**Emx1-Cre-mediated inactivation of PDK1 prevents plaque deposition in an Alzheimer’s disease-like mouse model**

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**Abstract**

BX912, an inhibitor for 3-phosphoinositide-dependent protein kinase 1 (PDK1), has been shown to produce beneficial effects in mouse models of Alzheimer’s disease (AD). To test the hypothesis that early inhibition on PDK1 may prevent neuropathology in a 5xFAD mouse model, we employed a genetic approach to generate a mutant line, termed as Pdk1 cKO/5xFAD, in which PDK1 is inactivated, specifically in the developing cortex of 5xFAD mice through Emx1-Cre-mediated gene recombination. We discovered that the Pdk1 cKO/5xFAD mice exhibited a massive reduction of plaque pathology compared with their 5xFAD littermates. We also demonstrated that gliosis was remarkably attenuated in Pdk1 cKO/5xFAD cortices and amyloid precursor protein levels were significantly lower in Pdk1 cKO/5xFAD cortices compared with 5xFAD littermates. This study suggests that early inhibition on PDK1 may effectively prevent AD-like neuropathology.

**Keywords:** Alzheimer's disease; Plaque deposition; PDK1; Neuroinflammation; Amyloid precursor protein; Conditional knockout

1. Introduction

Alzheimer’s disease (AD) is the most common type of dementia in the elderly. The typical pathological hallmarks include extracellular amyloid β-protein (Aβ) deposition, intracellular tangles formed by phosphorylated tau, and gliosis⁴⁻⁵. The mechanisms underlying AD pathogenesis remain to be determined. A number of factors, including Aβ⁶ and tau⁷⁻¹⁰, have been identified as important factors for the etiology of AD. It is believed that Aβ accumulation may induce inflammation and cause neuronal death in the brain⁶. Many studies have demonstrated that Aβ is a major target for the treatment of AD⁷⁻¹².
3-phosphoinositide-dependent protein kinase 1 (PDK1) is a well-known member in the phosphoinositide 3 kinase (PI3K) pathway. The PI3K signaling pathway has important physiological roles, including cell proliferation, cell metabolism, angiogenesis, and so on. Evidence has suggested that PDK1 may be involved in AD. First, it has been shown that AD patients exhibit enhanced PDK1 activity in the brain compared with control subjects without AD. Second, a recent transcriptomic study demonstrated a significant increase in PDK1 mRNA levels in excitatory neurons in the prefrontal cortex of AD patients compared with control subjects. Third, it has been found that PDK1 activity is enhanced in several lines of amyloid precursor protein (APP) transgenic (Tg) mice, including Tg2576, APP/presenilin-1 (PS1), and 3×Tg-AD mice. Moreover, previous evidence has shown that BX912, a potent PDK1 inhibitor, significantly diminishes amyloid plaques and improves spatial memory in mouse models of AD, raising a possibility that PDK1 may serve as a therapeutic target for AD.

Pietri et al. explored the mechanisms underlying BX912-induced beneficial effects in mouse models of AD. They found that BX912 increases the activity of α-secretase through tumor necrosis factor-α-converting enzyme (TACE), promotes the trans-localization of TACE into the plasma membrane, restores α-secretase-dependent cleavage of APP, and attenuates Aβ-induced neurotoxicity. The study suggests that the inhibition of PDK1 may be effective in halting AD. However, serious side effects have been reported from the inhibition of PDK1 pharmacologically or genetically. First, long-term treatment with BX912 results in the death of adult APP Tg mice. Second, a straight knockout (KO) of PDK1 causes embryonic lethality in mice. Third, neural progenitor cell (NPC)-specific inactivation of PDK1 results in apoptosis of cortical neurons and impairs spatial learning in mice. Fourth, interneuron- or oligodendrocyte-specific deletion of PDK1 or its substrate Akt leads to postnatal death in mice before the age of day 21 (P21).

