



# Omaveloxolone Ameliorates Cognitive Deficits by Inhibiting Apoptosis and Neuroinflammation in APP/PS1 Mice

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Received: 21 February 2024 / Accepted: 9 July 2024

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## Abstract

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease associated with aging, characterized by progressive cognitive impairment and memory loss. However, treatments that delay AD progression or improve its symptoms remain limited. The aim of the present study was to investigate the therapeutic effects of omaveloxolone (Oma) on AD and to explore the underlying mechanisms. Thirty-week-old APP/PS1 mice were selected as an experimental model of AD. The spatial learning and memory abilities were tested using the Morris water maze. Amyloid-beta (A $\beta$ ) deposition in the brains was measured using immunohistochemistry. Network pharmacological analyses and molecular docking were conducted to gain insights into the therapeutic mechanisms of Oma. Finally, validation analyses were conducted to detect changes in the associated pathways and proteins. Our finding revealed that Oma markedly rescued cognitive dysfunction and reduced A $\beta$  deposition in the brains of APP/PS1 mice. Network pharmacological analysis identified 112 intersecting genes, with CASP3 and MTOR emerging as the key targets. In vivo validation experiments indicated that Oma attenuated neuronal apoptosis by regulating apoptotic proteins, including caspase 3, Bax, and Bcl-2. Moreover, Oma suppressed neuroinflammation and induced autophagy by inhibiting the phosphorylation of mTOR. These findings highlight the therapeutic efficacy of Oma in AD and that its neuroprotective effects were associated with inhibiting neuronal apoptosis and regulating neuroinflammation.

**Keywords** Alzheimer's disease · APP/PS1 · Omaveloxolone · Therapeutic agent · Animal study

## Introduction

Alzheimer's disease (AD) is the most common dementia type, and it is characterized by progressive cognitive decline and psychiatric symptoms [1, 2]. AD affects approximately 10 to 30% of people aged > 65 years, posing a significant challenge for both individuals and society [3]. The pathological changes in AD include the accumulation of amyloid-beta (A $\beta$ ) aggregates, neurofibrillary tangles, neuroinflammation, and loss of neurons [4]. Nevertheless, the key mechanisms underlying its pathogenesis and progression remain poorly understood. Therefore, medications that delay AD progression or improve its symptoms are still limited.

Triterpenoids, including oleanolic acid, are a large family of compounds synthesized in some plants; they comprise pentacyclic motifs and potent immune-modulating activities [5, 6]. To increase the utility of natural triterpenoids, researchers have developed several synthetic derivatives [5]. Omaveloxolone (Oma) is a second-generation semi-synthetic oleanane triterpenoid with anti-cancer and anti-inflammation activities [6, 7]. To note, Oma can achieve

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meaningful concentrations in the brain and exert therapeutic effects on neurological diseases, such as epilepsy and amyotrophic lateral sclerosis [8–10]. Moreover, in 2023, the FDA has approved Omav as the first treatment for Friedreich's ataxia [11]. Although the possibility of using Omav for neurological diseases has been raised, studies in AD are scarce.

Network pharmacology is an approach that helps elucidate the mechanisms of drugs. It aims to refine the treatment strategies by studying the intricate biological network of disease characteristics and drug targets connected to each other [12]. Molecular docking, on the other hand, is a theoretical technique that explores the interaction and recognition between ligands and proteins; it holds significant importance in understanding the molecular mechanisms underlying pharmacological activities and predicting the structure of protein–ligand complexes [13]. The integrated use of these two methods has gained attention in providing a comprehensive framework for drug discovery and optimization.

In this study, we aimed to investigate whether Omav ameliorated cognitive impairment in APP/PS1 mice. Utilizing network pharmacology, we predicted the potential targets and biological pathways of Omav for AD treatment. Finally, we prioritized two of the predicted targets for further validation using molecular docking and *in vivo* experiments, to understand the underlying mechanisms.

## Materials and Methods

### Animals and Drug Treatment

A total of 16 APP/PS1 double transgenic mice and 8 wild-type (WT) mice with the same genetic background of 30-week-old were purchased from Beijing Zhishan Co., Ltd. (Beijing, China). These mice were maintained in cages with three to four per cage, ensuring free access to food and water. They were housed under monitored conditions, maintaining a constant temperature range of 20–24 °C and a regular 12-h light/dark cycle. After a week of adaptation to the environment, APP/PS1 mice were assigned randomly into two treatment groups: APP/PS1-vehicle (APP/PS1) and APP/PS1-Omav (APP/PS1-Omav). Omav (20 mg/kg) or vehicle (corn oil) was administered daily via oral gavage for 4 weeks. The Omav dose was selected based on previous literature and dosage conversion from human to mice using a calculator [14, 15]. After the 4-week period, all mice were subjected to behavioral testing, and the administrations were continued throughout the test.

### Morris Water Maze Test

The Morris water maze tests, as described by Vorhees and William [16], were conducted in a circular tank containing

a submerged platform (diameter, 10 cm). The water temperature was controlled at  $21 \pm 1$  °C. The experiments were conducted in two sections: (i) spatial acquisition section (days 1–5) and (ii) probe trial section (day 6). In the spatial acquisition section, each mouse was released into the water facing the wall and allowed to find the platform within 60 s. The escape latency was recorded as the time taken to find the platform. Mice that successfully found the platform within the time limit were permitted to remain for 5 s; otherwise, they were gently placed on the platform and allowed to stay for 20 s. Each mouse underwent four trials daily, and the escape latency on each training day was averaged over four trials. For the probe trial, the platform was removed, and mice were released into the water from the opposite quadrant and recorded for 60 s to evaluate the spatial memory ability. The time spent and the number of crossings in the target zone were recorded. Trajectories were video-tracked and analyzed using SMART3 software (Panlab Harvard, USA).

### Sample Collection

After behavioral testing, the mice were anesthetized and transcardially perfused with cold PBS. On an ice plate, brains were carefully removed and dissected in half. One half was fixed in 4% polyformaldehyde for morphological studies, whereas the remaining half was frozen at  $-80$  °C for biochemical analyses.

### Network Pharmacology and Molecular Docking

The chemical structure and SMILES information of Omav were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Potential targets of Omav were predicted using two databases: Super-PRED [17] and Pharmmaper [18]. Targets with a probability  $\geq 60\%$  or a norm fit  $> 0.5$ , respectively, were selected for further analysis. The potential targets of AD were obtained from the GeneCards database (<https://www.genecards.org/>). AD targets were selected with a relevance score  $> 10$ . A Venn diagram was drawn to obtain the intersection genes. The STRING database was used to construct a protein–protein interaction (PPI) network [19]. Then the PPI network was imported into Cytoscape 3.10.1 for further analyses and visualization. Key targets were obtained using the Centiscape 2.2 plugin according to three topological features (degree, betweenness, and closeness). The graph was arranged and optimized based on degree values. The DAVID database was then used for GO and KEGG enrichment analysis to elucidate the core mechanisms and pathways of Omav against AD [20]. Finally, the AutoDock Vina software was used to perform molecular docking between the candidate proteins with Omav. A binding energy of less than  $-5.0$  kcal/mol  $-7.0$  kcal/

mol indicates a good or strong binding activity, respectively [21]. The docking results were visualized using the PyMOL software.

### Immunohistochemistry and Immunofluorescence

Brains were fixed in 4% polyformaldehyde for 24 h and underwent a gradient dehydration process before being paraffin-embedded. Serial 5- $\mu$ m coronal sections were prepared, deparaffinized, hydrated, and treated with 0.01 M citric acid for antigen retrieval. After three washes with PBS, sections were submerged in 3% H<sub>2</sub>O<sub>2</sub> for 30 min. For blocking, the sections were incubated with 5% goat serum for 1 h. Next, they were incubated with primary antibody (anti-A $\beta$  1–42, Abcam, ab201060) at 4 °C overnight. After rinsing three times with PBS, horseradish peroxidase-labeled secondary antibody was used for incubation for 1 h, followed by colorization using a DAB kit. Images were captured using an optical microscope (Olympus, Tokyo, Japan). Three non-overlapping visual fields were randomly selected, and the number of senile plaques in each field was measured using the ImageJ software (Image Pro Plus software, USA). For immunofluorescence, the sections were incubated with the primary antibody (anti-Iba-1, Invitrogen, MA527726) overnight at 4 °C, followed by incubation with CoraLite488-conjugated secondary antibody for 2 h. DAPI was used for nuclear staining, and images were captured using an Olympus fluorescence microscope (Olympus, Japan).

