established by injecting lentivirus into hippocampus. Morris water maze test was used to evaluate the learning and memory ability of mice. The biochemical assays were used to detect the expression of Nrf2, mitochondrial biogenesis-related genes, and Aβ protein.Transmission electron microscopy was used to observe the number of mitochondria and synaptic structure in neurons. Chromatin immunoprecipitation was used to observe the binding of Nrf2 protein to the PGC1α promoter; Co-Immunoprecipitation was used to observe the interaction between PPARγ protein and PGC1α protein.

Results: Downregulation of Nrf2 reduced mitochondrial biogenesis, aggravated Aβ protein deposition and synaptic damage, and in turn aggravated cognitive impairment in mice. Compared with control mice, AD model mice had reduced levels of Nrf2, PPARγ, PGC1α, NRF1, TFAM protein, mitochondrial number and MAP2, increased Aβ protein deposition, and worsened synaptic damage and cognitive impairment. Lentivirus-induced Nrf2 downregulation downregulates PPARγ, PGC1α, NRF1, and TFAM protein expression, reduces mitochondrial number and MAP2 levels, and aggravates Aβ protein deposition, synaptic damage, and cognitive impairment. Nrf2 protein bound to the PGC1α gene promoter, and PPARγ protein interacted with PGC1α protein.

Conclusion: Nrf2 can directly regulate PGC1α transcription, and can also regulate PPARγ followed by binding to the PGC1α protein, thereby modulating mitochondrial biogenesis.Nrf2 downregulation reduces the expression of PPARγ and PGC1α proteins, thereby reducing their interaction. This suppression impairs mitochondrial biogenesis, exacerbates mitochondrial dysfunction, intensifies Aβ deposition and synaptic damage, and ultimately worsens cognitive impairment in AD mice.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and the leading cause of dementia. Its typical clinical manifestations are cognitive decline, mental and behavioral symptoms, and decreased ability to live daily. This condition is swiftly emerging as one of the most costly, lethal, and burdensome health crises of our time. Although there are many hypotheses about the mechanism of AD, and they have all contributed to understanding its onset and progression [1]. Unfortunately, there is currently no treatment that can effectively reverse the disease, and all treatments have a severely limited capacity to decelerate its progression [2].Therefore, it is crucial to elucidate the mechanisms of

* Corresponding author at: Department of neurology, Hebei Medical University Third Hospital, Shijiazhuang, Hebei 050000, China.
E-mail address: renhuiling@hebmu.edu.cn (H. Ren).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.bbr.2025.115805
Received 9 March 2025; Received in revised form 3 September 2025; Accepted 4 September 2025
Available online 5 September 2025

AD cognitive impairment, identify therapeutic targets, and conduct early intervention.

Mitochondrial dysfunction is considered an early event in AD [3,4]. Mitochondrial dysfunction is characterized by impaired mitochondrial biogenesis, reduced capacity of mitochondrial respiratory complexes, and decreased cellular mitochondrial membrane potential and ATP levels [5]. The balance of mitochondrial turnover processes, especially mitophagy and biogenesis, is crucial for cellular resilience and longevity [6]. It was found in alive and post-mortem brain tissue of AD patients that mitochondrial function was impaired [7]. In AD mouse models, impaired mitochondrial biogenesis precedes mitochondrial dysfunction and AD pathology, and impaired mitochondrial biogenesis signaling may contribute to the manifestation of mitochondrial dysfunction and AD pathology at later ages [8].Peroxisome proliferator-activated receptor γ coactivator 1-α(PGC1α) is a key regulator of mitochondrial biogenesis [9].A longitudinal study showed that all genes involved in mitochondrial biogenesis (including PGC1α) were significantly reduced in AD model mice compared with wild-type mice of the same age, indicating that mitochondrial biogenesis was impaired in AD mice [5].In addition, research indicates that activation of PGC1α can promote mitochondrial biogenesis, reduce amyloid beta(Aβ) deposition, and alleviate cognitive impairment in AD mice demonstrating the importance of PGC1α and mitochondrial biogenesis in the cognitive function of AD mice [10]. Therefore, exploring the mechanism of mitochondrial biogenesis in AD provides the possibility of intervening in the early stages of the disease.

Nuclear factor erythroid2-related factor 2 (Nrf2), as an important transcription factor in the body, regulates the expression of key components in mitochondrial biogenesis, autophagy, oxidative stress, mitochondrial function, and mitophagy in the central nervous system [11]. An increasing number of studies indicate that the level of Nrf2 transcription is closely related to aging and neurodegenerative diseases, especially Alzheimer's disease [12].The level of nuclear Nrf2 in the hippocampus and even cortex of AD patients is reduced [13]. Animal experimental studies indicate that Nrf2 is directly related to AD pathology, and the cognitive deficits of AD model mice lacking Nrf2 are exacerbated [14,15]. Moreover, inducing or activating Nrf2 can alleviate cognitive impairment in AD model mice [16,17].Studies have shown that dimethyl fumarate exerts a neuroprotective effect by activating the Nrf2 pathway and promoting mitochondrial autophagy in a Parkinson's mouse model [18]. However, the intrinsic molecular mechanism of Nrf2 related to mitochondrial biogenesis in AD still needs to be further elucidated. Therefore, this study used lentivirus to downregulate Nrf2 levels in AD mouse model, evaluate its effect on cognitive impairment in AD mice, and further explore its potential mechanism in PGC1α-dependent mitochondrial biogenesis, providing an effective target for the future treatment of AD.

2. Materials and methods

2.1. Animals

The animal experimental procedures were approved by the Experimental Animal Ethics Committee of the Third Hospital of Hebei Medical University (approval number: Z2022–004–2). Both 6-month-old male C57BL/6 J and APP/PS1 mice were provided by Beijing HFK Bioscience Co., Ltd. Animals had free access to food and water. Mice were housed under a 12-h light/dark cycle at room temperature, The temperature was 23 ± 2℃ and the humidity was 50 ± 5 %.

2.2. Experimental groups

C57BL/6 J and APP/PS1 mice were randomly divided into 2 groups respectively, with 12 mice in each group: C57BL/6 J with control lentivirus (C57 +GFP), C57BL/6 J with Nrf2-RNAi-lentivirus(C57 +Nrf2 shRNA), APP/PS1 with control lentivirus (APP+GFP), and APP/PS1 with Nrf2-RNAi-lentivirus (APP+Nrf2 shRNA). Referring to our previous study [19], mice were anesthetized via intraperitoneal injection of pentobarbital sodium (40 mg/kg), positioned in a stereotaxic frame (51600, Stoelting, USA), and underwent a midline scalp incision between the ears. A burr hole was drilled in the right skull at coordinates: posterior 2.7 mm, lateral 3.1 mm, and ventral 2.4 mm relative to bregma. Using a syringe pump (51600z, Stoelting, USA) equipped with a 27-gauge needle, 2 μL of Nrf2-RNAi lentivirus (6×10^8 TU/mL,Genechem, Shanghai, China) or lentiGFP was infused into the right hippocampal CA3 region at 0.5 μL/min. Lentiviral vectors encoding GFP alone served as RNAi controls. The needle was left in situ for 10 min post-injection, the scalp incision sutured, and mice allowed to recover in their cages. The experiments were started after the mice were raised to 10 months of age and the expression of the lentivirus was confirmed. Post-experiment, GFP expression at the injection site was validated by fluorescence microscopy, and the efficacy of Nrf2-specific shRNA confirmed by RT-PCR.

2.3. Morris water maze (MWM) test

The MWM test was used to evaluate the cognitive levels of mice such as spatial learning and memory [20]. The place navigation test was carried out for 5 days. At a fixed time every morning, mice were placed in water at four different orientations facing the side wall. Different visual cues were placed around them. The time required for the mice to find and climb onto the platform in the pool was recorded, which was the escape latency. If the animal did not find the platform within 60 s, its escape latency was recorded as 60 s. The experimenter guided it to the platform and let it stay on the platform for 15 s. 24 h after the place navigation test, the spatial exploration test was carried out. The hidden platform was removed. Each mouse was placed in the water at the same random starting position and allowed to swim freely for 60 s. The swimming traces of the mouse within 60 s, the time of activity in the target quadrant, and the number of times it crossed the area where the platform was located before were recorded. The movement of the mouse in all MWM tests was recorded and analyzed using a video tracking system (a camera combined with dedicated tracking software).

2.4. Quantitative real-time polymerase chain reaction (RT-PCR)

The Nrf-2 expression was quantified by real-time PCR. Total RNA was extracted from the right hippocampal tissues with Trizol reagent. The miRNA reverse transcription kit reverse transcribes RNA into cDNA, and the miRNA fluorescent quantitative RT-PCR detection kit is used to perform qRT-PCR using cDNA as a template. Then, the RT-PCR protocol was performed which was as follows: 1 cycle for 30 s at 95 ∘C, 40 cycles for 5 s at 95 ∘C, and 34 s at 60 ∘C. Primer sequences are listed in Table 1. The 2-ΔΔCt method was used to determine the mRNA relative differences of Nrf-2 in each group of samples, and it was normalized using the β-actin housekeeping gene.

