N-myc downstream-regulated gene 2 deficiency aggravates memory impairment in Alzheimer’s disease

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\textbf{ABSTRACT}

Alzheimer’s disease (AD) is a chronic degenerative disease of the central nervous system and the most common dementia type in elderly people. \textit{N-myc downstream-regulated gene 2} (NDRG2), a cell stress response gene, is primarily expressed in astrocytes in mammalian brains. The hippocampal protein levels of NDRG2 in AD patients were significantly higher than those in healthy peers. However, whether the increase in NDRG2 is involved in the development of AD or is an endogenous protective response initiated by stress remains unknown. Here, we investigated the roles of NDRG2 in the development of memory impairment in AD using mouse models established by amyloid β injection or crossing of APP/PS1 mice. We found that NDRG2 deficiency worsened the memory impairment in AD mice. In addition, NDRG2 deletion induced downregulation of the proteasome functional subunit PSMB6 in AD mice. These findings suggest that NDRG2 is an endogenous neuroprotectant that participates in the pathological course of waste-clearing impairment and memory damage in AD. NDRG2 may be a therapeutic target for the intervention of AD memory degradation.

1. Introduction

As a neurodegenerative disease that can lead to many deficiencies in memory, executive ability, visual space, language communication, abstract thinking, learning and calculation, Alzheimer’s disease (AD) is one of the primary diseases causing disability and death worldwide [1,2]. AD is characterized by the extracellular senile plaques formed by aggregation of amyloid β and the intracellular neurofibrillary tangles formed by aberrant accumulation of tau protein [3], resulting in the primary degeneration and necrosis of neurons [4]. However, no effective treatment exists for AD, and a high failure rate in the clinical phase of new drug development has occurred in recent years [5]. Therefore, the identification of new key molecules and mechanisms underlying AD is urgently needed.

Human \textit{N-myc downstream-regulated gene 2} (NDRG2) is located on chromosome 14q11.2 and contains 16 exons and 15 introns [6]. It encodes a protein with 371 amino acid residues and has a molecular weight of approximately 41 kDa [7]. NDRG2 is reported to be highly expressed in terminally differentiated tissues such as the brain [8], heart [9], muscle [10] and salivary glands [11]. NDRG2 is mainly distributed in the outer cortex, choroid plexus and epithelium of fetal heart [9], muscle [10] and salivary glands [11]. NDRG2 is mainly expressed in astrocytes of adult mice [12]. The mRNA and protein levels of NDRG2 in the brain tissues from AD patients were significantly higher than those from the control elderly people [13]. NDRG2 was colocalized with plaques and neurofibrillary tangles in the brains of a mouse model of AD [14,15]. Here, we also found a gradual increase in the expression of NDRG2 in the mouse hippocampus with the pathological progression of AD. Based on these studies, we proposed the hypothesis that during the pathological development process of AD, the accumulated amyloid β, as a kind of cell stress, induces the upregulation of NDRG2.

To test this hypothesis, we generated two independent AD mouse models lacking NDRG2 expression and analyzed the memory ability in...
these models. We provide evidence that strongly suggests that NDRG2 is an endogenous neuroprotectant in the development of AD.

2. Materials and methods

2.1. Animals

APP/PS1 double-transgenic [B6.Cg-Tg(APPswe,PSEN1ΔE9)85Dbo/Mmjax] mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All APP/PS1 mice used in this study were hemizygous. All C57BL/6 mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The Ndrg2-knockout (Ndrg2−/−) mice were kindly provided by Dr. Libo Yao (Fourth Military Medical University). Two- to eleven-month-old APP/PS1 and C57BL/6 mice were used in the studies. The animals were group-housed in a room maintained at 23 ± 1 °C with a 12-h light/dark cycle and free access to water and food. All experimental protocols were reviewed and approved by the Ethics Committee of the Fourth Military Medical University. The experimenters conducting the molecular and behavioral phenotyping were blinded to the mouse genotyping and grouping.