In response to the findings of Pietri et al., we propose that early inhibition of PDK1 function may prevent AD-like pathology in APP Tg mice. To test this hypothesis, we employed a genetic strategy to inactivate PDK1 in the developing cortices of 5xFAD mice. The latter, which have been widely used in the field of AD, are known to express five mutations on human APP and PS1 genes. It has been reported that plaque pathology begins to become apparent in the cortex and the subiculum in 5xFAD mice at 2 months. On the other hand, Cre is known to be expressed mainly in NPCs in the developing cortex and in excitatory neurons in the postnatal forebrain of Emx1-Cre mice. In this study, Emx1-Cre mice were bred with 5xFAD (APP/PS1) and floxed Pdk1 mice to generate Pdk1f/f;Emx1-Cre;APP/PS1 mice, termed as Pdk1 cKO/5xFAD hereafter. Amyloid plaques were hardly detected in Pdk1 cKO/5xFAD cortices, and the population of ionized calcium-binding adaptor molecule 1 (Iba1)+ cells decreased significantly. Furthermore, there was a significant decrease in APP levels in Pdk1 cKO/5xFAD mice compared with their 5xFAD littermates. The above findings support the notion that early inhibition of PDK1 may effectively prevent AD-like pathology.

2. Materials and methods

2.1. Mice

The mouse work was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Nanjing University. The background strain for male and female mice was C57BL/6. All mouse lines, including Pdk1f/f, Emx1-Cre, and 5xFAD mice, are described in our previous studies. The mice were maintained in an animal facility at the Model Animal Research Center of Nanjing University. They were group-housed in a room without specific pathogens. The room temperature was maintained at 25 ± 1°C, and the mice had unrestricted access to food and water. The animal protocol (AP) for this study was approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing University.

The following primers were used for genotyping: Primers for floxed Pdk1 were TGTGCTTGTTGGATATTGT (forward) and AAGGAGGAGGAGGAGATGT (reverse); primers for human APP were AGGACTGACCACCTGACCAG (forward) and CGGGGTCTGATTCTGAG (reverse); primers for human PS1 were AATAGGACCGGACGG (forward) and GCCATGAGGGCACTAATCAT (reverse); and primers for Cre were GCCCTGCATTACCGTGCATGCAACACCATT (forward) and GCCATGAGGGCACTAATCAT (reverse).

2.2. Tissue preparation for biochemical and morphological studies

The mice were perfused with cold phosphate-buffered solution (PBS). The brain was cut sagittally into two hemispheres, in which the cortex was dissected from one hemisphere to prepare protein samples. The other hemisphere was fixed in 4% paraformaldehyde (PFA) (Aladdin Biochemical Technology, C104188) at 4°C overnight and then dehydrated using a series of ethanol solutions (Nanjing Reagent, C0691510225). The dehydrated brain hemispheres were embedded in a paraffin machine (Leica Biosystems, 39601095) for the preparation...
of paraffin blocks. There were four brain hemispheres in each paraffin block from four different genotype groups, including the control, Pdk1 cKO, 5×FAD, and Pdk1 cKO/5×FAD.

2.3. Immunohistochemistry (IHC)

We chose two brain sections, spaced at 400 μm, from each mouse for IHC. The sections were deparaffinized and then rehydrated with degraded ethanol solutions. They were treated with boiled sodium citrate buffer (Xilong Scientific, 160012) for 25 min. The sections were cooled down at room temperature and then blocked with 3% hydrogen peroxide (Sinopharm Chemical Reagent, 10011218) for 20 min. They were subsequently treated with 5% BSA solution (bovine serum albumin dissolved in PBS) (Best Biological, BA0029) for 20 min.

The sections were incubated with primary antibodies at 4°C overnight and then washed out with PBS 3 times before incubation with a secondary antibody at 4°C for 1 h. The DAB Kit (Vector Laboratories, SK-4100) was then used for the processing of the sections. After dehydration, each section was sealed using neutral resin.

All the primary antibodies are listed in Table 1.

For fluorescence IHC, the following secondary antibodies were obtained from Jackson ImmunoResearch Laboratories: Alexa Fluor 488 (715-545-150) and Alexa Fluor 594 (711-585-152). Fluorescence images and IHC images were captured using a confocal microscope from Leica (SP5) and an Olympus microscope (BX53), respectively.