### Western Blot

For protein collection, mouse brain tissues were lysed in a RIPA buffer comprising a cocktail of protease and phosphatase inhibitors. After a brief sonication, the lysates were centrifuged at 12,000 g for 15 min (4 °C). The samples were then prepared by mixing with 5 $\times$  loading buffer and boiled for 5 min. Equal amounts of proteins were added into each well of the SDS-PAGE gels for electrophoresis and transferred onto 0.45- $\mu$ m PVDF membranes. After incubation with blocking buffer, membranes were incubated with the following primary antibodies: cleaved caspase 3 (Wanleibio, WL02117), Bax (Proteintech, 50599-2-Ig), Bcl-2 (Proteintech, 26593-1-AP),  $\beta$ -actin (Proteintech, 66009-1-Ig), p-mTOR (Abcam, ab109268), t-mTOR (Abcam, ab134903), LC3 (Abcam, ab192890), and P62 (MedChemExpress, HY-P80518) at 4 °C overnight. Membranes were then incubated with secondary antibody for 1 h. Protein bands were visualized using ECL reagent, and the abundance of proteins was analyzed using the ImageJ software.

### Hematoxylin–Eosin (H&E) Staining

H&E staining was performed using a commercial kit (Solarbio, Beijing, China). Briefly, after deparaffinization and hydration, the brain sections were dyed with a hematoxylin solution for 10 min, washed, and differentiated for 30 s. Then, they were re-dyed with eosin solution for 1 min. After another wash with tap water, the sections underwent dehydration, transparentization, and sealing procedures for observation.

### Enzyme-linked Immunosorbent Assay (ELISA)

Briefly, brain tissues were homogenized in cold PBS consisting of a cocktail of protease and phosphatase inhibitors. After a brief sonication, the homogenates were centrifuged at 5000 g for 10 min (4 °C). The levels of tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , and IL-6 in the supernatants were determined using commercial mouse ELISA kits (Elabscience, China) according to the manufacturer's instruction.

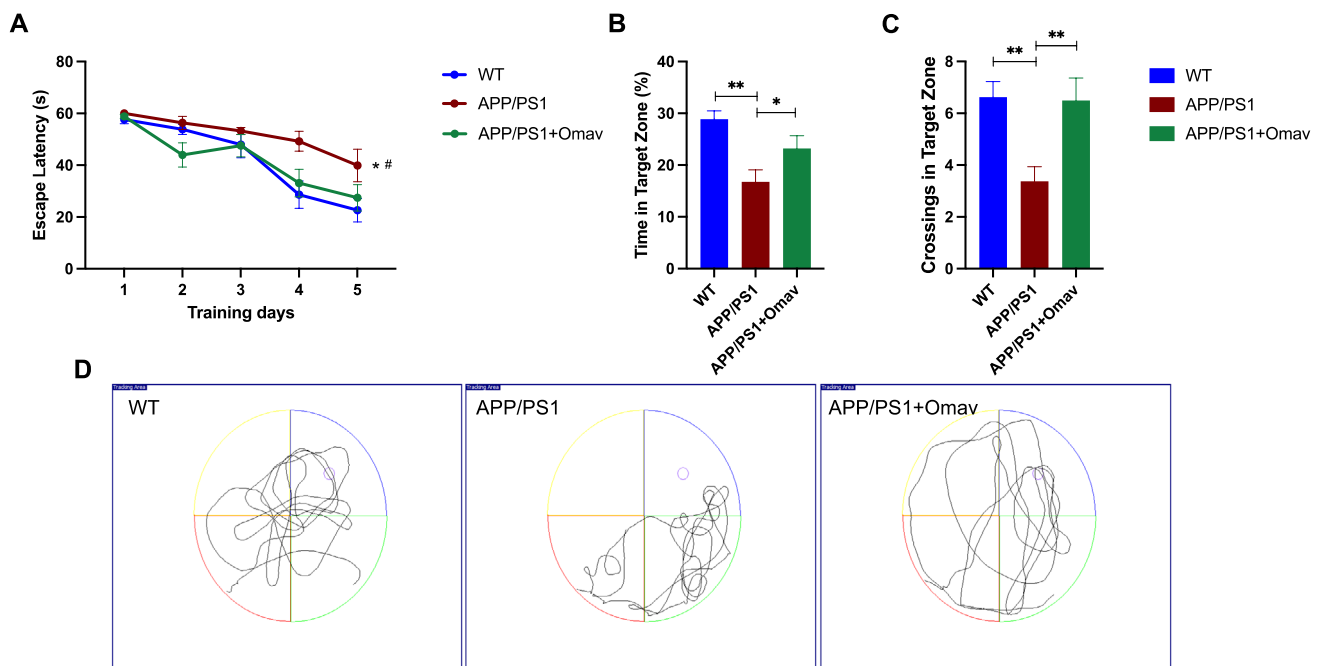
### Statistical Analysis

All values were presented as mean  $\pm$  SEM. The escape latencies in the Morris water maze were calculated using the area under the curve, and group differences were analyzed using one-way analysis of variance (ANOVA). All other data were analyzed using a *t*-test or one-way ANOVA, followed by the LSD post hoc test. *P* < 0.05 indicated statistical significance. All statistical analyses were performed using SPSS 26.0 (SPSS Corp., Armonk, NY, USA).

## Results

### Omap Treatment Ameliorates Cognitive Dysfunction in APP/PS1 Mice

The spatial learning and memory abilities of the mice were evaluated using the Morris water maze test. In the spatial acquisition section, APP/PS1 mice exhibited a longer time searching for the platform compared with the WT littermates. The administration of Omap significantly improved the impaired learning ability in APP/PS1 mice (Fig. 1A). Moreover, the probe trial performed on the last day suggested that APP/PS1 mice spent less time in the target zone and had fewer crossovers than the WT littermates. However, Omap-treated mice demonstrated an increase in the time spent in the target zone and had more crossovers compared with the APP/PS1 controls (Fig. 1B, C). Figure 1D



**Fig. 1** Omav improved cognitive deficits in APP/PS1 mice. The Morris Water Maze test was used to evaluate the effect of Omav on APP/PS1 mice. **A** Escape latency during the spatial acquisition section. The number sign (#) indicates  $P < 0.05$  between the APP/PS1 and WT

group. The asterisk (\*) indicates  $P < 0.05$  between the APP/PS1 and APP/PS1+Omav group. **B** and **C** Time and crossings in the target zone. \* $P < 0.05$ ; \*\* $P < 0.01$ . Data presented as mean  $\pm$  SEM. **D** Representative tracing graphs during the probe trials

illustrates the representative trajectories in the probe trial. These results suggested that Omav treatment rescued cognitive dysfunction in AD mice.