2.2. Immunofluorescence

Mice were anesthetized with a mixture of chloral hydrate (3 mg/10 g of body weight), perfused with 0.01 M phosphate-buffered saline (PBS), fixed with 4% cold paraformaldehyde phosphate buffer (PFA, pH 7.4) and dehydrated with 30% sucrose solution. The brains were quickly extracted and frozen in OCT (optimal cutting temperature) Compound (Fisher Scientific). Briefly, blocks were stored at −20 °C for 30 min before slicing on a cryostat at a 16 μm thickness. Slices were postfixed for 20 min in ice-cold 4% PFA before a quick wash with 0.01 M PBS. Sections were then incubated for 2 h in 5% normal donkey serum (NDS) in 0.01 M PBS before overnight incubation with primary antibodies (rabbit anti-NDRG2, 1:100, Abcam, ab174850; mouse anti-glial fibrillary acidic protein (GFAP), 1:300, Cell Signaling, #3670) in 1% NDS and 0.01 M PBS. After three washes in PBS for 10 min each, sections were incubated with anti-rabbit-594 and anti-mouse-488 secondary antibodies (rabbit anti-NDRG2, 1:100, Abcam, ab174850; mouse anti-GFAP, 1:300, Cell Signaling, #3670) overnight. The membranes were blocked with 3% (w/v) nonfat milk in 0.01 M PBS for 1 h-42 (1:1,000, mouse, Millipore, MABN878) or polyacrylamide gels) and transferred to PVDF membranes (Roche). The membranes were blotched with 3% (w/v) nonfat milk in 0.01 M PBS for 2 h and further incubated with primary antibodies against NDRG2 (1:1,000, rabbit, Cell Signaling, #5667), GFAP (1:1,000, mouse, Cell Signaling, #3670), Aβ1-42 (1:1,000, mouse, Millipore, MABN878) or GAPDH (1:1,000, rabbit, Cell Signaling, #5174) overnight. After 4 washes in Tris-buffered saline supplemented with Tween 20 (TBST), membranes were incubated for 2 h with secondary antibodies (1:5,000, Invitrogen). Membranes were washed four more times with TBST, and then antibody binding was detected by a chemiluminescence apparatus (Tanon, Shanghai, China) and quantified by densitometry with ImageJ (ImageJ 7.0 software).

2.3. Immunoblotting

Brain samples were collected and homogenized in RIPA lysis buffer containing phosphatase and protease inhibitor cocktails (Roche). For protein extraction, the samples were centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was removed to a new tube. Protein levels were assessed with a Bradford assay with BSA as the standard. Equal amounts of protein samples were separated by SDS-PAGE (4-20% polyacrylamide gels) and transferred to PVDF membranes (Roche). The membranes were blocked with 3% (w/v) nonfat milk in 0.01 M PBS for 2 h and further incubated with primary antibodies against NDRG2 (1:1,000, rabbit, Cell Signaling, #5667), GFAP (1:1,000, mouse, Cell Signaling, #3670), Aβ1-42 (1:1,000, mouse, Millipore, MABN878) or GAPDH (1:1,000, rabbit, Cell Signaling, #5174) overnight. After 4 washes in Tris-buffered saline supplemented with Tween 20 (TBST), membranes were incubated for 2 h with secondary antibodies (1:5,000, Invitrogen). Membranes were washed four more times with TBST, and then antibody binding was detected by a chemiluminescence apparatus (Tanon, Shanghai, China) and quantified by densitometry with ImageJ (ImageJ 7.0 software).

2.4. Novel object recognition test

The open field apparatus was constructed of a box measuring 40 cm × 40 cm × 35 cm. In the novel test situation, each animal was placed in the bottom right corner of the test apparatus and videotaped for 5 min using a video camera located 100 cm away from the arena. During each interval between the phases of experiments, the arena was cleaned with cotton soaked in 70% alcohol. The experimenters conducting the genotyping were blinded to the identities of the mice. The first day, the following behaviors were recorded for each mouse: the number of lines crossed by the mouse over 5 min intervals, total distance traveled and average speed. On the second day, two identical Lego blocks were placed in the open field 5 cm away from the wall. Each animal was placed in the bottom right corner of the test apparatus and allowed to explore for 10 min. During each interval between the phases of experiments, the arena was cleaned with cotton soaked in 70% alcohol. One hour later, one of the two identical Lego blocks was replaced with a new differently shaped Lego block made of the same material. Each animal was placed in the bottom right corner of the test apparatus and allowed to explore for 5 min. After 24 h, one of the two Lego blocks was replaced with another new differently shaped Lego block. Each animal was placed in the bottom right corner of the test apparatus and allowed to explore for 5 min. All videotapes were analyzed by an experimenter. Novel object preference was calculated as follows: percentage = time spent exploring the novel object / (time spent exploring the novel object + time spent exploring the familiar object). Object exploration was defined as each instance in which a mouse’s nose touched the object or was oriented toward the object and came within 2 cm of it. Chewing the object or climbing onto the object did not qualify as exploration.