2.4. Western blotting

Cortical tissues were homogenized using cold radioimmunoprecipitation assay (RIPA) buffer to prepare cortical protein lysates. The gradients were as follows (in mM): 20 mM tris-hydrochloride (HCl), pH 7.4, 150 mM sodium chloride (NaCl), 1 mM ethylenediaminetetraacetic acid (EDTA), 1% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS, Yifeixue Biotechnology, YS0005-500). The RIPA lysis buffer contained protease and phosphatase inhibitors. Protein concentration was obtained using a method reported recently. Cortical samples were normalized to determine the loading volume for each sample (total proteins = 40 μg). Samples were loaded into 10% SDS-PAGE running gel. Nitrocellulose membranes were used for protein transfer. After the membranes were blocked using a non-fat milk solution (Sangon Biotech, A600669) (5% w/v) for 1 h, they were then probed using the primary antibodies listed in Table 1 at 4°C overnight. The membranes were washed with PBS 3 times (15 min each time) and incubated with the following secondary antibodies: IRdye800 and IRdye680. The membranes were scanned using the LI-COR Imaging System.

2.5. Counting methods for cells positive for different markers

For each mouse, we chose two brain sections (spaced 400 μm) to perform IHC for Aβ, glial fibrillary acidic protein (GFAP), or Iba1. First, IHC images for Aβ were taken with the ×10 objective lens in the BX53 microscope, and amyloid plaques (diameter of plaques >5 μm) were counted in each IHC image (an area of 1.364 μm × 1.021 μm). Second, the same microscopy settings were used to capture IHC images for GFAP and Iba1. In each IHC image, GFAP+ cells or Iba1+ cells in the cortex were counted manually.

2.6. Statistical analysis

Data were shown as average ± standard error of mean (SEM). Two-tailed Student’s t-test was used to analyze the differences in protein levels, cell numbers, and plaque

Table 1. Primary antibodies used for Western blotting and immunohistochemistry.

| Antibodies                  | Suppliers                  | Catalogue number |
|-----------------------------|----------------------------|-----------------|
| Anti-PDK1 antibody          | Abcam                      | ab52893         |
| Anti-NeuN antibody          | Millipore                  | RRID: AB_881962|
| Anti-Aβ (1-16) antibody     | Biologen                   | 803001          |
| Anti-Aβ antibody (D54D2)    | Cell Signaling Technology  | 8243            |
| Anti-amyloid precursor      | Sigma-Aldrich              | A8717           |
| Anti-GFAP antibody          | ABclonal                   | A14673          |
| Anti-Iba1 antibody          | FUJIFILM Wako Shibayagi    | 016-20001       |
| Anti-phospho-S6 (Ser235/236)| Cell Signaling Technology  | 2211            |
| Anti-phospho-4E-BP1 (Ser65) | Cell Signaling Technology  | 9451            |
| Anti-BACE1 antibody         | Abcam                      | Ab183612        |
| Anti-ADAM10 antibody        | Millipore                  | AB19026         |
| Anti-β-actin antibody       | Genex                      | 4060            |
| Anti-GAPDH antibody         | CW Biotech                 | cw0100          |

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numbers between Pdk1 cKO/5×FAD mice and their 5×FAD littermates. *P < 0.05 indicated a significant effect.

3. Results

3.1. Molecular analysis of the 5×FAD model with PDK1 deficiency through Emx1-Cre-mediated gene recombination

It has been shown that long-term treatment of adult Tg2576 mice with BX912 decreases amyloid plaques but causes lethal effect in mice[22]. To investigate whether the inactivation of PDK1, beginning from the embryonic stage, could prevent AD-like pathology in 5×FAD mice[30], we generated Pdk1<sup>f/f</sup>;Emx1-Cre/5×FAD (Pdk1 cKO/5×FAD) mice, in which the deletion of PDK1 begins at embryonic day 10.5 (E10.5) in the forebrain neurons. The four different genotypes are shown in Figure 1A.

To determine the expression pattern of Cre, Rosa26-LSL-tdTomato mice[16,47] were crossed to Emx1-Cre to generate Emx1-Cre;Rosa26-LSL-tdTomato, in which tdTomato is expressed in Cre-positive (Cre+) cells. The brain sections of the double mutant mice at P0 were used to perform costaining for tdTomato and NeuN. We observed abundant tdTomato+/NeuN+ cells in the cortex (Figure 1B). The cell counting results showed that ~92% of NeuN+ cells were positive for tdTomato. Subsequently, we performed fluorescence IHC for PDK1. We found that PDK1 immunoreactivity was qualitatively reduced in the Pdk1 cKO/5×FAD cortex at 2.5 months compared with 5×FAD littermates (Figure 1C). Western blotting was performed for PDK1 using cortical lysates from mice at 2.5 months. PDK1 levels were notably decreased in the Pdk1 cKO/5×FAD cortex compared with 5×FAD mice (Figure 1D), suggesting efficient inactivation of PDK1 in Pdk1 cKO/5×FAD mice.