2.5. Western blotting

The right hippocampus was extracted after deep anesthesia with

Table1
Primer sequences.

Name	Sequences
Nrf2-F	CCAGCACATCCAGACAGACAC
Nrf2-R	GATATCCAGGGCAAGCGACTC
β-actin -F	CGTGTTCCTACCCCCAATGT
β-actin -R	TGTCATCATACTTGGCAGGTTTCT
PGC1α−1-F	CCCTCATTGACTCAGGAACGAC
PGC1α−1-R	TTGAATAAACATTGGACTCCAA
PGC1α−2-F	CCAAAGGCCAAGTGTTTCCTTT
PGC1α−2-R	GGCACCTGTCTTACTACAGTCC

pentobarbital sodium (40 mg/kg i.p.). Total protein was extracted with a tissue and cell total protein extraction kit (P1250–50, Applygen, China), and protein concentrations were determined by the BCA method.The protein samples underwent separation using 10 % SDS-PAGE electrophoresis, after which they were transferred onto polyvinylidene fluoride (PVDF) membranes. These membranes were subsequently blocked with a 5 % skimmed milk solution for one hour. Following this, the membranes were treated with primary antibodies and left to incubate overnight in a refrigerator set at 4 °C. The primary antibodies used were as follows: anti-Aβ(1:1000, Cat No. 25524–1-AP, Proteintech), anti-Nrf2 (1:4000,Cat No.16396–1-AP,Proteintech),anti-PPARγ (1:1000,Cat. no. YT3836, Immunoway), anti-PGC1-α(1:3000, Cat No. 66369–1-Ig,Proteintech),anti-NRF1(1:1000, Cat No.12482–1-AP, Proteintech),anti-TFAM(1:1000, Cat No. 82745–1-RR, Proteintech),and anti-β-actin (1:1000, Cat No. 66009–1-Ig, Proteintech). The membranes were washed and incubated with goat anti-rabbit antibody(1:3000, 926–32211, LI-COR Biosciences)and goat anti-mouse antibody (1:3000,926–68070, LI-COR Biosciences) at 37°C for 2 h. ImageJ software analyzed grayscale values of target and β-Actin protein bands, calculating ratios for statistical com-parisons.

2.6. Immunofluorescence staining (IF)

Mouse brain tissue was fixed with 4 % paraformaldehyde, dehydrated with sucrose, embedded and frozen at −80°C. After permeabilization with 0.25 % Triton X-100 at 37°C for 30 min, sections were blocked with 10 % BSA for 1 h. Brain slices were incubated with anti-MAP2 antibody (1: 200, 8707 T, Cell Signaling) or anti-PGC1α antibody (1: 200, Cat No. 66369–1-Ig,Proteintech,) at 4°C overnight. Sections were then incubated with fluorescent secondary antibodies at 37°C for 2 h, and then the nuclei were stained with DAPI. Finally, The sections were visualized using fluorescence microscopy and quantitative analysis was performed using ImageJ.

2.7. Immunohistochemical staining

The brain tissue was fixed, sliced, dewaxed, and hydrated. The slices were incubated with anti-Aβ primary antibody (1:200, Cat No. 25524–1-AP, Proteintech) at 4°C overnight. After washing three times with PBS, the slices were incubated with biotin-labeled goat anti-rabbit immunoglobulin G (1:1 000 , 926–32211, LI-COR Biosciences) secondary antibody at 37°C for 30 min. After washing with PBS, the slices were incubated with avidin-peroxidase complex for 30 min. After hematoxylin counterstaining and further dehydration, they were covered with gum. The area of the right hippocampus was photographed with an optical microscope. Image-J software was used to detect the positive area of Aβ expression in hippocampus.

2.8. Transmission electron microscopy

Transmission electron microscopy was used to observe the number of mitochondria and synaptic ultrastructure in hippocampal neurons. Hippocampal tissue blocks (1 mm × 1 mm × 3 mm) were fixed in 4 % glutaraldehyde at 4 °C for 2–4 h, dehydrated with gradient acetone, embedded in epoxy resin at 37°C for 5 h, and polymerized in an oven. Ultrathin sections (50 nm) were cut with an ultramicrotome, stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (JEOL JEM-1230).

2.9. Chromatin immunoprecipitation (ChIP) assay

ChIP assay kit (Epigentek, p-2002–2) was used according to the manufacturer's instructions. Cross-linked chromatin was sonicated into fragments and then immunoprecipitated with Nrf2 antibody (1:200, CST, #12721). IgG was used as a negative control. DNA fractions were analyzed by qRT-PCR. The primers sequences for ChIP-PCR were listed in Table 1.

2.10. Co-Immunoprecipitation(co-IP)

The tissue was lysed with NP-40 (Beyotime, P0013F) lysis buffer. The supernatant (500 μl) after centrifugation was incubated with anti-PPARγ antibody (Cat No.16643–1-ap, Proteintech) and the antigen-antibody mixture was slowly shaken overnight at 4℃. Then 30 μl agarose protein A+G (Beyotime, P2055) was added and reacted at 4℃ for 3 h. The complex bound to protein A/G conjugate was washed. It was dissolved in SDS-PAGE loading buffer and analyzed by Western blotting.

2.11. Statistical analysis

Data are presented as mean ± standard deviation (SD) and analyzed using SPSS 25.0 software package. The escape latency data in the place navigation test in the water maze were compared among multiple groups using repeated analysis of variance (ANOVA), and the other data were performed using one-way analysis of variance. Multiple post hoc comparisons of data were performed using Tukey's test. ChIP results were analyzed using *t*-test. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Lentivirus downregulated Nrf2 levels in mouse hippocampus

To detect the Nrf2 expression level in 10-month-old C57BL/6 J and APP/PS1 mice and the down-regulation effect of lentivirus on Nrf2, we performed reverse transcription polymerase reaction and western blot analysis on the right hippocampus. The results showed that compared with the C57 +GFP group, the mRNA level and protein content of Nrf2 in the hippocampus of the APP+GFP group decreased, indicating that the Nrf2 expression level in AD model mice would decrease (Fig. 1 and Fig. 4A,B). The Nrf2 mRNA level and protein content in the hippocampus of the C57 +Nrf2 shRNA group were lower than those in the

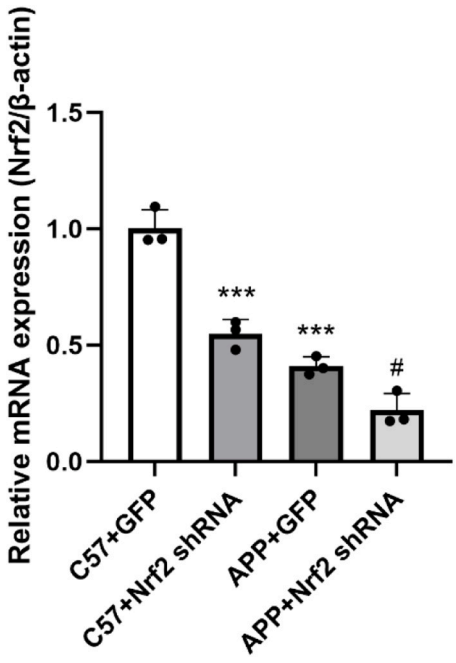

Fig. 1. Lentivirus downregulated the expression level of Nrf2 in mouse hippocampus ($F = 77.366$, $P < 0.001$)($n = 3$). The data were presented as mean ± SD, one-way ANOVA with post hoc Tukey test was performed. *** $p < 0.001$ versus C57 +GFP,# $p < 0.05$ versus APP+GFP.

C57 +GFP group. Compared with the APP+GFP group, the Nrf2 mRNA level and protein content in the hippocampus of the APP+Nrf2 shRNA group decreased. The above results indicate that lentiviral injection successfully reduced the Nrf2 expression level in the hippocampus of C57BL/6 J and APP/PS1 mice.

3.2. Lentivirus-induced downregulation of Nrf-2 exacerbated cognitive impairment in mice

Morris water maze test was used to assess spatial learning and memory deficits in mice. In the place navigation test (days 1–5), spatial learning ability was assessed by escape latency (Fig. 2A). The escape latency of the four groups gradually shortened, and the escape latency of the C57 +Nrf2 shRNA group and the APP+GFP group was longer than that of the C57 +GFP group. The escape latency of the APP+Nrf2 shRNA group was longer than that of the APP+GFP group. The spatial probe test was conducted in day 6 to assess retrograde reference memory (Fig. 2B-D). Compared with the C57 +GFP group, the C57 +Nrf2 shRNA group and the APP+GFP group crossed the removed platform area less frequently. The APP+Nrf2 shRNA group crossed the removed platform area less frequently than the APP+GFP group. Compared with the C57 +GFP group, the C57 +Nrf2 shRNA group and the APP+GFP group spent less time in the target quadrant after the platform was removed, and the APP+Nrf2 shRNA group spent less time in the target quadrant than the APP+GFP group. There was no statistical difference in the average speed among the four groups of mice(Fig. 2E).