### Omav Treatment Reduces A $\beta$ Deposition in APP/PS1 Mice

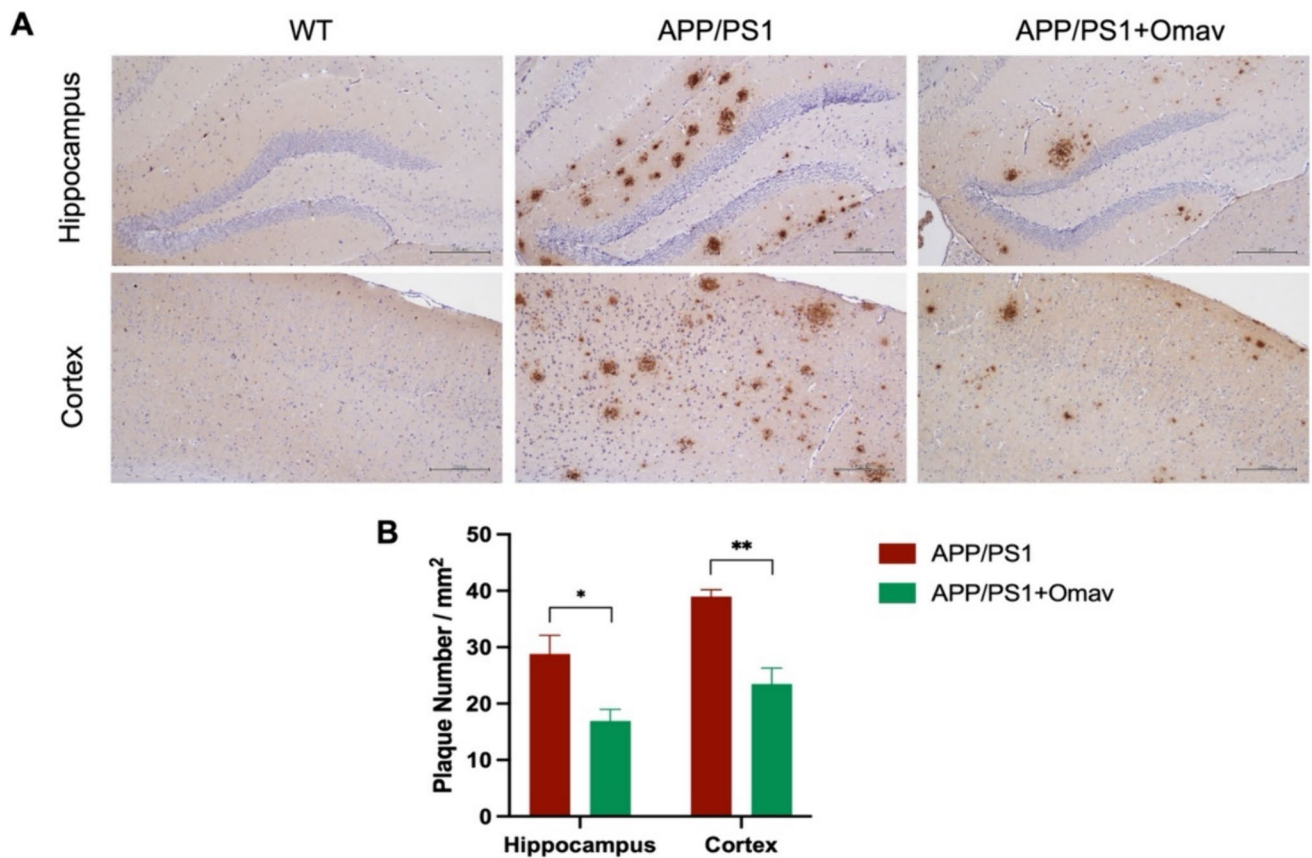
To investigate the effects of Omav treatment on A $\beta$  deposition in APP/PS1 mice, immunohistochemical staining was conducted using a specific antibody against A $\beta$  1–42. The results revealed a significant elevation in the number of plaques in the hippocampus and cortex of APP/PS1 mice compared with WT littermates. Notably, Omav treatment dramatically reduced A $\beta$  deposition compared with APP/PS1 mice (Fig. 2A, B). These results suggested that Omav treatment decreased A $\beta$  burden in APP/PS1 mice.

### Network Pharmacological Analysis of Omav Against AD

We obtained 94 and 120 genes from Super-PRED and Pharmmapper, respectively. After eliminating the duplicates, 208 genes were retained for further analysis. Additionally, we selected 2022 genes with a relevance score  $> 10$  by searching the GeneCards database. Targets of both Omav and AD were subjected to a Venn diagram, and 112 common genes were screened (Fig. 3A). The PPI network indicated

110 nodes and 1229 edges. The key targets were screened using Centscape 2.2 plugin. Finally, the key target subnetwork with 26 nodes and 268 edges was obtained based on a closeness  $\geq 0.005$  (average), betweenness  $\geq 100.364$  (average), and degree  $\geq 22.345$  (average) (Fig. 3B). The size and darkness of a node directly correlate with its degree value, with larger and darker nodes indicating higher degree values.

GO enrichment analysis included biological progress (BP), cellular components (CC), and molecular functions (MF). A total of 319 BP, 57 CC, and 91 MF satisfied the required  $p$ -value  $< 0.05$ . The leading 10 BP, CC, and MF catalogs were visualized (Fig. 3C). Results of BP analysis suggested that the function of Omav against AD was primarily focused on positive regulation of cell migration, inflammatory response, positive regulation of gene expression, negative regulation of apoptotic process, and protein phosphorylation. The inflammatory response and negative regulation of apoptotic process were the leading BP items; thus, Omav might exert therapeutic effects by regulating neuroinflammation and apoptosis. In addition, we identified 142 signaling pathways enriched by KEGG analysis ( $p$ -value  $< 0.05$ ) and selected the leading 20 for visualization (Fig. 3D). To note, Alzheimer's disease was also one of the top pathways enriched by KEGG, supporting the potential role of Omav in the treatment of AD. Next, we tried to select key targets for further validation. We found that CASP3 and MTOR were the top two key targets obtained by PPI network, and were also involved in the inflammatory



**Fig. 2** Omav reduced A $\beta$  burden in APP/PS1 mice. **A** Representative images of A $\beta$ 42 deposits in the hippocampus and cortex using an antibody against A $\beta$ 42. Scale bar: 200  $\mu$ m. **B** Quantification of plaque

numbers in the hippocampus and cortex. \* $P < 0.05$ ; \*\* $P < 0.01$ . Data presented as mean  $\pm$  SEM

response and negative regulation of apoptotic process in the GO analysis as well as the Alzheimer's disease pathway in the KEGG analysis. Therefore, we selected the leading two targets, CASP3 and MTOR, for further verification.