2.5. Stereotaxic surgery

All surgeries were performed under aseptic conditions using isoflurane (1.4%) as the anesthetic. The skull surface was exposed, and 30-gauge syringe needles were used to infuse 0.5 μL of Aβ oligomers or saline into the left lateral ventricle at a rate of 0.1 μL/min. The coordinates of the stereotaxic apparatus were as follows: 0.22 mm posterior to the bregma, 1.0 mm to the left of the midline and 1.4 mm in depth. All mice were allowed to recover for 2 weeks.

2.6. RT-PCR analysis

Mouse brain samples were collected and homogenized in TRIzol reagent (Life Technologies), and total RNA was extracted according to the manufacturer’s protocol. Then, the RNA samples were quantified using an ultraviolet spectrophotometer at 260 nm. Equal amounts of RNA were reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Target cDNA levels were determined by RT-PCR (Thermo Fisher, Wilmington, USA) using SYBR Green (Takara) assays. Amplification assays were performed in 25 μL reaction mixtures containing 2 μL of the cDNA solution, 12.5 μL of TB Green Premix, 1 μL of each primer (10 μM), and 8.5 μL of water. The PCR profile was 30 s at 95 °C, 40 cycles of 5 s at 95 °C, and 30 s at 55 °C. The cDNA was normalized to the amount of GAPDH amplified with mouse SYBR qRT-PCR primers. The forward and reverse PCR primers were as follows: PSMB5: 5′-GGCTGCTATGGGCCACCATG-3′ and 5′-TGAGGAGGCCGTCCGAGAG-3′; PSMB6: 5′-CAAGAGCAGAATGTCGCTGAGT-3′ and 5′-ACTCCTGAAATTGGCTGGCAAG-3′; PSMB7: 5′-CTGGCTCCTTGCGAGCAAT-3′ and 5′-GCCAGCTGAATACTCCTCAC-3′.

2.7. Statistical analysis

The data are represented as the mean ± SEM. Statistical significance was evaluated using Student’s t test analysis or one-way ANOVA followed by the Tukey-Kramer post hoc test (GraphPad Prism
Fig. 1. The expression levels of NDRG2 change with the age of AD mice. (A) Fluorescent staining for NDRG2, Aβ1-42, and GFAP in the hippocampal regions of AD mice from 2 months to 11 months. Scale bar = 100 μm. (B) The protein expression levels of NDRG2, Aβ1-42\right), and GFAP in the hippocampus of AD mice from 2 months to 11 months by immunoblotting analysis. Data are expressed as the mean ± SEM from three independent determinations and evaluated using one-way ANOVA followed by Tukey-Kramer’s post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001. n = 3 biological replicates.
7.0 software). P < 0.05 was used to determine significance where indicated.

3. Results

3.1. The expression levels of NDRG2 increased with age in AD mice

The hippocampus is the first and most profoundly affected brain region with respect to AD pathology [16]. The loss of neurons and synapses in the hippocampus and other regions can explain why some of the first symptoms of AD are related to defects in declarative memory [17]. We first investigated the expression of NDRG2, GFAP, and Aβ1-42 with age in the hippocampus of AD mice, hemizygous APP/PS1 double-transgenic mice. Immunofluorescence staining was first performed, and as shown in Fig. 1A, the expression of NDRG2 increased with age in the hippocampus of APP/PS1 mice. Immunoblotting analysis was also carried out using hippocampal tissues. Consistent with the results of immunofluorescence staining, the protein levels of NDRG2 were significantly enhanced in aged mice compared to young mice (Fig. 1B). Similar protein expression patterns were also found for GFAP and Aβ1-42 (Fig. 1A and 1B). In addition, in healthy mice, no changes in NDRG2 protein levels were observed in the hippocampus, and similar protein expression patterns were also found for GFAP and Aβ1-42 (Fig. 1C). These data suggested that NDRG2 might participate in the pathologic development of AD.