3.3. Lentivirus-induced Nrf2 downregulation increased Aβ protein deposition in the mouse hippocampus

Aβ protein deposition is an important pathophysiological mechanism for the occurrence and development of AD. We used Western blotting and immunohistochemistry to detect the Aβ protein content in the hippocampus of mice. Compared with the C57 +GFP group, the Aβ protein content in the hippocampus of the C57 +Nrf2 shRNA group and the APP+GFP group increased, and the Aβ protein content in the hippocampus of the APP+Nrf2 shRNA group was higher than that of the APP+GFP group (Fig. 3A, B). The immunohistochemistry results showed that compared with the C57 +GFP group, the Aβ-positive area in the hippocampus of the C57 +Nrf2 shRNA group and the APP+GFP group increased, and the Aβ-positive area in the hippocampus of the APP+Nrf2 shRNA group was higher than that of the APP+GFP group (Fig. 3C, D).

3.4. Lentivirus-induced Nrf2 reduced PPARγ, mitochondrial biogenesis-related proteins and mitochondrial number

PGC1α is a key protein in mitochondrial biogenesis, and the number of mitochondria combined with mitochondrial biogenesis markers can be used as an indicator of mitochondrial biogenesis [21]. NRF1/TFAM is a downstream signaling molecule of PGC-1α, and after PGC-1α activation, it upregulates TFAM expression through NRF1, which together promotes mitochondrial biogenesis [22].Western blotting was used to detect the content of peroxisome proliferator activated receptor-γ(PPARγ) and mitochondrial synthesis-related proteins in

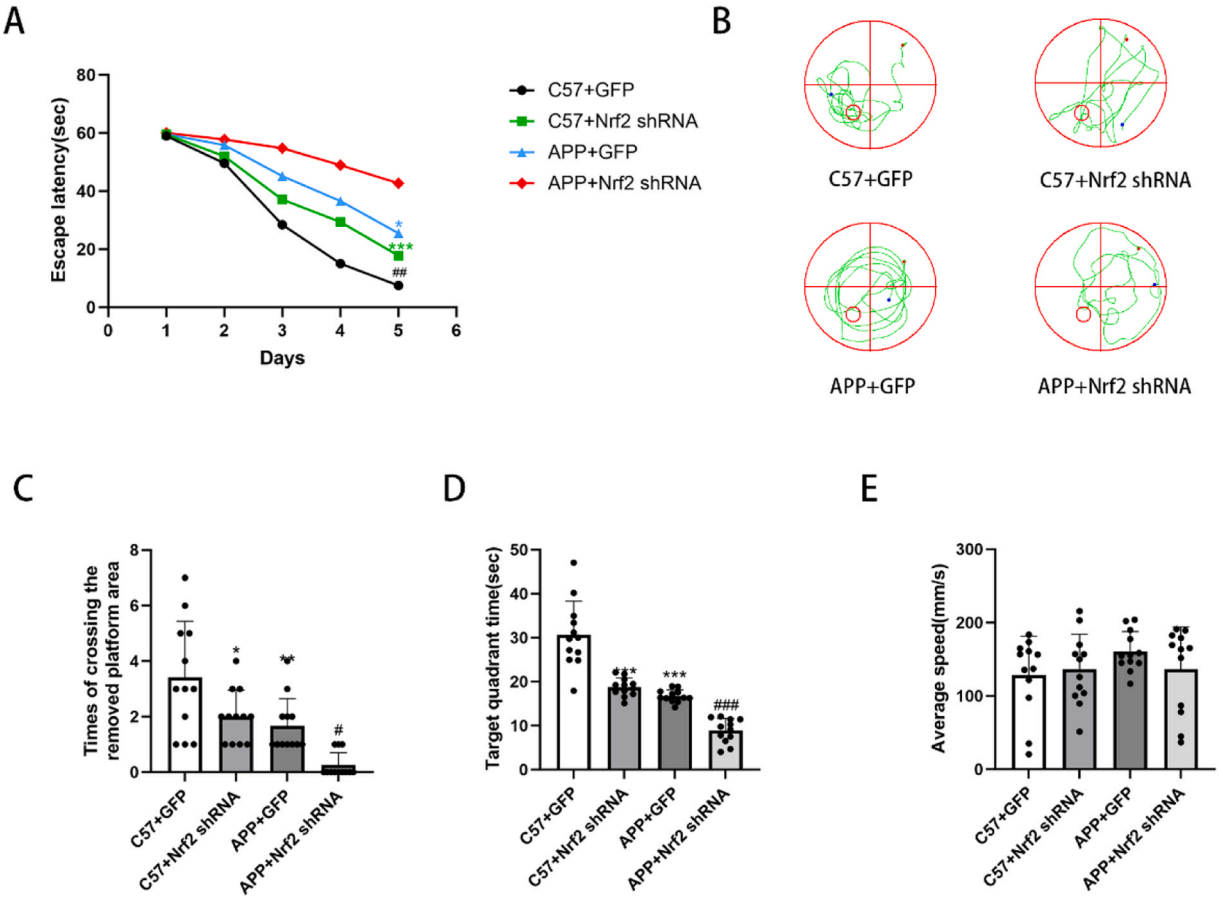

Fig. 2. Lentivirus-induced downregulation of Nrf2 exacerbated cognitive impairment in mice (n = 12). (A) The place navigation test($F = 9.409$, $P < 0.001$). (B) Representative swimming traces during the spatial probe test. (C) Times of crossing the removed platform area($F = 13.153$, $P < 0.001$). (D) Time spent in target quadrant where the platform was previously placed($F = 53.831$, $P < 0.001$). (E) Average speed($F = 1.015$, $P = 0.395$).The data were presented as mean ± SD, one-way ANOVA with post hoc Tukey test was performed.*$p < 0.05$ versus C57 +GFP, **$p < 0.01$ versus C57 +GFP,*** $p < 0.001$ versus C57 +GFP,# $p < 0.05$ versus APP+GFP, ###$p < 0.001$ versus APP+GFP.