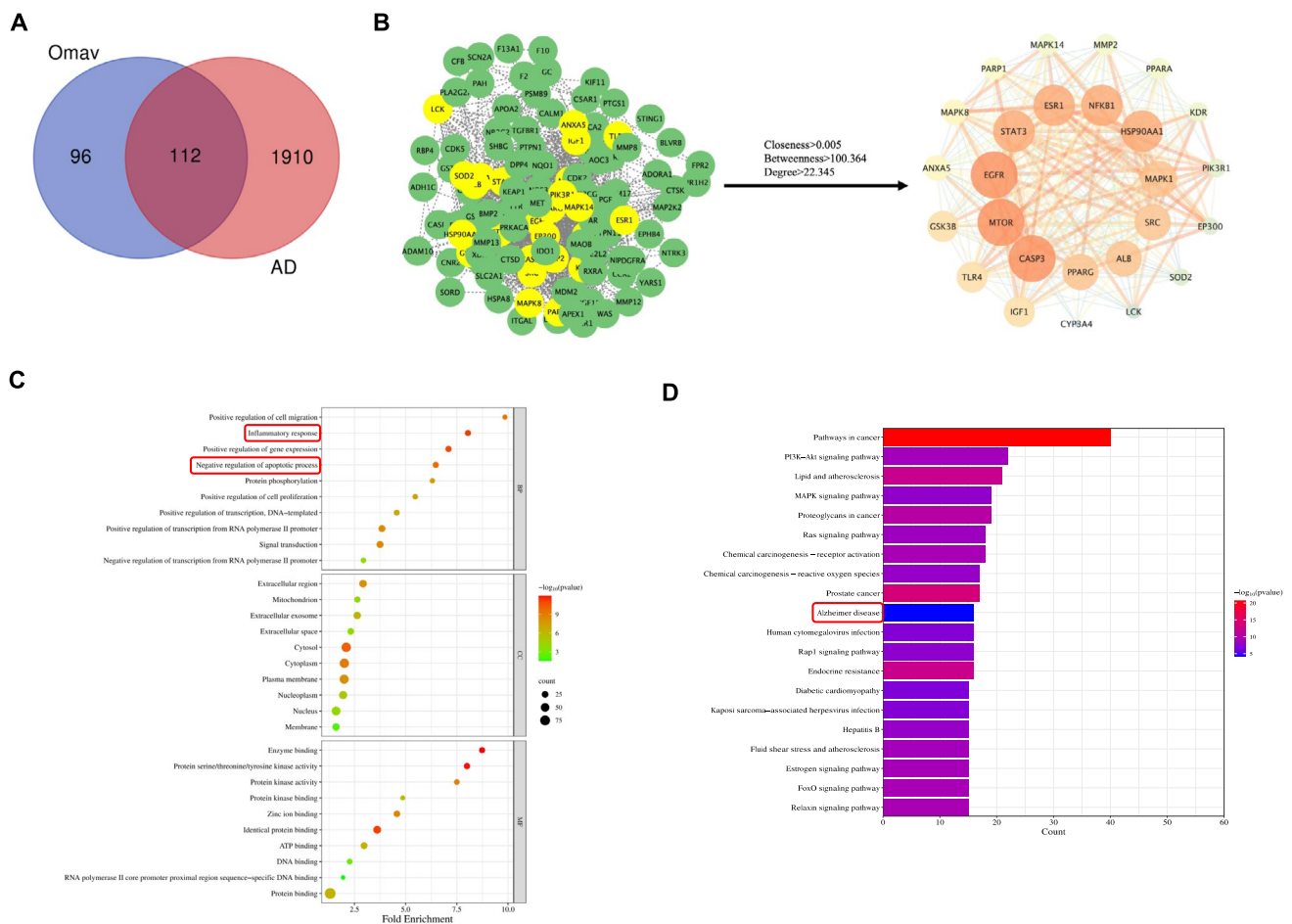
### Molecular Docking Analysis

Molecular docking was performed to identify the binding ability of the candidate proteins to Omav for verification. The binding energies of Omav for CASP3 and MTOR were  $-10.2$  and  $-9.8$  kcal/mol, respectively. Notably, both binding energies were less than  $-7$  kcal/mol, indicating strong binding affinities. Figure 4 presents the three-dimensional docking conformations of the ligands with their respective targets, providing a visual representation of the molecular interactions.

### Omav Ameliorates Neuronal Apoptosis and Damage in APP/PS1 Mice

H&E staining was performed to evaluate the effects of Omav on neuronal damage. Neurons in the WT group

displayed a normal structure; they were neatly arranged, and uniformly stained, with round nuclei and obvious nucleoli (Fig. 5A). In contrast, the APP/PS1 group exhibited smaller numbers of neurons, irregular neuronal arrangements, and abnormal neuronal morphology, including a dark appearance, the lack of clear nuclei, and triangulated and shrunken neuronal bodies. These pathological changes were mitigated upon the administration of Omav. Caspase 3, the key target explored by network analyses, is one of the executing enzymes in apoptosis, and is regarded as a proximate mediator of apoptosis [22, 23]. Therefore, we examined the levels of cleaved caspase 3 and other apoptosis-related proteins, including Bax and Bcl-2, in the brains. The results showed that the cleaved caspase 3 and Bax (a pro-apoptotic protein) were highly expressed in the brains of APP/PS1 mice than in WT littermates, whereas Bcl-2 (an anti-apoptotic protein) was downregulated. Omav treatment downregulated the expression of cleaved caspase 3 and Bax and upregulated that of Bcl-2 (Fig. 5B). Taken together, Omav regulated apoptosis and alleviated neuronal damage in APP/PS1 mice.

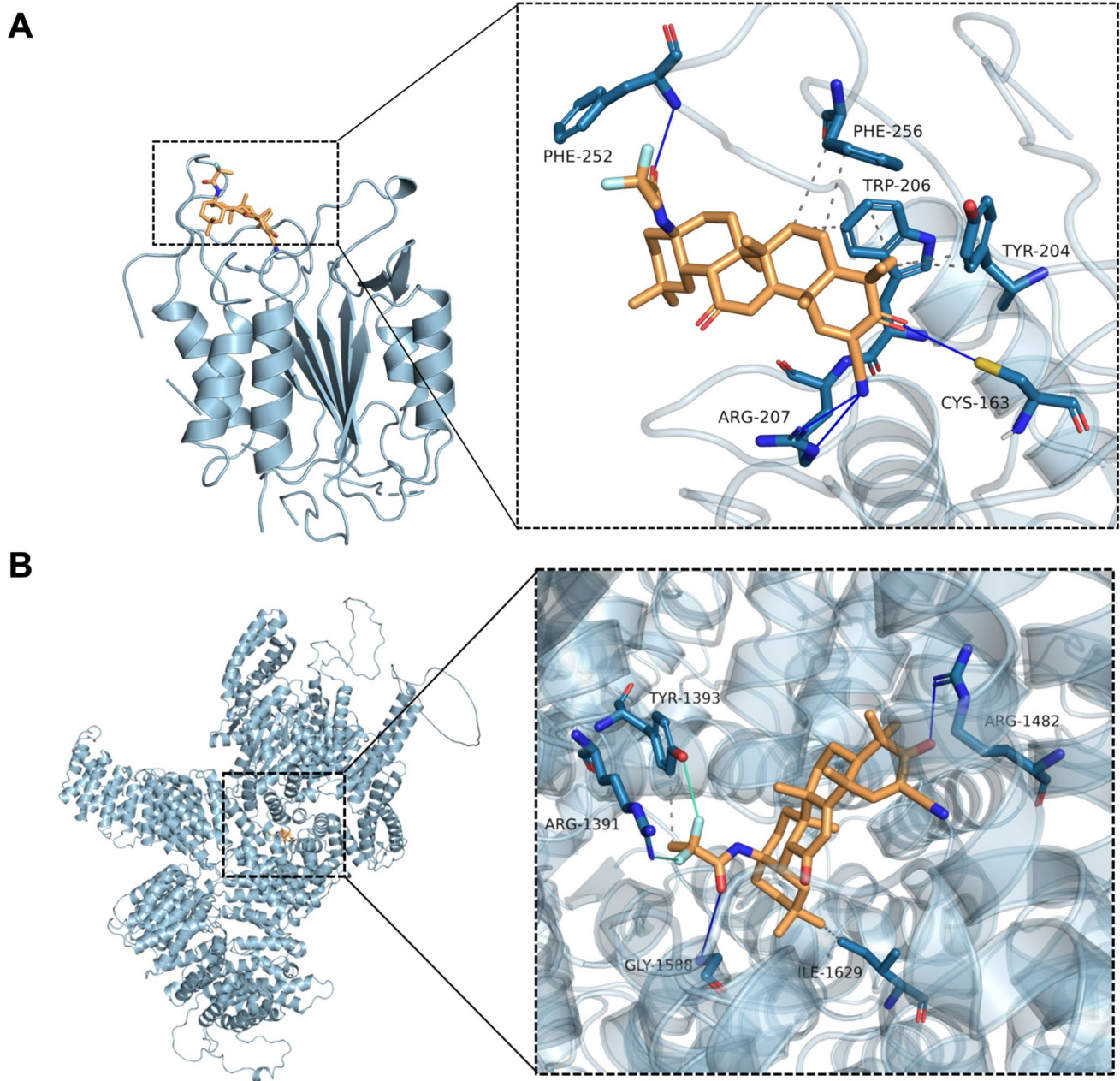


**Fig. 3** Network pharmacology of Omav against AD. **A** The Venn diagram showed 112 common targets between Omav and AD. **B** PPI network analyses revealed 26 key targets. **C** Bubble graph of GO enrichment analysis. **D** Bar chart of KEGG enrichment analysis