3.2. Plaque deposition is prevented in 5×FAD mice with PDK1 deficiency

To investigate whether PDK1 deletion affects plaque deposition in 5×FAD mice, IHC was performed for Aβ using brain sections from control, Pdk1 cKO, 5×FAD, and Pdk1 cKO/5×FAD mice at three different ages (2.5, 4, and 6 months). First, there were a few amyloid plaques in the 5×FAD cortex at 2.5 months but abundant amyloid plaques at 4 and 6 months compared with non-Tg littermate controls (Figure 2A). In contrast, amyloid plaques were hardly seen in the Pdk1 cKO/5×FAD cortex at the aforementioned ages.

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**Figure 1.** Characterization of forebrain-specific Pdk1 cKO/5×FAD mice. (A) Breeding strategies to generate Pdk1 cKO/5×FAD mice. Pdk1<sup>f/f</sup> and Pdk1<sup>f/+</sup>;Emx1-Cre as the control group, Pdk1<sup>f/+</sup>;APP/PS1 and Pdk1<sup>f/+</sup>;Emx1-Cre;APP/PS1 as 5×FAD, Pdk1<sup>f/+</sup>;Emx1-Cre as Pdk1 cKO, and Pdk1<sup>f/+</sup>;Emx1-Cre;APP/PS1 as Pdk1 cKO/5×FAD. (B) Representative images of tdTomato/NeuN costaining. Abundant tdTomato+ and tdTomato+/NeuN+ cells were detected in the cortex of Emx1-Cre;Rosa26-LSL-ttdTomato mice. (C) Representative images of fluorescence IHC for PDK1. Sagittal brain sections at 2.5 months were used. The immunoreactivity of PDK1 was qualitatively different between 5×FAD and Pdk1 cKO/5×FAD mice. The scale bar is 100 μm. (D) Western blotting for PDK1. Cortical samples were prepared from 5×FAD and Pdk1 cKO/5×FAD mice aged at 2.5 months (n = 3 mice per group; *P < 0.05).
(Figure 2A and B). Second, quantitative results revealed significant reductions in the average number of plaques in the Pdk1 cKO/5×FAD cortex at 2.5, 4, and 6 months compared to that in 5×FAD (Figure 2C; P < 0.001 at 2.5 months, P < 0.01 at 4 months, and P < 0.001 at 6 months). Indeed, amyloid plaques were hardly seen in the Pdk1 cKO/5×FAD cortex (Figure 2A–C). The results suggest that PDK1 deletion may prevent plaque deposition in APP Tg mice.

3.3. Gliosis is alleviated in 5×FAD mice with PDK1 deficiency

Neuroinflammation is a typical feature in mouse models of AD\textsuperscript{[30,36]}. To investigate whether microglia are affected in the Pdk1 cKO/5×FAD cortex, we performed IHC for Iba1 using brain sections from mice at 2.5, 4, and 6 months. First, Iba1 cortical immunoreactivity did not differ between the control and Pdk1 cKO mice at each age tested (Figure 3A). However, it was increased in the 5×FAD group at 2.5, 4, and 6 months compared with the control group (Figure 3A and 3B). Second, the average number of Iba1+ cells was significantly higher in the 5×FAD cortex than in the control at 2.5, 4, and 6 months (Figure 3C; P < 0.001), but it was significantly lower in the Pdk1 cKO/5×FAD group at 4 and 6 months compared with the 5×FAD group (Figure 3C; P < 0.001). These data suggest that PDK1 deletion may ameliorate microgliosis in APP Tg mice.