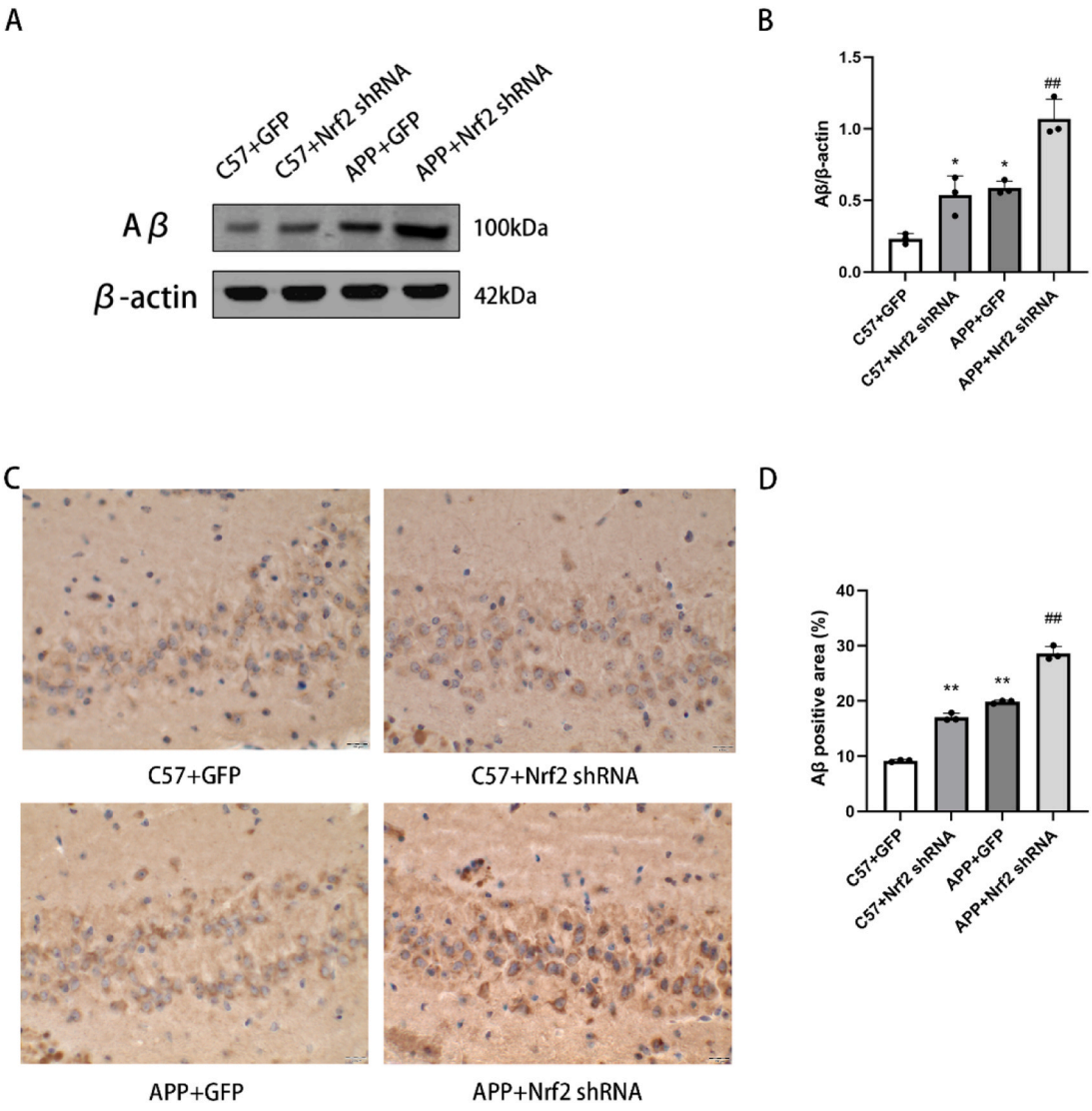

Fig. 3. Lentivirus-induced Nrf2 downregulation increases Aβ protein deposition in the mouse hippocampus (n = 3). (A) Western blot images of Aβ protein content in mouse hippocampus. (B) Quantitative analysis of Aβ protein expression($F=42.348$, $P < 0.001$). (C) Immunohistochemical images of Aβ in hippocampus. 400 ×, Scale bar = 20 μm. (D)Quantitative analysis of Aβ-positive area in hippocampus($F=42.348$, $P < 0.001$). The data were presented as mean ± SD, one-way ANOVA with post hoc Tukey test was performed. *$p < 0.05$ versus C57 +GFP, **$p < 0.01$ versus C57 +GFP, ##$p < 0.01$ versus APP+GFP.

mouse hippocampal tissue, immunofluorescence was used to detect the PGC1α protein content in mouse hippocampal tissue, and transmission electron microscopy was used to observe the number of mitochondria in hippocampal tissue.Compared with the C57 +GFP group, the PPARγ protein content in hippocampal tissues of the C57 +Nrf2 shRNA group and the APP+GFP group was reduced, and the PPARγ protein content in hippocampal tissues of the APP+Nrf2 shRNA group was lower than that of the APP+GFP group (Fig. 4A, C). Regarding proteins related to mitochondrial synthesis, compared with the C57 +GFP group, the levels of PGC1α, nuclear respiratory factor 1(NRF1)and TFAM proteins in the hippocampus of the C57 +Nrf2 shRNA group and the APP+GFP group were reduced, and the levels of PGC1α, NRF1 and mitochondrial transcription factor A (TFAM)proteins in the hippocampus of the APP+Nrf2 shRNA group were lower than those in the APP+GFP group (Fig. 4A, D, E and Fig. 5A, B). Immunofluorescence results showed that compared with the C57 +GFP group, the immunofluorescence intensity of PGC1α in the hippocampus of the C57 +Nrf2 shRNA group and the APP+GFP group was lower, and the immunofluorescence intensity of PGC1α in the hippocampus of the APP+Nrf2 shRNA group was lower than that of the APP+GFP group (Fig. 5C, D). Compared with the C57 +GFP group, the number of mitochondria in the hippocampal tissue of the C57 +Nrf2 shRNA group and the APP+GFP group decreased under transmission electron microscopy. The number of mitochondria in the hippocampal tissue of the APP+Nrf2 shRNA group were lower than those in the APP+GFP group (Fig. 5E, F). Chromatin immunoprecipitation (ChIP) results indicated that Nrf2 could bind with the site 2 of PGC1α promoter (Fig. 4F). The results of the co-IP experiment showed that PPARγ protein and PGC1α protein interacted with each other(Fig. 4G).Nrf2 is involved in regulating mitochondrial biogenesis.

The data were presented as mean ± SD. ChIP results were analyzed using *t*-test,and the other data were performed using one-way analysis of variance. Multiple post hoc comparisons of data were performed using Tukey's test. **$p < 0.01$ versus C57 +GFP,*** $p < 0.01$ versus C57 +GFP, #$p < 0.05$ versus APP+GFP,# #$p < 0.01$ versus APP+GFP. (F)**$p < 0.01$ versus IgG.

3.5. Lentivirus downregulation of Nrf2 aggravated synaptic damage and reduced MAP2 content in hippocampal tissue

Synaptic damage is an important mechanism of cognitive decline in

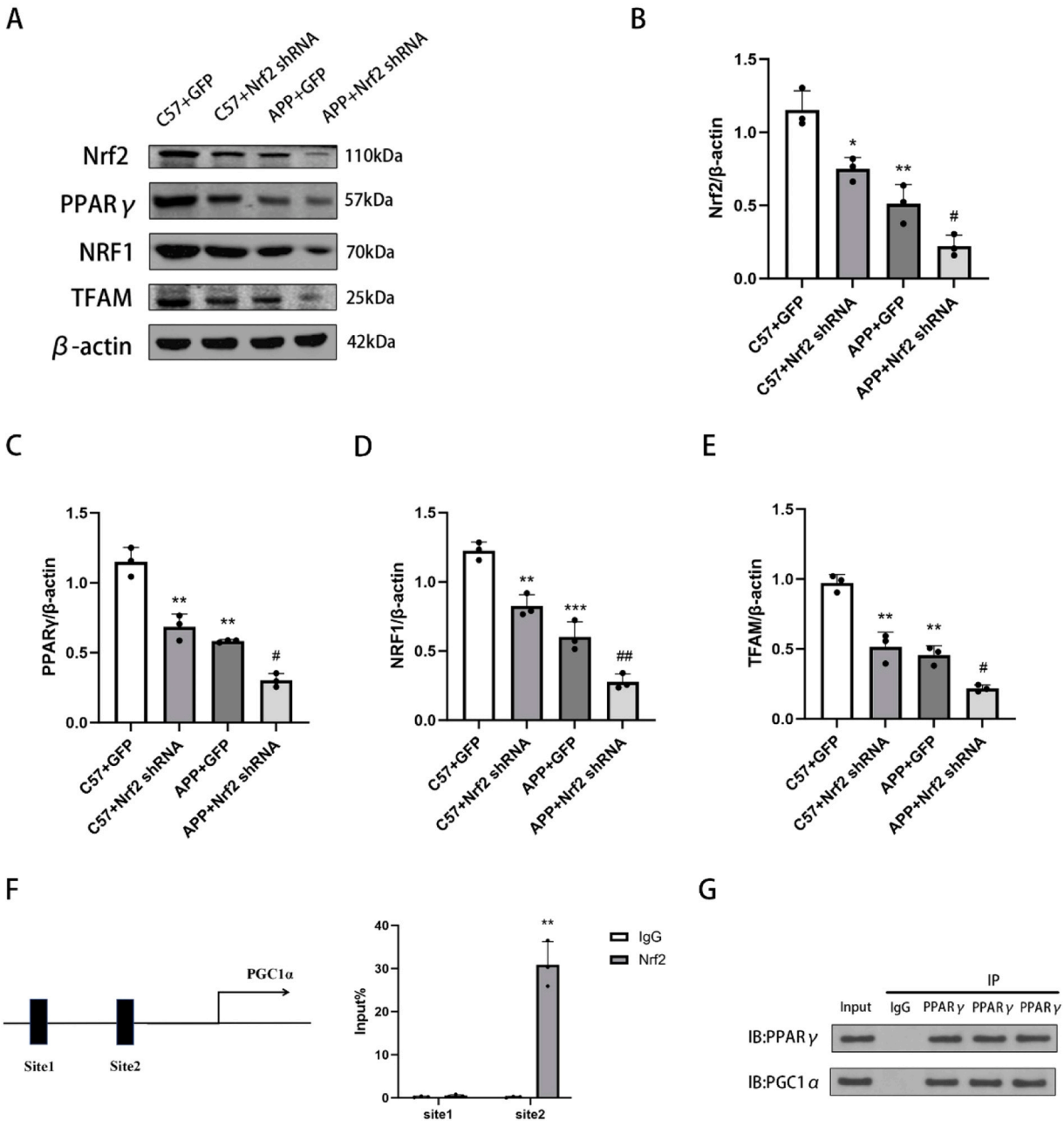

Fig. 4. Effects of lentivirus on Nrf2, PPARγ and mitochondrial synthesis-related proteins(n = 3). (A) Western blot images of Nrf2,PPARγ,NRF1 and TFAM protein content in mouse hippocampus. (B-E) Quantitative analysis of Nrf2(F=38.448, P < 0.001), PPARγ(F=48.357, P < 0.001), NRF1(F=77.277, P < 0.001) and TFAM (F=38.869, P < 0.001) protein expression. (F) Chromatin immunoprecipitation (ChIP) results indicated the binding of Nrf2 on the site 2 of PGC1aα promoter[site1 (t = -1.512, P = 0.205) ; site2(t = -9.992, P = 0.001)].(G) Co-immunoprecipitation(co-IP) demonstrated that PPRAγ protein and PGC1α protein interacted with each other.