### Omav Alleviates Inflammatory Response and Induces Autophagy in APP/PS1 Mice

We assessed whether Omav administration regulated the pro-inflammatory cytokine levels in APP/PS1 mice. The levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were markedly increased in APP/PS1; whereas the administration of Omav significantly reduced the elevation (Fig. 6A). Activated glia was reported to upregulate the expression of pro-inflammatory cytokines [24]. Thus, immunofluorescence staining was performed to assess microglial reactivity using an anti-Iba1 (a microglial cell marker) antibody. Results showed a marked increase in the immunoreactivity of Iba1 in APP/PS1 mice compared with WT littermates, whereas the staining was less intense in Omav-treated mice (Fig. 6B). Then, we examined the effects of Omav on mTOR activation, since mTOR was the key gene in the PPI network and was also enriched in the GO analysis

of the inflammatory response. Western blot analysis suggested that mTOR phosphorylation was significantly increased in the APP/PS1 group and was downregulated by Omav administration (Fig. 6C), indicating the inhibitory effect of Omav on the mTOR signaling pathway. mTOR is a pivotal negative regulator of autophagy, and previous study reported that impaired autophagy could result in inflammation [25, 26]. Therefore, we examined the expressions of autophagy-related proteins, including LC3 (a hallmark of autophagy activation) and P62 (a classic autophagy substrate). We observed a significant decrease in the LC3-II/LC3-I ratio and an increase in P62 expression in APP/PS1 mice, compared with their WT littermates. Notably, Omav treatment enhanced the ratio of LC3-II/LC3-I and decreased P62 expression, indicating an increase in autophagy flux (Fig. 6C). These results showed that Omav could alleviate neuroinflammation and induce autophagy through the inhibition of mTOR phosphorylation.



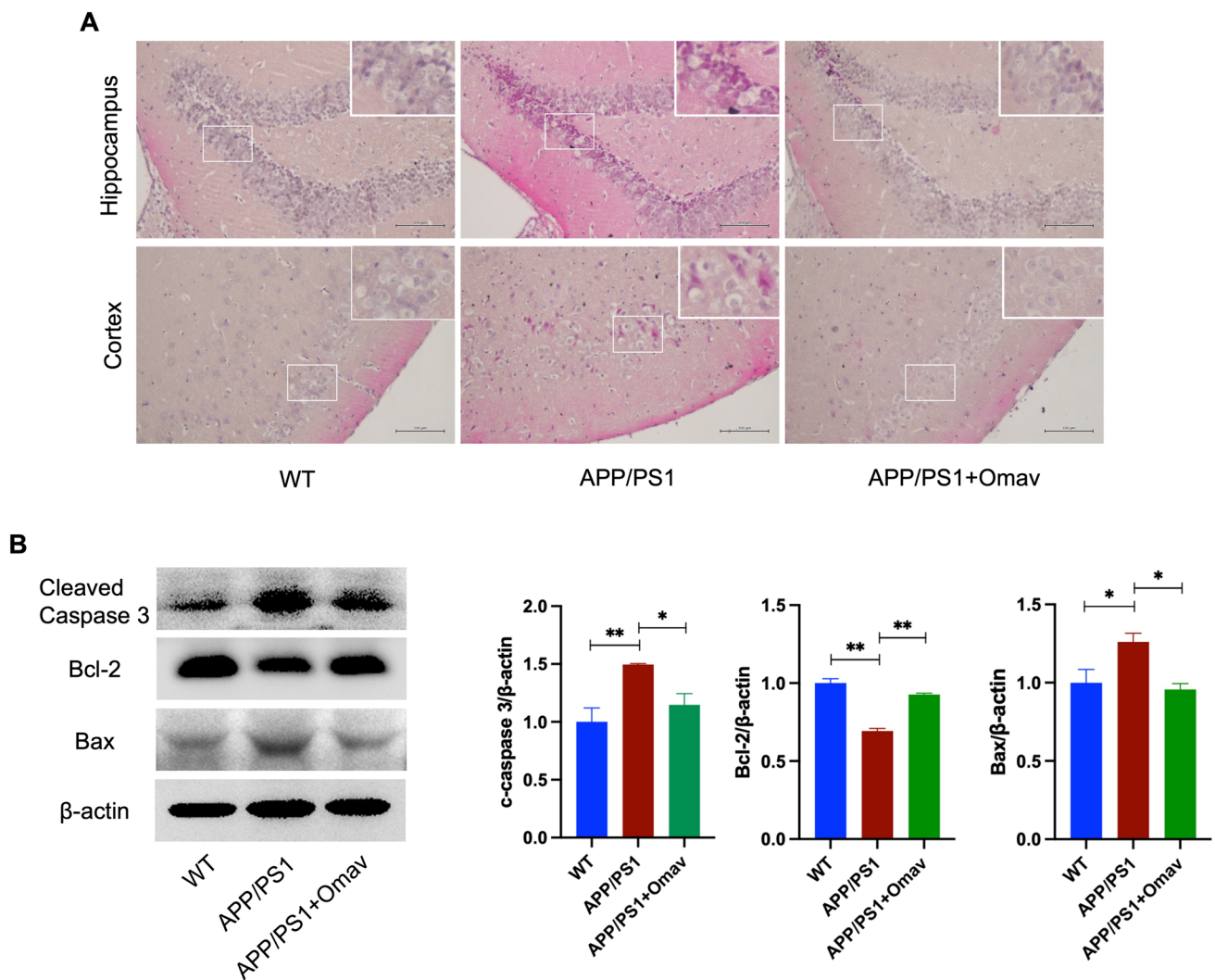
**Fig. 4** Docking results of Omav with CASP3 (**A**) and MTOR (**B**)

## Discussion

AD is a progressive neurological disease with an increasing prevalence, and it affects the most sensitive areas of the brain [27]. However, the development of AD drugs is challenging, with only two innovative drugs approved by the FDA since 2003 [28]. Safe and effective therapeutic strategies are urgently needed to treat AD. In the present study, Omav significantly alleviated cognitive dysfunction and reduced A $\beta$  deposition in APP/PS1 mice. Additionally, based on the results of network pharmacology, Omav ameliorated

neuronal apoptosis and inflammatory response by reducing the activation of caspase 3 and mTOR, respectively. This study presents compelling evidence supporting the role of Omav as a future treatment option for patients with AD.

Omav, also termed RTA-408 or CDDO-DFPA, is a novel semi-synthetic oleanane triterpenoid [29]. Partly because of its brain-penetrating properties, increasing evidence has indicated its pharmacological role in treating neurodegenerative diseases [10]. For example, Omav treatment significantly improved neurological function in patients with Friedreich ataxia and, thus, became the