3.2. NDRG2 knockout exacerbated memory impairment in mimic AD mice generated with Aβ oligomer injection

To further verify whether NDRG2 could participate in the pathology of AD, two-month-old Ndrg2−/− mice or Ndrg2−/+ mice were injected with amyloid β peptide through the lateral ventricle to mimic AD development (Fig. 2A). The levels of Aβ in the Ndrg2−/− and Ndrg2−/+ mouse brains were detected 14 days after Aβ oligomer injection. Amyloid β peptide accumulation in the Ndrg2−/− hippocampus was higher than that in the Ndrg2+/+ hippocampus (Fig. 2B).

We next evaluated several neurobehaviors in these mimic AD mice generated with Aβ oligomer injection. First, the locomotor activities of these mice were assessed by open field testing. As we reported previously, two-month-old Ndrg2−/− mice exhibited significant increases in total distance traveled, average speed and number of crossings in the open field test compared with the Ndrg2−/+ mice [18]. We found Ndrg2−/+ mice injected with Aβ oligomers showed a significant reduction in total distance traveled, average speed and number of crossings in the open field test compared with Ndrg2−/− mice that received a saline injection (Fig. 2C). AD is well known to be a progressive disease that involves memory impairment [19]. Therefore, to test whether the loss of NDRG2 could aggravate memory impairment in Aβ oligomer-injected mice, we administered a novel object recognition test. No difference was observed between Ndrg2−/− and Ndrg2−/+ mice that received injection of Aβ oligomers or saline in the exploration time ratio between the two identical objects. Subsequently, in an interseesion interval ( ISI) of 1 h, 24 h or 48 h, we replaced one of the identical objects with a novel object and then allowed the mice to explore in the open field again to detect the effect of NDRG2 on short-term and long-term memory (Fig. 2D). During the ISI 1 h experiment, we found that the recognition time for novel objects in both Ndrg2−/− and Ndrg2−/+ mice injected with Aβ oligomers significantly decreased compared with the mice injected with saline. However, NDRG2 deficiency did not aggravate the short-term memory damage after Aβ oligomer injection. During the ISI 24 h experiment, we found that Ndrg2−/− mice injected with Aβ oligomers had a significant decrease in exploration time of the novel object compared with Ndrg2−/+ mice injected with Aβ oligomers. During the ISI 48 h experiment, we also found that the mice injected with Aβ oligomers had a significant decrease in exploration time of the novel object compared with mice injected with saline. Thus, NDRG2 deficiency aggravated memory damage after Aβ oligomer injection in ISI 24 h and 48 h, but not 1 h. In addition, saline-injected Ndrg2−/− mice exhibited impaired memory in ISI 1 h, 24 h, and 48 h compared with saline-injected Ndrg2−/+ mice, which is consistent with our previous study [18]. Together, these data suggest that NDRG2 is required for maintaining long-term memory in mimic AD mice generated with Aβ oligomer injection.

3.3. NDRG2 deficiency worsened memory damage in APP/PS1 mice

To further dissect the role of NDRG2 loss in AD-like behaviors, we crossed Ndrg2−/− and hemizygous APP/PS1 double-transgenic mice to obtain four phenotypes, Ndrg2−/−/APP/PS1, Ndrg2−/+/APP/PS1, Ndrg2−/−/APP and Ndrg2−/+/APP (Fig. 3A). The genotypes of the hybridized mice were identified (Supplementary Fig. 1), and we then detected the Aβ1-42 levels in Ndrg2−/−/APP/PS1 and Ndrg2−/+/APP/PS1 mouse brains. The protein levels of Aβ in Ndrg2−/−/APP/PS1 mice were increased compared to those in the Ndrg2−/+/APP/PS1 mice (Fig. 3B).

To examine whether loss of NDRG2 worsened memory damage in APP/PS1 mice, we performed a novel object recognition test in eight-month-old Ndrg2−/−/APP/PS1 and Ndrg2−/+/APP/PS1 mice. Ndrg2−/−/APP/PS1 mice traveled a significantly greater total distance in the open field and exhibited increased average speed and number of crossings in the open field test (Fig. 4A). Consistent with the results of Aβ oligomer-injected mice, no difference in the exploration time ratio to the novel object was observed between Ndrg2−/−/APP/PS1 and Ndrg2−/+/APP/PS1 mice with ISI 1 h. However, the novel object exploration time in Ndrg2−/−/APP/PS1 mice was significantly decreased compared to Ndrg2−/+/APP/PS1 mice with ISI 24 h (Fig. 4B). Taken together, these results suggest that NDRG2 deficiency were implicated an exacerbated memory impairment in AD mice.