AD, and microtubule-associated protein 2 (MAP2) is a cytoskeletal protein that can reflect the structural integrity of nerve cells. Therefore, we used transmission electron microscopy to observe the intracellular synaptic structure and immunofluorescence to detect the content of hippocampal MAP2. The presynaptic membrane, postsynaptic membrane and gap structures of the C57 +GFP group were clear, the connection sites between synapses were obvious, and the number of synaptic vesicles was abundant. The synaptic structures of the C57 +Nrf2 shRNA and APP+GFP groups were unclear, and the synaptic vesicles were sparse. The presynaptic membrane of the APP+Nrf2 shRNA group was swollen and destroyed, and the synaptic gap disappeared, indicating that the function of the synapse was relatively low (Fig. 6A). The immunofluorescence results showed that compared with the C57 +GFP group, the immunofluorescence intensity of MAP2 in the hippocampal tissue of the APP+GFP group was lower, indicating that aging has affected the cytoskeletal function (Fig. 6B, C). The immunofluorescence intensity of MAP2 in the hippocampal tissue of the C57 +Nrf2 shRNA group was lower than that of the C57 +GFP group, and the immunofluorescence intensity of MAP2 in the hippocampal tissue of the APP+Nrf2 shRNA group was lower than that of the APP+GFP group, indicating that the structural integrity of neurons was significantly reduced when Nrf2 was downregulated.Fig. 7

4. Discussion

AD is a common, age-related, irreversible neurodegenerative disease

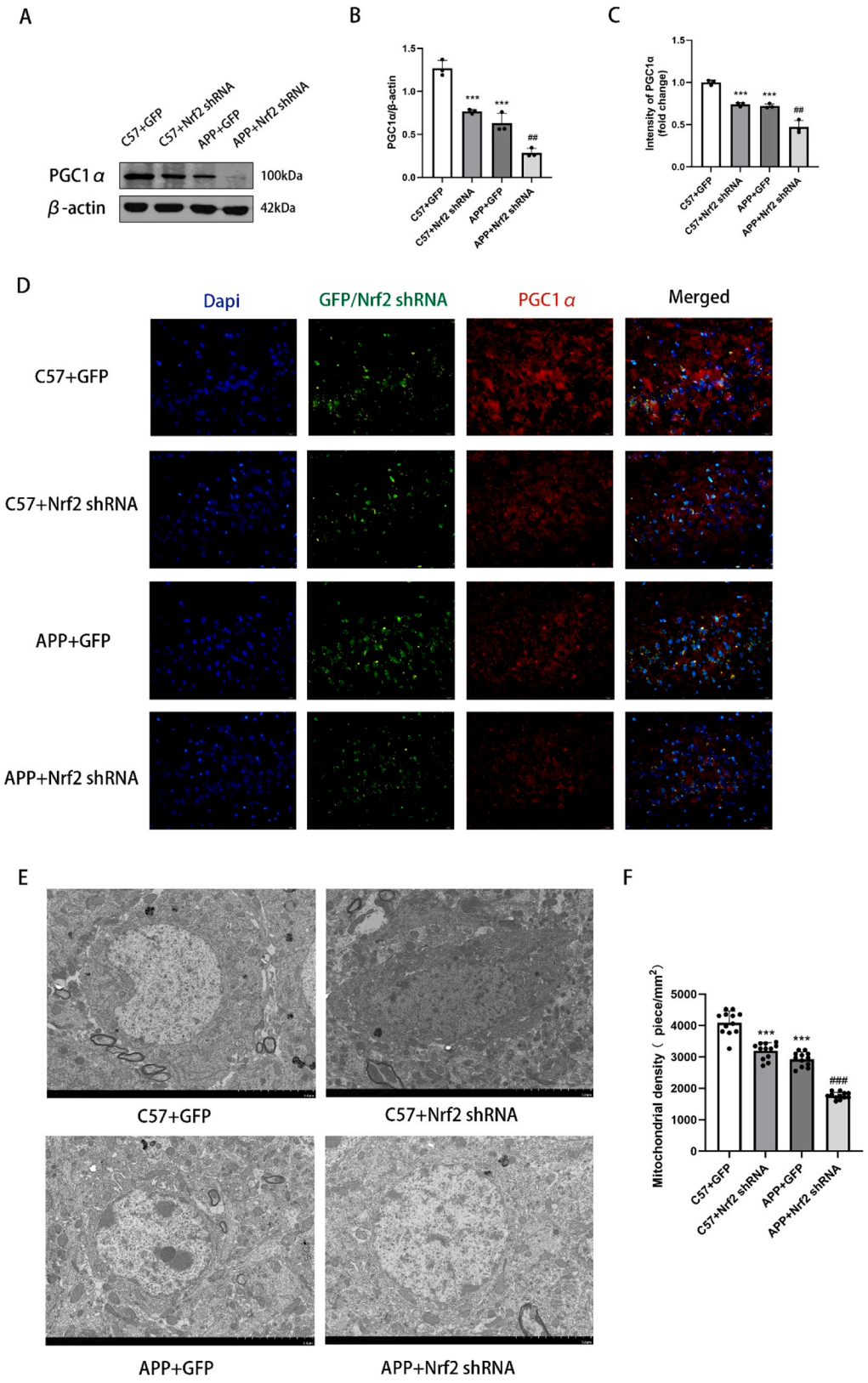

Fig. 5. Lentivirus-induced downregulation of Nrf-2 decreased PGC1α protein content and mitochondrial number (n = 3). (A) Western blot images of PGC1α protein content in mouse hippocampus. (B) Quantitative analysis of PGC1α protein expression($F=80.902$, $P < 0.001$). (C) Immunofluorescence images of PGC1α in the hippocampus. 400 × , Scale bar = 20 μm. (D) Quantitative analysis of PGC1α fluorescence intensity in the hippocampus($F=68.411$, $P < 0.001$). (E) Transmission electron microscope image of the number of mitochondria. (F) Quantification of mitochondrial numbers under transmission electron microscopy($F=161.729$, $P < 0.001$).The data were presented as mean ± SD, one-way ANOVA with post hoc Tukey test was performed. *** $p < 0.001$ versus C57 +GFP,# #$p < 0.01$ versus APP+GFP, ###$p < 0.001$ versus APP+GFP.