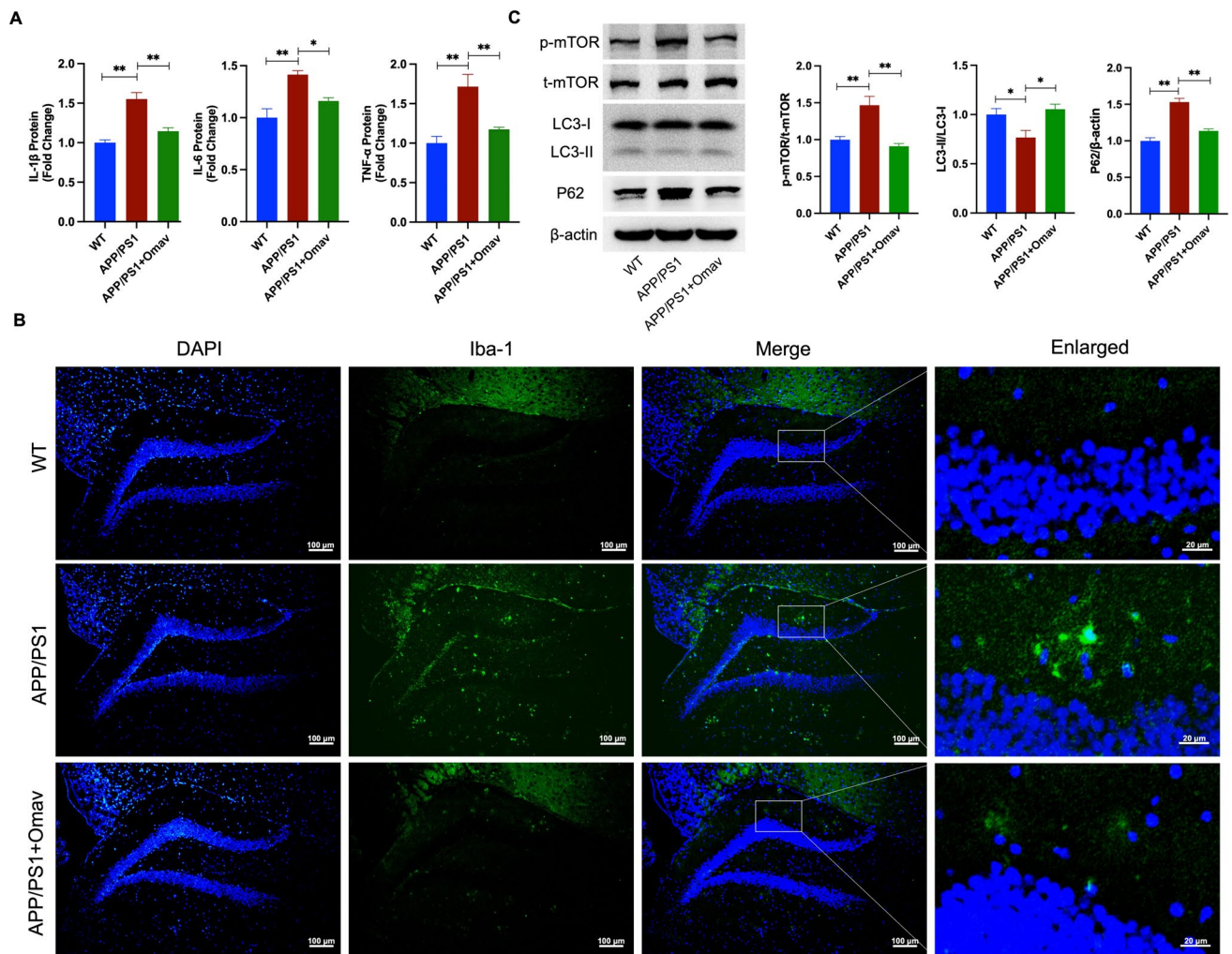
**Fig. 5** OmaV ameliorated neuronal apoptosis and damage in APP/PS1 mice. **A** Representative images of H&E staining; scale bar: 100  $\mu$ m. **B** Representative western blots for cleaved caspase 3, Bcl-2, and Bax.

The relative density was normalized to  $\beta$ -actin. \* $P$  < 0.05; \*\* $P$  < 0.01. Data presented as mean  $\pm$  SEM

first-ever drug approved by the FDA for its treatment [11, 30]. Additionally, Yang et al. reported that OmaV alleviated neuronal degeneration in mice with amyotrophic lateral sclerosis harboring the hSOD1<sup>G93A</sup> mutation [9]. In addition to inducing neuroprotection in neurodegenerative disorders, OmaV exerts protective effects against other neurological diseases, including epilepsy and secondary brain injury after intracerebral or subarachnoid hemorrhage [8, 29, 31]. Despite reports on the neuroprotective role, its effects on AD and related critical mechanisms require clarification. The current study demonstrates that OmaV significantly enhanced cognitive performance of APP/PS1 mice and also reduced A $\beta$  deposition in both the hippocampus and cortex. These findings highlight the unique potential of triterpenoid derivatives, such as OmaV, for the treatment of AD.

Network pharmacology was performed to identify the possible targets and signaling pathways regulated by OmaV. The results showed that caspase 3 displayed the highest degree value, indicating its pivotal role in the treatment of AD with OmaV. Caspase 3 is one of the key proteases that initiates the caspase cascade, and the stimulation of caspase 3 can result in apoptosis, which is considered to underlie the pathological manifestations associated with AD [23, 32]. To date, OmaV has been proved to decrease the expression of cleaved caspase 3 in other pathological conditions including subarachnoid hemorrhage, diabetic cardiomyopathy, and H<sub>2</sub>O<sub>2</sub>-induced cell injury [31, 33, 34]. Consistent with previous studies, we observed an elevation in cleaved caspase 3 levels in the brains of APP/PS1 mice [22, 35], which was attenuated by OmaV treatment. Furthermore, to further investigate the influence of OmaV on apoptosis, we evaluated





**Fig. 6** Omap alleviated neuroinflammation and induced autophagy in APP/PS1 mice. **A** ELISA analysis of pro-inflammatory cytokines including IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ; \* $P$ <0.05; \*\* $P$ <0.01. **B** Repre-

sentative immunostaining of Iba-1 in the different groups. **C** Representative western blots for p-mTOR, LC3, and P62. \* $P$ <0.05; \*\* $P$ <0.01. Data presented as mean  $\pm$  SEM

additional apoptosis-related proteins. Our findings revealed that Omap treatment led to an upregulation of Bcl-2 expression and a downregulation of Bax expression, suggesting that Omap effectively mitigated apoptosis in APP/PS1 mice.

Inflammatory response is another enriched BP item in GO pathway enrichment analysis and has been implicated to play a substantial role in AD [36, 37]. Microglia, the immune-derived cells within the brain, are activated in AD and release several pro-inflammatory cytokines, such as IL-1 $\beta$  and TNF- $\alpha$ , triggering neuroinflammation [38]. In the present study, we investigated the effect of Omap on the expression of pro-inflammatory cytokines and microglial activation. The results demonstrated that Omap treatment attenuated the expression of pro-inflammatory cytokines and suppressed microglial activation.

mTOR, a top-ranked gene in the PPI network that was enriched in inflammatory response, emerged as another key

target in the treatment of Omap against AD. mTOR is a serine/threonine kinase, which belongs to the phosphoinositide kinase-related family [39]. As a master regulator of numerous cellular behaviors, mTOR plays a significant role in aging-related processes, such as protein synthesis, ribosome biogenesis, and cell proliferation [40]. Additionally, mTOR was also reported to hold a central position in autophagy; it hinders autophagy by inhibiting the autophagy-initiating complex [41]. Previous studies have reported that Omap and other oleanane triterpenoid derivatives could inhibit the phosphorylation of mTOR and promote autophagy [42, 43].

Interestingly, there is a complex relationship between neuroinflammation and autophagy. In the pathological process of AD, autophagy in microglia is inhibited, accompanied by an increase in mTOR signaling pathway activation [44, 45]. The compromised autophagy could lead to sustained inflammation. On the one hand, defect autophagy

may increase inflammatory phenotype in AD and disrupt the degradation of various mediators involved in inflammatory activation or pro-inflammatory cytokines [40, 46, 47]. On the other hand, dysregulation of autophagy accelerates amyloidosis and tau pathology, which, in turn, exacerbates microglial activation and neuroinflammation [48, 49]. In the present study, compared with APP/PS1 mice, we found a significant reduction in the phosphorylation of mTOR and an increased autophagic level in those treated with Omav. Improving autophagic dysfunction may contribute to the attenuation of neuroinflammation observed with Omav treatment.

Our results hold crucial translational implications, offering direct evidence of the anti-apoptotic and anti-inflammatory properties of Omav in APP/PS1 mice via the inhibition of caspase 3 and mTOR, respectively. Given its ability to target multifactorial mechanisms underlying neurological injuries, Omav emerges as a promising therapeutic candidate for AD.

## Conclusions

The administration of Omav resulted in significant improvements in cognitive dysfunction and reduced A $\beta$  deposition in APP/PS1 mice. Furthermore, it attenuated neuronal apoptosis and suppressed neuroinflammation. Taken together, these results underscore the potential of Omav as an effective treatment for AD.