Figure 2. Prevention of Aβ deposition in the cortex of Pdk1 cKO/5×FAD mice. (A) Representative images of Aβ immunostaining. Brain sections at 2.5, 4, and 6 months were used. Images were captured from the cortex of four different groups of mice, including the control, Pdk1 cKO, 5×FAD, and Pdk1 cKO/5×FAD groups. The scale bar is 20 μm. (B) Enlarged images for boxed regions in (A). The scale bar is 25 μm. (C) Quantification results showing the total number of amyloid plaques in the cortex. There were significant differences between 5×FAD and Pdk1 cKO/5×FAD mice at 2.5 (**P < 0.01), 4 (**P < 0.01), and 6 months (**P < 0.001) (n = 3–5 mice per group per age).
To study whether there are changes to astrocytes in Pdk1 cKO/5×FAD mice, IHC was performed for GFAP. First, it was observed that GFAP immunoreactivity was qualitatively higher in the Pdk1 cortex and the 5×FAD cortex at 2.5, 4, and 6 months compared with the littermate controls (Figure 4A). GFAP immunoreactivity was reduced in the Pdk1 cKO/5×FAD cortex compared with the 5×FAD cortex (Figure 4A and B). Second, the total number of GFAP+ cells in the 5×FAD cortex at 4 or 6 months was more than that in the control cortex (Figure 4C; P < 0.001), but it was smaller in the Pdk1 cKO/5×FAD cortex than in the 5×FAD cortex (Figure 4C; P < 0.001). These results suggest that astrogliosis may be attenuated by PDK1 deletion in 5×FAD mice.

3.4. mTOR signaling is inhibited in 5×FAD mice with PDK1 deficiency

In the mTOR signaling pathway, S6 kinase (S6K) phosphorylates S6 at Ser235 and Ser236 (Ser235/236) following its activation by mTOR[49,50]. We investigated whether this pathway is affected by PDK1 deletion. First, we performed Western blotting for pS6Ser235/236. We found that the levels of pS6Ser235/236 were significantly lower in the Pdk1 cKO/5×FAD cortex compared with the 5×FAD cortex (Figure 5A and B). Second, we performed Western blotting for phosphorylated 4E-BP1, an important member of the mTOR pathway[51]. We found that the levels of p4E-BP1Ser70 were lower in the Pdk1 cKO/5×FAD cortex compared with 5×FAD mice (Figure 5A and B). Third, we performed IHC
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3.5. Expression of APP is decreased in 5×FAD mice with PDK1 deficiency

To determine the molecular mechanisms by which PDK1 deletion reduces Aβ deposition, we focused on APP. Since Aβ is known to be generated from APP through sequential cleavages by β- and γ-secretases[52], Western blotting on APP was performed using cortical samples from four different genotypes of mice (Figure 6A). Total APP levels were robustly higher in the 5×FAD cortex than in the control cortex (Figure 6B) and significantly lower in Pdk1 cKO/5×FAD mice than in 5×FAD mice (Figure 6B). Next, IHC for APP was performed. The results showed that APP immunoreactivity was qualitatively reduced in Pdk1 cKO/5×FAD mice than in 5×FAD mice (Figure 6C).

We also performed Western blotting to examine the products of APP cleaved by α- and γ-secretases: Secreted (s)APPα and APP-C-terminal fragment (CTF) (Figure 6D). Our results revealed that protein levels for sAPPα and APP-CTF were significantly lower in Pdk1 cKO/5×FAD mice than in 5×FAD mice.
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Figure 5. Reduced mTOR activity in Pdk1 cKO/5×FAD mice. (A and B) Western blotting for pS6\(^{Ser235/236}\) and p4E-BP1\(^{Ser65}\). (A) Cortical samples from 5×FAD and Pdk1 cKO/5×FAD mice were used. (B) There were significant differences in the relative levels of pS6\(^{Ser235/236}\) and p4E-BP1\(^{Ser65}\) between 5×FAD and Pdk1 cKO/5×FAD mice at 4 months (n = 3–4 mice per group; * P < 0.05, ** P < 0.01). (C) Fluorescence IHC for pS6\(^{Ser235/236}\). Brain sections at 4 months were used. The immunoreactivity of pS6\(^{Ser235/236}\) was qualitatively different between 5×FAD and Pdk1 cKO/5×FAD mice. The scale bar is 50 μm.

4. Discussion

The recent study by Petri et al. suggests that pharmacological inhibition on PDK1 may be beneficial to AD. The aim of this study was to test whether early inhibition of PDK1 may prevent AD-like pathology in APP Tg mice. In this study, Emx1-Cre-mediated Pdk1 cKO/5×FAD mice were used. We demonstrated that PDK1 deletion prevents plaque deposition and attenuates gliosis in the 5×FAD cortex. In addition, PDK1 deletion inhibits the mTOR signaling pathway and downregulates APP expression in 5×FAD mice. Overall, these findings are consistent with the notion that PDK1 could be a promising therapeutic target for AD.