3.4. NDRG2 deficiency increased astrogial activation in mimic AD mice

NDRG2 is mainly expressed in astrocytes but not in neurons in rodents [12]. NDRG2 is implicated in an astrogial activation-associated inflammation response in a cortical stab injury mouse model [20]. Thus, we wondered whether the astrogial response is involved in NDRG2 loss-mediated memory damage. The expression of GFAP is referred to as reactive astrogliosis or astrogial activation [21]. Despite Aβ oligomer injection-induced reactive astrogliosis in both Ndrg2−/−/APP and Ndrg2−/+/APP mice, there was more fluorescent staining of GFAP in the hippocampus of Ndrg2−/+ mice than in Ndrg2−/−/APP mice (Fig. 5A). Immunoblotting analysis was also carried out to quantify astrogial activation in the hippocampus. The protein levels of GFAP were significantly increased in Ndrg2−/+ mice injected with Aβ oligomers compared with Ndrg2−/+ mice (Fig. 5B). Therefore, these data indicate that Aβ oligomer stress can induce reactive astrogliosis and that NDRG2 deficiency enhances astrogial activation.

3.5. NDRG2 is required for the stabilization of the proteasome functional subunit

The ubiquitin-proteasome system (UPS), a major intracellular protein, is well known to be related to AD pathogenesis [22]. The proteasome complex is a major regulator and an essential proteolytic enzyme for the degradation of Aβ and tau proteins [23]. PSMB5, PSMB6, and PSMB7 subunits belong to the 20S proteasomal subunit family and play important roles in protein degradation [24–29]. We found that the levels of Aβ were significantly accumulated in Ndrg2−/− mice injected with Aβ oligomers as well as in Ndrg2−/+ APP/PS1 mice. Thus, we speculated that NDRG2 silencing may affect the function of the proteasome subunit and the degradation of the Aβ protein. Next, we tested the expression levels of the proteasome subunits, including PSMB5, PSMB6, and PSMB7, in eight-month-old APP/PS1 mice. The results of qRT-PCR showed that the mRNA levels of the proteasome subunit...
PSMB6 were decreased in the hippocampus of Ndrg2−/− APP/PS1 mice compared with Ndrg2+/− APP/PS1 mice. However, the expression levels of PSMB5 and PSMB7 were not significantly altered between Ndrg2−/− APP/PS1 and Ndrg2+/− APP/PS1 mice (Fig. 6). Together, these results suggest that reduced PSMB6 expression may be involved in NDRG2 deficiency-mediated deteriorations in AD memory impairment. However, why and how NDRG2 deficiency leads to abnormal expression of PSMB6 need to be further investigated.

4. Discussion

In the present study, we found that the expression of NDRG2 increased with age in AD mice and that NDRG2 deficiency resulted in a deterioration in memory impairment of AD mice.
Memory loss is well known to be the defining presenting symptom in patients with AD [30], and Aβ accumulation plays a leading role in the pathogenesis of AD [31–33]. Interestingly, NDRG2 is an early-stage stress response gene in AD progression [14]. A previous study reported that NDRG2 was upregulated in the brains of patients with AD [13]. Here, we found APP/PS1 mice showed an increase in the expression levels of NDRG2 with age, which is consistent with results from AD patients [13]. However, whether the increase in NDRG2 is associated...
Fig. 5. *Ndrg2−/−* mice show an altered astrocytic response following Aβ oligomer injections. (A) Fluorescent staining of GFAP in the hippocampal regions of *Ndrg2−/−* and *Ndrg2−/+* mice, as well as *Ndrg2−/−* and *Ndrg2−/+* mice injected with the Aβ oligomer. Scale bar = 100 μm. (B) The protein levels of GFAP were analyzed by immunoblotting, and the data are expressed as the mean ± SEM. Statistical significance was evaluated using one-way ANOVA followed by Tukey-Kramer’s post hoc test, *p < 0.05, **p < 0.01. n = 3 biological replicates.

Fig. 6. The influence of NDRG2 silencing on proteasome functional subunits in AD mice. The mRNA levels of (A) PSMB5, (B) PSMB6, and (C) PSMB7 in the hippocampus of *Ndrg2−/−APP/PS1* and *Ndrg2−/+APP/PS1* mice. Statistical significance was evaluated using Student’s t test, *p < 0.05. n = 3 biological replicates.
with progressive disease or a protective response in AD remains unknown.