**Author Contribution** All authors contributed to the research concept and the study design. Zhaojun Liu performed and conducted experiments, analyzed the data, drew figures, and wrote the manuscript. Jianping Jia obtained funding and revised the manuscript critically.

**Funding** This study was supported by the Key Project of the National Natural Science Foundation of China [grant number U20A20354]; Beijing Brain Initiative from Beijing Municipal Science & Technology Commission [grant numbers Z201100005520016, Z201100005520017]; STI2030- Major Projects [grant number 2021ZD0201802, 2021ZD0201803]; the National Key Scientific Instrument and Equipment Development Project [grant number 31627803]; the Key Project of the National Natural Science Foundation of China [grant number 81530036].

**Data Availability** The data supporting the findings of this study are available on request from the corresponding author.

## Declarations

**Ethics Approval** The experimental procedures were approved by the Institutional Animal Care and Use Committee of Capital Medical University.

**Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

**Competing Interests** The authors declare no competing interests.

## References

1. Qi P, Li J, Gao S, Yuan Y, Sun Y, Liu N, Li Y, Wang G et al (2020) Network pharmacology-based and experimental identification of the effects of quercetin on Alzheimer's disease. *Front Aging Neurosci* 12:589588. <https://doi.org/10.3389/fnagi.2020.589588>
2. Li Q, Jia C, Wu H, Liao Y, Yang K, Li S, Zhang J, Wang J et al (2022) Nao Tan Qing ameliorates Alzheimer's disease-like pathology by regulating glycolipid metabolism and neuroinflammation: a network pharmacology analysis and biological validation. *Pharmacol Res* 185:106489. <https://doi.org/10.1016/j.phrs.2022.106489>
3. Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL (2015) Alzheimer's disease. *Nat Rev Dis Primers* 1:15056. <https://doi.org/10.1038/nrdp.2015.56>
4. Kumar A, SinghEkavali A (2015) A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol Rep* 67(2):195–203. <https://doi.org/10.1016/j.pharep.2014.09.004>
5. Wang YY, Yang YX, Zhe H, He ZX, Zhou SF (2014) Bardoxolone methyl (CDDO-Me) as a therapeutic agent: an update on its pharmacokinetic and pharmacodynamic properties. *Drug Des Devel Ther* 8:2075–2088. <https://doi.org/10.2147/DDDT.S68872>
6. Wei HJ, Pareek TK, Liu Q, Letterio JJ (2017) A unique tolerizing dendritic cell phenotype induced by the synthetic triterpenoid CDDO-DFPA (RTA-408) is protective against EAE. *Sci Rep* 7(1):9886. <https://doi.org/10.1038/s41598-017-06907-4>
7. Probst BL, Trevino I, McCauley L, Bumeister R, Dulubova I, Wigley WC, Ferguson DA (2015) RTA 408, A novel synthetic triterpenoid with broad anticancer and anti-inflammatory activity. *PLoS ONE* 10(4):e0122942. <https://doi.org/10.1371/journal.pone.0122942>
8. Shekh-Ahmad T, Eckel R, Dayalan Naidu S, Higgins M, Yamamoto M, Dinkova-Kostova AT, Kovac S, Abramov AY et al (2018) KEAP1 inhibition is neuroprotective and suppresses the development of epilepsy. *Brain* 141(5):1390–1403. <https://doi.org/10.1093/brain/awy071>
9. Yang B, Pan J, Zhang XN, Wang H, He L, Rong X, Li X, Peng Y (2023) NRF2 activation suppresses motor neuron ferroptosis induced by the SOD1(G93A) mutation and exerts neuroprotection in amyotrophic lateral sclerosis. *Neurobiol Dis* 184:106210. <https://doi.org/10.1016/j.nbd.2023.106210>
10. Reisman SA, Gahir SS, Lee CI, Proksch JW, Sakamoto M, Ward KW (2019) Pharmacokinetics and pharmacodynamics of the novel Nrf2 activator omaveloxolone in primates. *Drug Des Devel Ther* 13:1259–1270. <https://doi.org/10.2147/DDDT.S193889>
11. Subramony SH, Lynch DL (2023) A milestone in the treatment of ataxias: approval of omaveloxolone for Friedreich ataxia. *Cerebellum*. <https://doi.org/10.1007/s12311-023-01568-8>
12. Wu Q, Li X, Jiang XW, Yao D, Zhou LJ, Xu ZH, Wang N, Zhao QC et al (2022) Yuan-Zhi decoction in the treatment of Alzheimer's disease: an integrated approach based on chemical profiling, network pharmacology, molecular docking and experimental evaluation. *Front Pharmacol* 13:893244. <https://doi.org/10.3389/fphar.2022.893244>
13. Chen G, Seukep AJ, Guo M (2020) Recent advances in molecular docking for the research and discovery of potential marine drugs. *Mar Drugs* 18 (11). <https://doi.org/10.3390/md18110545>
14. Cohen-Nowak AJ, Cohen AJ, Correia ED, Portocarrero CP, South AP, Nikbakht N (2022) Omaveloxolone attenuates squamous cell carcinoma growth and disease severity in an Epidermolysis