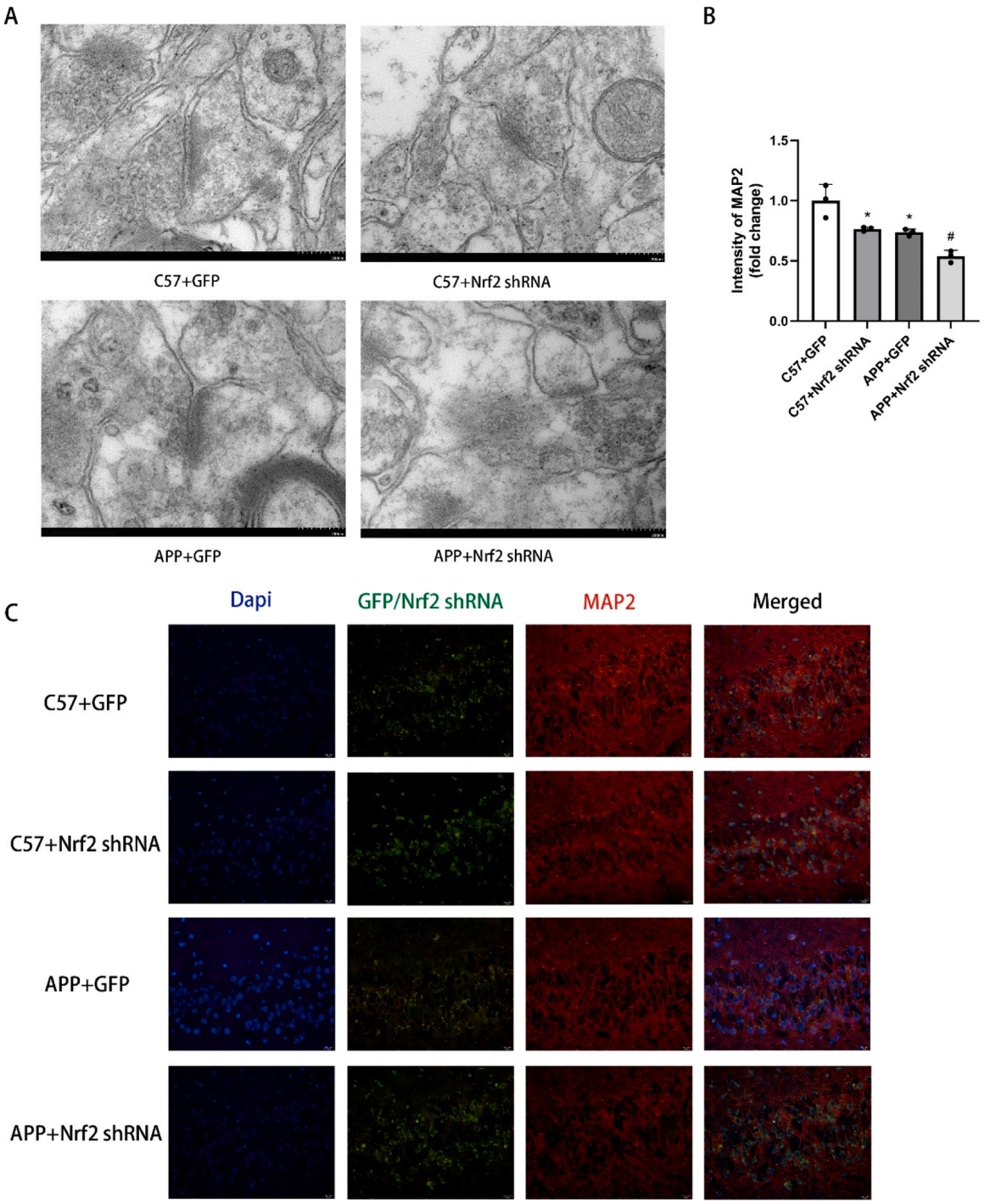

Fig. 6. Lentivirus-induced downregulation of Nrf2 reduced the number of mitochondria and MAP2 content. (A) Transmission electron microscope image of neural synapse. (B) Quantitative analysis of MAP2 fluorescence intensity in the hippocampus($F=19.819$, $P < 0.001$). (B) Immunofluorescence images of MAP2 in the hippocampus. 400 × , Scale bar = 20 μm. The data were presented as mean ± SD, one-way ANOVA with post hoc Tukey test was performed. * $p < 0.05$ versus C57 +GFP, #$p < 0.05$ versus APP+GFP.

characterized by multiple cognitive impairments and memory loss. The disease process involves mitochondrial dysfunction, synaptic damage, and activation of glial cells/astrocytes, in addition to the well-recognized accumulation of Aβ and hyperphosphorylated tau (p-tau) [23]. Despite many advances in molecular biology, genetics, and pharmaceutical science, there are still no effective drugs to reverse, even slow the neurodegeneration process. Neurons' intense energy needs make them highly reliant on mitochondrial function [24,25]. Mitochondrial dysfunction has long been recognized as a prominent pathological characteristic of neurodegenerative diseases such as AD [26]. At present, some studies have provided evidence that PPARγ/PGC1α can upregulate the expression of genes essential for mitochondrial biogenesis in neurons [27]. In the process of oxidative stress in the body, Nrf2 is the upstream transcription factor of PPARγ. Recent research indicates that the activation of Nrf2 can improve the cognitive impairment of AD mice [28], but its mechanism in mitochondrial function remains to be studied. Therefore, in this study, we established an Alzheimer's disease (AD) mouse model with

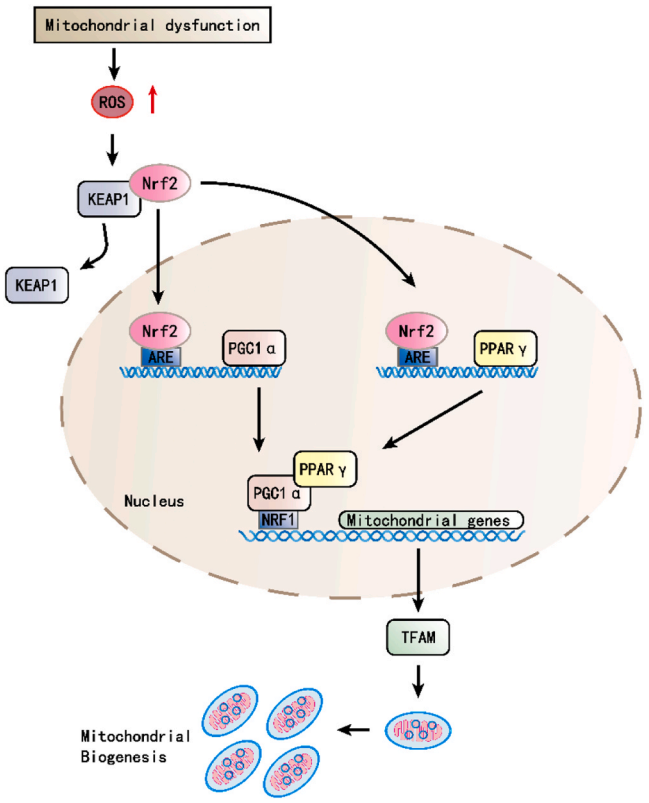

Fig. 7. Diagram of the mechanism of Nrf2 acting on mitochondrial biogenesis.

downregulated Nrf2 expression via hippocampal lentiviral injection. We demonstrated that Nrf2 downregulation reduces the expression of PPARγ and PGC1α proteins, thereby reducing their interaction. This suppression impairs mitochondrial biogenesis, exacerbates mitochondrial dysfunction, intensifies Aβ deposition and synaptic damage, and ultimately worsens cognitive impairment in AD mice.

The neurocognitive system is a complex system that requires the normal dynamics of neural signals and the synaptic connections of hundreds of millions of neurons to function properly. Neurons are vulnerable to mitochondrial dysfunction owing to their intricate structure and elevated energy demands. Therefore, mitochondrial dysfunction is a core characteristic of neurodegenerative diseases [29]. Mitochondrial dysfunction includes mitochondrial DNA damage, abnormal mitochondrial dynamics and morphology, abnormal substrate catabolism, and imbalance in the production/clearance of reactive oxygen species. Among them, mitochondrial biogenesis may maintain homeostasis by supporting metabolic function and cell viability [30]. Mitochondrial biogenesis refers to the process of synthesizing new mitochondria in cells during cellular stress, when many different signaling pathways are activated [31]. Among various signaling pathways, PGC1α has been identified as the main regulator of mitochondrial biogenesis [32]. As a downstream nuclear transcription factor of PGC1α, NRF1 can promote the expression of TFAM, thereby initiating the replication process of mitochondrial DNA [33,34].In AD, brain mitochondria show reduced biogenesis. Reduced levels of PGC1α, NRF1, NRF2 and TFAM were found in APP transgenic mice at 6 and 12 months of age, indicating that mitochondrial biogenesis function is defective in AD [35,36]. Studies on primary hippocampal neurons of mutant APP mice found that the mRNA and protein levels of mitochondrial biogenesis genes PGC1α, NRF1, NRF2 and TFAM were reduced [37]. Lentivirus vector-induced high expression of PGC1α in the cerebral cortex and hippocampus of APP23 mice can improve the spatial and recognition memory of mice, while significantly reducing Aβ deposition [38], indicating the improvement of PGC1α on mouse cognition. Our results also showed that compared with the control group, the expression of PGC1α, NRF1, and TFAM proteins was reduced in the AD model group, indicating that the level of mitochondrial biogenesis was reduced in the AD model group.

As a key transcription factor of oxidative stress, Nrf2 has cytoprotective effects against oxidative damage and reducing mitochondrial damage, and has become a potential therapeutic target for AD [39]. Our results showed that Nrf2 downregulation led to aggravated cognitive impairment in both control and AD mice, and a decrease in mitochondrial biogenesis proteins such as PGC1α, NRF1, and TFAM, accompanied by a decrease in the number of mitochondria under electron microscopy. In a study investigating the effects of Nrf2 deficiency on motor and cognitive performance in aged and mature mice, the results showed that Nrf2 deficiency led to impaired performance on memory tasks in both mature and aged mice [40], which is consistent with our results. Piantadosi et al. first proposed a link between Nrf2 and mitochondrial biogenesis. Their study showed that Nrf2 upregulates NRF1 transcription, leading to activation of mitochondrial biogenesis genes [41]. Studies have shown that activation of Nrf2-mediated antioxidant enzymes can enhance mitochondrial biogenesis in mice and humans [42,43].These studies and our research results all prove that Nrf2 is involved in the intrinsic mechanism of mitochondrial biogenesis.

Our results show that Nrf2 can not only directly activate PGC1α to promote mitochondrial biogenesis, but also activate PPARγ and promote its binding to PGC1α, thereby promoting mitochondrial biogenesis and improving cognitive impairment in AD. PPARγ is a ligand-activated transcription factor that is involved in regulating various genes and metabolic processes, such as adipogenesis, redox balance, mitochondrial biogenesis, and insulin sensitivity [44]. Previous studies have demonstrated through bioinformatics analysis and research results that PPARγ is a downstream molecule of Nrf2 and is driven by Nrf2 [45,46]. Current studies have shown that drugs can activate the Nrf2/PPARγ signaling pathway, inhibit oxidative stress and inflammatory response, and thus improve cognitive impairment in AD [47]. In cardiomyocyte studies, results showed that Nrf2 and PGC1α cooperate in transcription to promote mitochondrial biogenesis [48]. PPARγ and PGC1α synergistically upregulate the expression of genes related to neuronal mitochondrial biogenesis [27]. In AD, PPAR agonists can activate PPARγ, which in turn activates PGC1α, thereby exerting a protective effect against AD [49,50]. Our results, combined with previous studies, indicate that downregulation of Nrf2 reduces the gene expression of PPARγ and PGC1α, thereby reducing the interaction between the two and aggravating cognitive impairment.