We previously reported that Ndrg2+/− mice exhibited attention-deficit/hyperactivity disorder (ADHD)-like symptoms characterized by attention deficits, hyperactivity, impulsivity, and impaired memory [18]. Interstitial glutamate levels and excitatory transmission were markedly increased in the brains of Ndrg2−/− mice due to reduced astroglial glutamate clearance, which may be associated with the ADHD-like behaviors in Ndrg2−/− mice. Here, we found that two-month-old Ndrg2−/− mice injected with Aβ oligomers had aggravated memory deficit compared with Ndrg2−/− mice injected with saline. However, the hyperactivity in Ndrg2−/− mice was significantly decreased after intracerebroventricular injection with Aβ oligomers. We hypothesized that accumulated Aβ oligomers in Ndrg2−/− mice affect not only memory but also locomotor activity.

Ndrg2−/− APP/PS1 mice showed a significantly worse memory compared to Ndrg2+/− APP/PS1 mice with ISI 24 h. We noticed that the novel object exploration time in Ndrg2−/− APP/PS1 mice with ISI 24 h (ratio is around 0.6) was relatively higher compared with the APP/PS1 mice in previous reports (ratio is around 0.5) [34]. We speculated this baseline value variation is owing to different experimental systems and mice lines, although both mice were control mice. In addition, there is a very low probability of obtaining littermates APP/PS1 and Ndrg2+/− APP/PS1 mice. Thus, we used Ndrg2−/− APP/PS1 as a control group instead of Ndrg2−/− APP/PS1 mice. However, we previously found that Ndrg2−/− and Ndrg2+/− are comparable with regard to general behaviors such as locomotor activity. Therefore, we speculate that although the protein levels of NDRG2 may be different between Ndrg2+/− APP/PS1 or Ndrg2−/− APP/PS1 mice, Ndrg2−/− APP/PS1 mice still have the ability to express NDRG2 and are appropriate as control mice.

Our data suggest that NDRG2 deficiency could exacerbate long-term memory impairment in two AD mice models. We speculate that NDRG2 deficiency may affect the expression or function of AD-associated long-term memory proteins such as kinase M zeta [35,36] and brain-derived neurotrophic factor [37,38], although this needs to be further studied.

Recent studies have revealed that a malfunction of the proteasome complex plays an important role in AD development [39]. Although the exact mechanism of the pathological process of AD is not fully clarified, the dysfunction of the protein degradation mechanisms has been proposed to play an important role in AD [40]. The ubiquitin-proteasome system (UPS) is essential for protein repair, turnover and degradation and is perturbed in AD [41]. The level of Aβ1-42 was significantly increased in Ndrg2−/− mice injected with Aβ oligomers compared with Ndrg2+/− mice injected with Aβ oligomers, suggesting a problem in the Aβ1-42 protein degradation process. Therefore, we hypothesize that the loss of NDRG2 may affect the proteasome system, which is the major intracellular protein quality control system. PSMB5, PSMB6 and PSMB7 subunits belong to the 20S proteasomal subunit family, which plays an important role in protein degradation and repair. Our data showed decreased expression of PSMB6 in NDRG2-deficient AD mice. PSMB6 is a member of the proteasome β-type family and participates in the formation of proteolytic centers of the proteasome machinery [42].

NDRG2 has protective effects in both acute and chronic brain disorders such as ischemia and depression [43–45]. In the present study, we found that NDRG2 silencing induced Aβ accumulation and worsened memory impairment, which was contradictory to another report on the role of NDRG2 in AD [15]. In that study, human APP695 SK-N-SH cells were used in vitro to mimic AD, and NDRG2 silencing significantly reduced the levels of Aβ in these cultured cells. However, in our study, we found that Aβ expression levels in Ndrg2−/−/APP/PS1 mice were significantly increased compared to those in Ndrg2+/− APP/PS1 mice. This discrepancy could be attributed to the difference between in vivo and in vitro pathological models.

In summary, NDRG2 deficiency affects memory in AD mice and may be associated with the malfunction of the proteasome. Therefore, NDRG2 is a promising neuroprotectant for AD.

Author contribution statement

YL conceived and designed the experiments. LT, YZ, RW, JH, YM, HG, WT, and LZ performed the experiments. YZ, ZF, AY, and WH interpreted the data and prepared the figures. YL, LT and YZ wrote and revised the manuscript.

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