As previously reported, long-term treatment with BX912 results in the death of Tg2576 mice\(^{27}\). In line with this, straight KO of Pdk1 leads to early embryonic lethality in mice\(^{27}\). In contrast, Emx1-Cre-mediated PDK1 deletion does not cause any lethal effect in mice. We did not observe any abnormal deaths in Pdk1 cKO/5×FAD mice or in Pdk1 cKO mice. Nevertheless, the pharmacological method\(^{22}\) and genetic approach used in the present study may efficiently inhibit PDK1 function. However, it is known that Emx1-Cre-mediated PDK1 deletion disrupts the formation of cortex in mice\(^{29}\). Therefore, we cannot exclude the possibility that a reduced cortex size may play a role in improving AD-like pathology in Pdk1 cKO/5×FAD mice.

Genetic approaches have been widely used in neurodegenerative diseases\(^{34}\). First, a gene therapy-based clinical trial has revealed high treatment efficacy in spinal muscular atrophy (SMA) type 1\(^{39}\). Second, genetic approaches have been tested in animal models for amyotrophic lateral sclerosis\(^{56}\) and Huntington’s disease\(^{57}\). Third, the CRISPR-Cas9 technology was employed in a study to inactivate the mutant human APP gene in 5×FAD mice, resulting in a significant alleviation of AD-like pathology\(^{38}\). Based on our finding that early inhibition of PDK1 prevents AD-like pathology in 5×FAD mice, we propose the development of a genetic approach that targets neuronal PDK1 as a potential treatment strategy for AD.

To identify the underlying molecular mechanisms in Pdk1 cKO/5×FAD mice, we examined the expression of APP and APP processing enzymes, including α-secretase
and BACE1. Since the levels of APP were significantly reduced in the Pdk1 cKO/5×FAD cortex, we reason that this may be responsible for the prevention of plaque deposition. In agreement with this, Duan et al. have reported that CRISPR-Cas9-mediated KO of mutant human APP gene reduces amyloid plaques and improves spatial working memory in 5×FAD mice.

On the other hand, no changes have been observed in the levels of ADAM10, BACE1, and sAPPα in Pdk1 cKO/5×FAD cortices, suggesting a possibility that PDK1 deletion may not enhance the activity of α-secretase or β-secretase. In contrast, evidence has shown that BX912 may promote α-secretase-dependent cleavage of APP\textsuperscript{[22]}. We reason that the above discrepancy reflects the fact that pharmacological and genetic approaches exhibit different target selectivity and cell specificity\textsuperscript{[37]}. For instance, BX912 is known to inhibit a spectrum of kinases, including PDK1\textsuperscript{[59,60]}. In contrast, Emx1-Cre-mediated gene recombination permits conditional inactivation of PDK1 in neurons in the postnatal forebrain.
Since ribosomal kinase S6 is a key member of the mTOR signaling pathway\(^{[13,16,21]}\), the decreased pS6 levels suggest that the activity of mTOR may be reduced in the Pdk1 cKO/5xFAD cortex. Since previous evidence has shown that S6 is essential for ribosome biogenesis\(^{[61]}\), it is likely that ribosome biogenesis may be regulated by PDK1 via S6\(^{[37]}\). In essence, the following molecular and cellular mechanisms may be responsible for the prevention effects on AD-like pathology as observed in Pdk1 cKO/5xFAD mice. First, PDK1 deletion causes a reduction in mTOR signaling and subsequently inhibits the activity of S6. Second, the deactivation of S6 may impair ribosome-dependent protein synthesis, which then downregulates APP expression at the translational level\(^{[37]}\). Third, Aβ generation from APP is repressed, and gliosis is ameliorated in the forebrain of Pdk1 cKO/5xFAD mice.

5. Conclusion

Deletion of PDK1 in the developing cortex prevents plaque deposition and ameliorates gliosis in 5xFAD mice. PDK1 deletion inhibits mTOR signaling activity and downregulates APP expression. PDK1 may serve as a potential target for the treatment of AD.

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Conflict of interest

The authors have no conflicts of interest to declare.

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