Cognitive dysfunction is closely related to Aβ deposition and synaptic integrity. Mitochondrial dysfunction drives the production of Aβ [51]. Abnormal mitochondrial metabolism leads to insufficient synaptic energy, which in turn leads to synaptic damage and neurodegenerative changes [10]. Mitochondria in dendrites support synaptic density and plasticity. The loss of mitochondria in these areas inhibits synaptic transmission due to insufficient ATP supply or altered Ca2 + dynamics during intense synaptic activity [52]. In this study, AD model mice had increased Aβ protein, more severe synaptic damage and cognitive impairment compared with the control group. After lentiviral downregulation of Nrf2, Aβ protein increased, and synaptic damage and cognitive impairment were aggravated.

However, this study has certain limitations. First, we conducted in vivo experiments and did not conduct in vitro experiments to further verify the signaling pathway. In addition, Nrf2 and PPARγ act as multi-effect regulators of antioxidant regulation, anti-inflammation, adipogenesis, and microglial polarization in vivo, and we did not consider the mutual influence of their effects in vivo. In addition, the hippocampus is only the main structure that causes cognitive impairment, and the brain is a whole. We plan to use the entire brain as a research object to explore the effect of mitochondrial disorders on AD cognitive impairment.

In summary, downregulation of Nrf2 reduces both the production and interaction of PPARγ and PGC1α proteins. This leads to decreased

mitochondrial biogenesis, resulting in reduced mitochondrial quantity, exacerbated mitochondrial dysfunction, increased Aβ deposition, aggravated synaptic deficits, and ultimately worsened cognitive impairment in AD mice. This study demonstrated the important role of Nrf2 in mitochondrial biogenesis and the occurrence and development of AD cognitive impairment, providing a potential therapeutic target for the future treatment of AD.

Ethics statement

All animal experimental protocols were approved by the Animal Ethics Review Committee of the Third Hospital of Hebei Medical University.

CRediT authorship contribution statement

Yuling Wang: Validation, Supervision, Software, Conceptualization. **Jinyang Wang:** Visualization, Software, Methodology. **Guisong Zhang:** Visualization, Software, Methodology, Conceptualization. **Yujuan Dong:** Validation, Supervision, Software, Conceptualization. **Weigang Luo:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Wei Bu:** Writing – original draft, Project administration, Investigation, Formal analysis. **Huiling Ren:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Yanan Jia:** Visualization, Software, Methodology. **Cuicui Liu:** Software, Methodology, Conceptualization. **Xiaokai Hu:** Visualization, Methodology, Conceptualization.

Funding

This study was supported by 2024 Government-funded Clinical Medicine Talent Training Project (ZF2024090).

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgments

Not applicable.

Data availability

Data will be made available on request.

References

[1] M. Nasb, W. Tao, N. Chen, Alzheimer's disease puzzle: delving into pathogenesis hypotheses, Aging Dis. 15 (1) (2024) 43–73.
[2] W. Wang, F. Zhao, X. Ma, G. Perry, X. Zhu, Mitochondria dysfunction in the pathogenesis of alzheimer's disease: recent advances, Mol. Neurodegener. 15 (1) (2020) 30.
[3] S. Hauptmann, I. Scherping, S. Drose, U. Brandt, K.L. Schulz, M. Jendrach, et al., Mitochondrial dysfunction: an early event in alzheimer pathology accumulates with age in AD transgenic mice, Neurobiol. Aging 30 (10) (2009) 1574–1586.
[4] A. Zia, A.M. Pourbagher-Shahri, T. Farkhondeh, S. Samarghandian, Molecular and cellular pathways contributing to brain aging, Behav. Brain Funct. 17 (1) (2021) 6.
[5] M. Reutzel, R. Grewal, A. Joppe, G.P. Eckert, Age-dependent alterations of cognition, mitochondrial function, and beta-amyloid deposition in a murine model of alzheimer's disease-a longitudinal study, Front. Aging Neurosci. 14 (2022) 875989.
[6] S. Khan, N. Bano, S. Ahamad, U. John, N.J. Dar, S.A. Bhat, Excitotoxicity, oxytosis/ferroptosis, and neurodegeneration: emerging insights into mitochondrial mechanisms, Aging Dis. (2024).
[7] J.S. Kerr, B.A. Adriaanse, N.H. Greig, M.P. Mattson, M.Z. Cader, V.A. Bohr, et al., Mitophagy and alzheimer's disease: cellular and molecular mechanisms, Trends Neurosci. 40 (3) (2017) 151–166.
[8] M.P. Singulani, C. Pereira, A. Ferreira, P.C. Garcia, G.D. Ferrari, L.C. Alberici, et al., Impairment of PGC-1alpha-mediated mitochondrial biogenesis precedes mitochondrial dysfunction and alzheimer's pathology in the 3xtg mouse model of alzheimer's disease, Exp. Gerontol. 133 (2020) 110882.
[9] K. Taskintuna, M.A. Bhat, T. Shaikh, J. Hum, N. Golestaneh, Sex-dependent regulation of retinal pigment epithelium and retinal function by pgc-1alpha, Front. Cell Neurosci. 18 (2024) 1442079.
[10] W.W. Jia, H.W. Lin, M.G. Yang, Y.L. Dai, Y.Y. Ding, W.S. Xu, et al., Electroacupuncture activates AMPKalpha1 to improve learning and memory in the APP/PS1 mouse model of early alzheimer's disease by regulating hippocampal mitochondrial dynamics, J. Integr. Med. 22 (5) (2024) 588–599.
[11] M. George, M. Tharakan, J. Culberson, A.P. Reddy, P.H. Reddy, Role of nrf2 in aging, alzheimer's and other neurodegenerative diseases, Ageing Res. Rev. 82 (2022) 101756.
[12] A. Uruno, M. Yamamoto, The KEAP1-NRF2 system and neurodegenerative diseases, Antioxid. Redox Signal (2023).
[13] C.P. Ramsey, C.A. Glass, M.B. Montgomery, K.A. Lindl, G.P. Ritson, L.A. Chia, et al., Expression of nrf2 in neurodegenerative diseases, J. Neuropathol. Exp. Neurol. 66 (1) (2007) 75–85.
[14] C. Branca, E. Ferreira, T.V. Nguyen, K. Doyle, A. Caccamo, S. Oddo, Genetic reduction of nrf2 exacerbates cognitive deficits in a mouse model of alzheimer's disease, Hum. Mol. Genet 26 (24) (2017) 4823–4835.
[15] G. Joshi, K.A. Gan, D.A. Johnson, J.A. Johnson, Increased alzheimer's disease-like pathology in the APP/ PS1DeltaE9 mouse model lacking nrf2 through modulation of autophagy, Neurobiol. Aging 36 (2) (2015) 664–679.
[16] M. Cheng, C. Yuan, Y. Ju, Y. Liu, B. Shi, Y. Yang, et al., Quercetin attenuates oxidative stress and apoptosis in brain tissue of APP/PS1 double transgenic AD mice by regulating keap1/nrf2/HO-1 pathway to improve cognitive impairment, Behav. Neurol. 2024 (2024) 5698119.
[17] M. Song, S. Zhang, W. Yu, X. Fan, Gomisin n rescues cognitive impairment of alzheimer's disease by targeting GSK3beta and activating nrf2 signaling pathway, Phytomedicine 132 (2024) 155811.
[18] P. Pinjala, K.P. Tryphena, A. Kulkarni, P.G. Goswami, D.K. Khatri, Dimethyl fumarate exerts a neuroprotective effect by enhancing mitophagy via the NRF2/BNIP3/PINK1 axis in the MPP(+) iodide-induced parkinson's disease mice model, J. Alzheimers Dis. Rep. 8 (1) (2024) 329–344.
[19] H.L. Ren, C.N. Lv, Y. Xing, Y. Geng, F. Zhang, W. Bu, et al., Downregulated nuclear factor e2-related factor 2 (nrf2) aggravates cognitive impairments via neuroinflammation and synaptic plasticity in the senescence-accelerated mouse prone 8 (SAMP8) mouse: a model of accelerated senescence, Med. Sci. Monit. 24 (2018) 1132–1144.
[20] J.Q. Mao, L. Cheng, Y.D. Zhang, G.J. Xie, P. Wang, Chinese formula guben-jiannao ye alleviates the dysfunction of circadian and sleep rhythms in APP/PS1 mice implicated in activation of the PI3k/AKT/mTOR signaling pathway, J. Ethnopharmacol. 335 (2024) 118696.
[21] L. Ma, Y. Wang, X. Li, Z. Wang, B. Zhang, Y. Luo, et al., Tom70-regulated mitochondrial biogenesis via TFAM improves hypoxia-induced dysfunction of pulmonary vascular endothelial cells and alleviates hypoxic pulmonary hypertension, Respir. Res. 24 (1) (2023) 310.
[22] Q. Zhao, Z. Tian, G. Zhou, Q. Niu, J. Chen, P. Li, et al., SIRT1-dependent mitochondrial biogenesis supports therapeutic effects of resveratrol against neurodevelopment damage by fluoride, Theranostics 10 (11) (2020) 4822–4838.
[23] J.A. Pradeepkiran, P.H. Reddy, Defective mitophagy in alzheimer's disease, Ageing Res. Rev. 64 (2020) 101191.
[24] P.R. Angelova, A.Y. Abramov, Role of mitochondrial ROS in the brain: from physiology to neurodegeneration, FEBS Lett. 592 (5) (2018) 692–702.
[25] W.T. Dong, L.H. Long, Q. Deng, D. Liu, J.L. Wang, F. Wang, et al., Mitochondrial fission drives neuronal metabolic burden to promote stress susceptibility in Male mice, Nat. Metab. 5 (12) (2023) 2220–2236.
[26] M.M. Klemmensen, S.H. Borrowman, C. Pearce, B. Pyles, B. Chandra, Mitochondrial dysfunction in neurodegenerative disorders, Neurotherapeutics 21 (1) (2024) e00292.
[27] S. Jamwal, J.K. Blackburn, J.D. Elsworth, PPARgamma/PGC1alpha signaling as a potential therapeutic target for mitochondrial biogenesis in neurodegenerative disorders, Pharm. Ther. 219 (2021) 107705.
[28] L. She, J. Sun, L. Xiong, A. Li, L. Li, H. Wu, et al., Ginsenoside RK1 improves cognitive impairments and pathological changes in alzheimer's disease via stimulation of the AMPK/nrf2 signaling pathway, Phytomedicine 122 (2024) 155168.
[29] T. Ashleigh, R.H. Swerdlow, M.F. Beal, The role of mitochondrial dysfunction in alzheimer's disease pathogenesis, Alzheimers Dement 19 (1) (2023) 333–342.
[30] C.A. Piantadosi, H.B. Suliman, Transcriptional control of mitochondrial biogenesis and its interface with inflammatory processes, Biochim Biophys. Acta 1820 (4) (2012) 532–541.
[31] T. Valero, Mitochondrial biogenesis: pharmacological approaches, Curr. Pharm. Des. 20 (35) (2014) 5507–5509.
[32] S.O. Abu, T. Arroum, S. Morris, K.B. Busch, PGC-1alpha is a master regulator of mitochondrial lifecycle and ROS stress response, Antioxidants 12 (5) (2023).
[33] Y. Hu, L. Zhang, C. Tian, F. Chen, P. Li, A. Zhang, et al., Molecular crosstalk and putative mechanisms underlying mitochondrial quality control: the hidden link with methylmercury-induced cognitive impairment, Ecotoxicol. Environ. Saf. 278 (2024) 116360.
[34] L.H. Khedr, N.N. Nassar, L. Rashed, E.D. El-Denshary, A.M. Abdel-Tawab, TLR4 signaling modulation of PGC1-alpha mediated mitochondrial biogenesis in the LPS-chronic mild stress model: effect of fluoxetine and pentoxiyfylline, Life Sci. 239 (2019) 116869.
[35] M. Manczak, R. Kandimalla, D. Fry, H. Sesaki, P.H. Reddy, Protective effects of reduced dynamin-related protein 1 against amyloid beta-induced mitochondrial