- Bullosa mouse model. *Exp Dermatol* 31(7):1083–1088. <https://doi.org/10.1111/exd.14564>
15. Janhavi P, Divyashree S, Sanjailal KP, Muthukumar SP (2022) DoseCal: a virtual calculator for dosage conversion between human and different animal species. *Arch Physiol Biochem* 128(2):426–430. <https://doi.org/10.1080/13813455.2019.1687523>
  16. Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* 1(2):848–858. <https://doi.org/10.1038/nprot.2006.116>
  17. Nickel J, Gohlke BO, Erehman J, Banerjee P, Rong WW, Goede A, Dunkel M, Preissner R (2014) SuperPred: update on drug classification and target prediction. *Nucleic Acids Res* 42 (Web Server issue):W26–31. <https://doi.org/10.1093/nar/gku477>
  18. Wang X, Shen Y, Wang S, Li S, Zhang W, Liu X, Lai L, Pei J et al (2017) PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res* 45(W1):W356–W360. <https://doi.org/10.1093/nar/gkx374>
  19. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT et al (2019) STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 47(D1):D607–D613
  20. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T, Chang W (2022) DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res* 50(W1):W216–W221. <https://doi.org/10.1093/nar/gkac194>
  21. Liu J, Liu J, Tong X, Peng W, Wei S, Sun T, Wang Y, Zhang B et al (2021) Network pharmacology prediction and molecular docking-based strategy to discover the potential pharmacological mechanism of Huai Hua San against ulcerative colitis. *Drug Des Devel Ther* 15:3255–3276. <https://doi.org/10.2147/DDDT.S319786>
  22. Bai D, Jin G, Yin S, Zou D, Zhu Q, Yang Z, Liu X, Ren L et al (2017) Antioxidative and anti-apoptotic roles of silibinin in reversing learning and memory deficits in APP/PS1 mice. *Neurochem Res* 42(12):3439–3445. <https://doi.org/10.1007/s11064-017-2389-3>
  23. He Y, Ruganzu JB, Lin C, Ding B, Zheng Q, Wu X, Ma R, Liu Q et al (2020) Tanshinone IIA ameliorates cognitive deficits by inhibiting endoplasmic reticulum stress-induced apoptosis in APP/PS1 transgenic mice. *Neurochem Int* 133:104610. <https://doi.org/10.1016/j.neuint.2019.104610>
  24. Gong Z, Huang J, Xu B, Ou Z, Zhang L, Lin X, Ye X, Kong X et al (2019) Urolithin A attenuates memory impairment and neuroinflammation in APP/PS1 mice. *J Neuroinflammation* 16(1):62. <https://doi.org/10.1186/s12974-019-1450-3>
  25. Aman Y, Schmauck-Medina T, Hansen M, Morimoto RI, Simon AK, Bjedov I, Palikaras K, Simonsen A et al (2021) Autophagy in healthy aging and disease. *Nat Aging* 1(8):634–650. <https://doi.org/10.1038/s43587-021-00098-4>
  26. Eshraghi M, Adlimoghaddam A, Mahmoodzadeh A, Sharifzad F, Yasavoli-Sharahi H, Lorzadeh S, Albensi BC, Ghavami S (2021) Alzheimer's disease pathogenesis: role of autophagy and mitophagy focusing in microglia. *Int J Mol Sci* 22 (7). <https://doi.org/10.3390/ijms22073330>
  27. Neha PS (2023) Emerging therapeutics agents and recent advances in drug repurposing for Alzheimer's disease. *Ageing Res Rev* 85:101815. <https://doi.org/10.1016/j.arr.2022.101815>
  28. Cummings J, Zhou Y, Lee G, Zhong K, Fonseca J, Cheng F (2023) Alzheimer's disease drug development pipeline: 2023. *Alzheimers Dement (N Y)* 9(2):e12385. <https://doi.org/10.1002/trc2.12385>
  29. Hu L, Cao Y, Chen H, Xu L, Yang Q, Zhou H, Li J, Yu Q et al (2022) The novel Nrf2 activator omaveloxolone regulates microglia phenotype and ameliorates secondary brain injury after intracerebral hemorrhage in mice. *Oxid Med Cell Longev* 2022:4564471. <https://doi.org/10.1155/2022/4564471>
  30. Lynch DR, Chin MP, Delatycki MB, Subramony SH, Corti M, Hoyle JC, Boesch S, Nachbauer W et al (2021) Safety and efficacy of omaveloxolone in Friedreich ataxia (MOXIe study). *Ann Neurol* 89(2):212–225. <https://doi.org/10.1002/ana.25934>
  31. Tsai TH, Lin SH, Wu CH, Tsai YC, Yang SF, Lin CL (2020) Mechanisms and therapeutic implications of RTA 408, an activator of Nrf2, in subarachnoid hemorrhage-induced delayed cerebral vasospasm and secondary brain injury. *PLoS ONE* 15(10):e0240122. <https://doi.org/10.1371/journal.pone.0240122>
  32. Kumari S, Dhapola R, Reddy DH (2023) Apoptosis in Alzheimer's disease: insight into the signaling pathways and therapeutic avenues. *Apoptosis* 28(7–8):943–957. <https://doi.org/10.1007/s10495-023-01848-y>
  33. Liu X, Ward K, Xavier C, Jann J, Clark AF, Pang IH, Wu H (2016) The novel triterpenoid RTA 408 protects human retinal pigment epithelial cells against H<sub>2</sub>O<sub>2</sub>-induced cell injury via NF-E2-related factor 2 (Nrf2) activation. *Redox Biol* 8:98–109. <https://doi.org/10.1016/j.redox.2015.12.005>
  34. Hao J, Zhou J, Hu S, Zhang P, Wu H, Yang J, Zhao B, Liu H et al (2024) RTA 408 ameliorates diabetic cardiomyopathy by activating Nrf2 to regulate mitochondrial fission and fusion and inhibiting NF- $\kappa$ B-mediated inflammation. *Am J Physiol Cell Physiol* 326(2):C331–C347. <https://doi.org/10.1152/ajpcell.00467.2023>
  35. Wu F, Huang M, Zuo X, Xie R, Liu J, Ke J, Li W, Wang Q et al (2023) Osthole/borneol thermosensitive gel via intranasal administration enhances intracerebral bioavailability to improve cognitive impairment in APP/PS1 transgenic mice. *Front Pharmacol* 14:1224856. <https://doi.org/10.3389/fphar.2023.1224856>
  36. Wiatrak B, Jawien P, Szelag A, Jeskowiak-Kossakowska I (2023) Does inflammation play a major role in the pathogenesis of Alzheimer's disease? *Neuromolecular Med* 25(3):330–335. <https://doi.org/10.1007/s12017-023-08741-6>
  37. Bachiller S, Jimenez-Ferrer I, Paulus A, Yang Y, Swanberg M, Deierborg T et al (2018) Microglia in neurological diseases: a road map to brain-disease dependent-inflammatory response. *Front Cell Neurosci* 12:488. <https://doi.org/10.3389/fncel.2018.00488>
  38. Novoa C, Salazar P, Cisternas P, Gherardelli C, Vera-Salazar R, Zolezzi JM, Inestrosa NC (2022) Inflammation context in Alzheimer's disease, a relationship intricate to define. *Biol Res* 55(1):39. <https://doi.org/10.1186/s40659-022-00404-3>
  39. Xu T, Sun D, Chen Y, Ouyang L (2020) Targeting mTOR for fighting diseases: a revisited review of mTOR inhibitors. *Eur J Med Chem* 199:112391. <https://doi.org/10.1016/j.ejmech.2020.112391>
  40. Rapaka D, Bitra VR, Challa SR, Adiuoku PC (2022) mTOR signaling as a molecular target for the alleviation of Alzheimer's disease pathogenesis. *Neurochem Int* 155:105311. <https://doi.org/10.1016/j.neuint.2022.105311>
  41. Kim YC, Guan KL (2015) mTOR: a pharmacologic target for autophagy regulation. *J Clin Invest* 125(1):25–32. <https://doi.org/10.1172/JCI73939>
  42. Wang YY, Yang YX, Zhao R, Pan ST, Zhe H, He ZX, Duan W, Zhang X et al (2015) Bardoxolone methyl induces apoptosis and autophagy and inhibits epithelial-to-mesenchymal transition and stemness in esophageal squamous cancer cells. *Drug Des Devel Ther* 9:993–1026. <https://doi.org/10.2147/DDDT.S73493>
  43. Yue W, Yupeng G, Jun C, Kui J (2023) Apatinib combined with olaparib induces ferroptosis via a p53-dependent manner in ovarian cancer. *J Cancer Res Clin Oncol* 149(11):8681–8689. <https://doi.org/10.1007/s00432-023-04811-1>
  44. Yang CZ, Wang SH, Zhang RH, Lin JH, Tian YH, Yang YQ, Liu J, Ma YX (2023) Neuroprotective effect of astragalosin via

- activating PI3K/Akt-mTOR-mediated autophagy on APP/PS1 mice. *Cell Death Discov* 9(1):15. <https://doi.org/10.1038/s41420-023-01324-1>
45. Pomilio C, Gorojod RM, Riudavets M, Vinuesa A, Presa J, Gregosa A, Bentivegna M, Alaimo A et al (2020) Microglial autophagy is impaired by prolonged exposure to beta-amyloid peptides: evidence from experimental models and Alzheimer's disease patients. *Geroscience* 42(2):613–632. <https://doi.org/10.1007/s11357-020-00161-9>
46. Cheng X, Wei Y, Qian Z, Han L (2023) Autophagy balances neuroinflammation in Alzheimer's disease. *Cell Mol Neurobiol* 43(4):1537–1549. <https://doi.org/10.1007/s10571-022-01269-6>
47. Plaza-Zabala A, Sierra-Torre V, Sierra A (2017) Autophagy and microglia: novel partners in neurodegeneration and aging. *Int J Mol Sci* 18 (3). <https://doi.org/10.3390/ijms18030598>
48. Wu AG, Zhou XG, Qiao G, Yu L, Tang Y, Yan L, Qiu WQ, Pan R et al (2021) Targeting microglial autophagic degradation in NLRP3 inflammasome-mediated neurodegenerative diseases. *Ageing Res Rev* 65:101202. <https://doi.org/10.1016/j.arr.2020.101202>
49. Zhang Z, Yang X, Song YQ, Tu J (2021) Autophagy in Alzheimer's disease pathogenesis: therapeutic potential and future perspectives. *Ageing Res Rev* 72:101464. <https://doi.org/10.1016/j.arr.2021.101464>

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