dysfunction and synaptic damage in alzheimer's disease, Hum. Mol. Genet 25 (23) (2016) 5148–5166.
[36] M. Manczak, R. Kandimalla, X. Yin, P.H. Reddy, Hippocampal mutant APP and amyloid beta-induced cognitive decline, dendritic spine loss, defective autophagy, mitophagy and mitochondrial abnormalities in a mouse model of alzheimer's disease, Hum. Mol. Genet 27 (8) (2018) 1332–1342.
[37] P.H. Reddy, X. Yin, M. Manczak, S. Kumar, J.A. Pradeepkiran, M. Vijayan, et al., Mutant APP and amyloid beta-induced defective autophagy, mitophagy, mitochondrial structural and functional changes and synaptic damage in hippocampal neurons from alzheimer's disease, Hum. Mol. Genet 27 (14) (2018) 2502–2516.
[38] L. Katsouri, Y.M. Lim, K. Blondrath, I. Eleftheriadou, L. Lombardero, A.M. Birch, et al., PPARgamma-coactivator-1alpha gene transfer reduces neuronal loss and amyloid-beta generation by reducing beta-secretase in an alzheimer's disease model, Proc. Natl. Acad. Sci. 113 (43) (2016) 12292–12297.
[39] T.F. Villavicencio, R.A. Quintanilla, Contribution of the nrf2 pathway on oxidative damage and mitochondrial failure in parkinson and alzheimer's disease, Antioxidants 10 (7) (2021).
[40] M.M. Gergues, A. Moiseyenko, S.Z. Saad, A.N. Kong, G.C. Wagner, Nrf2 deletion results in impaired performance in memory tasks and hyperactivity in mature and aged mice, Brain Res. 1701 (2018) 103–111.
[41] C.A. Piantadosi, M.S. Carraway, A. Babiker, H.B. Suliman, Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via nrf2-mediated transcriptional control of nuclear respiratory factor-1, Circ. Res. 103 (11) (2008) 1232–1240.
[42] G. Hayashi, M. Jasoliya, S. Sahdeo, F. Sacca, C. Pane, A. Filla, et al., Dimethyl fumarate mediates nrf2-dependent mitochondrial biogenesis in mice and humans, Hum. Mol. Genet 26 (15) (2017) 2864–2873.
[43] T.L. Merry, M. Ristow, Nuclear factor erythroid-derived 2-like 2 (NFE2l2, nrf2) mediates exercise-induced mitochondrial biogenesis and the anti-oxidant response in mice, J. Physiol. 594 (18) (2016) 5195–5207.
[44] Y. Tang, K. Wei, L. Liu, J. Ma, S. Wu, W. Tang, Activation of PPARgamma protects obese mice from acute lung injury by inhibiting endoplasmic reticulum stress and promoting mitochondrial biogenesis, PPAR Res. 2022 (2022) 7888937.
[45] H.Y. Cho, W. Gladwell, X. Wang, B. Chorley, D. Bell, S.P. Reddy, et al., Nrf2-regulated PPARgamma expression is critical to protection against acute lung injury in mice, Am. J. Respir. Crit. Care Med. 182 (2) (2010) 170–182.
[46] L. Li, J. Fu, D. Liu, J. Sun, Y. Hou, C. Chen, et al., Hepatocyte-specific nrf2 deficiency mitigates high-fat diet-induced hepatic steatosis: involvement of reduced PPARgamma expression, Redox Biol. 30 (2020) 101412.
[47] H. Qiu, X. Liu, Echinacoside improves cognitive impairment by inhibiting abeta deposition through the PI3k/AKT/nrf2/PPARgamma signaling pathways in APP/PS1 mice, Mol. Neurobiol. 59 (8) (2022) 4987–4999.
[48] Y. Li, Y.F. Feng, X.T. Liu, Y.C. Li, H.M. Zhu, M.R. Sun, et al., Songorine promotes cardiac mitochondrial biogenesis via nrf2 induction during sepsis, Redox Biol. 38 (2021) 101771.
[49] A. Justin, S. Mandal, P. Prabitha, S. Dhivya, S. Yuvaraj, P. Kabadi, et al., Rational design, synthesis, and in vitro neuroprotective evaluation of novel glitazones for PGC-1alpha activation via PPAR-gamma: a new therapeutic strategy for neurodegenerative disorders, Neurotox. Res. 37 (3) (2020) 508–524.
[50] Z. Li, Y. Zhang, Y. Zheng, W. Liu, X. Zhang, W. Li, et al., Intranasal 15d-PGJ2 ameliorates brain glucose hypometabolism via PPARgamma-dependent activation of PGC-1alpha/GLUT4 signalling in APP/PS1 transgenic mice, Neuropharmacology 196 (2021) 108685.
[51] R.H. Swerdlow, J.M. Burns, S.M. Khan, The alzheimer's disease mitochondrial cascade hypothesis: progress and perspectives, Biochim. Biophys. Acta 1842 (8) (2014) 1219–1231.
[52] Z.H. Sheng, Q. Cai, Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration, Nat. Rev. Neurosci. 13 (2) (2012) 77–93.