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*Original Article*

# Transforming growth factor β1 restores hippocampal synaptic plasticity and memory loss in an Alzheimer's disease model through the PI3K/Akt signaling pathway

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**Abstract:** Alzheimer's disease (AD) is a type of neurodegenerative disorder that exhibits gradual memory deprivation. The hallmarks of AD include the presence of neurofibrillary tangles, amyloid beta-plaques, and neuronal loss. Amyloid beta oligomers (AβOs) have been reported to trigger synaptotoxicity, eventually resulting in reduced dendritic spine density (DSD), thus confirming the speculation that synapsis impairment is a causative factor of AD development. Transforming growth factor (TGF) β1, an immunosuppressive cytokine, has been shown to play a neuroprotective in an AD model. Studies have described that TGFβ1 was positively relevant to the dendritic spine number in mice. Furthermore, other studies have indicated the role of the PI3K/Akt pathway is involved in synaptic plasticity (SP) and cognitive damage in mice with seizures. Several studies have also reported that TGFβ1 could regulate the PI3K signaling pathway. However, the underlying mechanisms involving TGFβ1 and the PI3K/Akt signaling pathway in altering memory and SP in AD models are not clear. In the present study, TGFβ1 was injected into the intracerebroventricle (ICV) and Aβ1-42 was injected into both sides of the hippocampus to assess the neuroprotective component of TGFβ1. TGFβ1 administration after the Aβ1-42 injection restored the memory loss, as investigated by the Morris water maze test, and the deterioration of SP, as detected by Golgi staining. Then, western blot was performed to evaluate the expression levels of p-Akt, Akt, and p-Ser-9-GSK3β. Results showed that TGFβ1 administration restored the memory loss and assisted in the reduction of DSD caused due to the Aβ1-42 injection in the hippocampus. Additionally, the ratio of p-Akt/Akt p-Ser-9-GSK3β was enhanced by the Aβ1-42 injection, which was later decreased by TGFβ1 treatment. Therefore, TGFβ1 prevented the decrease in DSD in the hippocampus and the memory deprivation through the PI3K/Akt signaling pathway.

**Keywords:** Transforming growth factor (TGF) β1, synaptic plasticity, memory loss, PI3K/Akt signaling pathway

## Introduction

Alzheimer's disease (AD), a neurodegenerative disorder, and is the most common cause of dementia in elderly people. The hallmarks of AD include the presence of neurofibrillary tangles, amyloid beta-plaques, and neuronal loss. Pathologically, AD is related to synapse/neuronal dysfunction, resulting in wide neurodegeneration in the hippocampus [1]. Therefore, there is speculation that synaptic impairment is associated with AD development. For example, high Aβ levels were confirmed to cause synaptic [2-4] and dendritic spine degeneration [3, 5].

Transforming growth factor (TGF) β1, a type of pleiotropic cytokine, can modulate the biological processes in different types of cells [6] and is part of the cascade involved in the brain's reactions to damage and inflammation [7]. TGFβ1 exhibits high expression levels in the hippocampus, substantia nigra, and brainstem [8]. It prevents neurons from excito-toxic-triggered impairments, ischemia, and trophic factor deprivation [9, 10]. Studies have found that TGFβ1 plays a key role in AD occurrence [11]. For instance, TGFβ1 levels in the plasma of patients with AD were decreased [11, 12], and the release of TGFβ1 into the blood circulation of these patients was similarly reduced [11]. More importantly, it has been found that TGFβ1 overexpression dramatically reduced plaque occurrence and Aβ accumulation in hAPP mice [7]. In other words, it has been found that TGFβ1 was positively relevant to the den-

dritic spine number in mice [13]. In addition, earlier research has shown that TGFβ1 increased the cell actin concentration and facilitated the formation of actin stress fibers [14], inducing long-term synaptic facilitation [15] and transmission [16]. However, the mechanism underlying the synaptic plasticity (SP) of TGFβ1 in AD remains unknown.

The phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway has the potential to modulate diverse intracellular functions, such as nutrient absorption, cell proliferation, growth, autophagy, apoptosis, and migration [17, 18]. Furthermore, a few studies have found that the PI3K/Akt pathway is involved in cognitive impairment and SP [19-21]. In another study, magnesium sulfate treatment (MST) reportedly stimulated Akt activity and shielded the cognitive functions and SP in a streptozotocin-triggered sporadic AD model [22]. Other studies have demonstrated that adiponectin supplements augmented the dendritic branch number and mushroom proportion and mitigated tau hyperphosphorylation at different AD-relevant sites by increasing the Akt and PI3K activity [21]. Some other studies have reported that TGF-family members could regulate PI3K/Akt [23, 24]. TGFβ1 induces the activity of PDK-1, a downstream target of PI3K starting at 60 s, and then phosphorylates and concentrates Akt, a kinase downstream of PDK-1, in the membrane fraction within 5 min [25]. However, the interplay between the PI3K/Akt pathway and TGFβ1 in altering SP and memory deprivation induced by AD has not yet been explored. Hence, we conducted this study to investigate the changes in memory and SP in an AD model and the mechanisms underlying the PI3K/Akt pathway and TGFβ1.

## Materials and methods

### *Animals*

Male SD rats (aged 4 months) were used as experimental animals. All animals were housed in the laboratory under a normal 12/12-h light-dark cycle, at 25°C ± 1°C, under a relative humidity (50% ± 5%) with food and water freely available. All procedures were approved by the Affiliated Shandong Provincial Hospital of Shandong University of Animal Care and Use Committee.

### *Morris water maze test*

The Morris water maze (MWM) test consisted of a circular pool with a diameter of 150 cm and a height of 60 cm filled with water (20°C ± 2°C) to 40-cm deep. The MWM test was performed as described by Pritchett D (2016) [26] and Yu et al. (2018) [27]. The behavior task consisted of platform location training and probe trial. In the first part, the rats were required (120 s) to find the location of a hidden platform (10 cm in diameter) under the surface of water (approximately 2.5 cm), in which 4 trials per day were performed from different release positions that varied systematically for 5 consecutive days. During the hidden platform training session, the rat that found the platform was allowed 0.5 min to stay on it. However, it was placed on the platform for 0.5 min, when it failed this task within 1 min. In the second part, the platform was removed, and then the rat was given 0.5 min to swim to seek it. The escape latency (EL, the first time that the rat crossed the location of the removed platform), the crossing number of the area of the removed platform, and the time in the target quadrant in the probe trial sessions were recorded and investigated.

### *Golgi staining*

All rats were decapitated, and the brains were removed. One hemisphere was stained with Golgi, and the other was used for western blot (WB).

The hemispheres were stained with Golgi-Cox using the Rapid Golgi Stain Kit (PK401A; FD Neuro-Technologies, USA). In brief, the tissue blocks were preserved in a mixed solution of A and B (1:1) at RT for a fortnight away from light before being added to solution C at 4°C for 2 d. Subsequently, they were cut into 120-μm coronal sections on a vibratome. The sections were washed with distilled water, cultured in a mixed solution of D, E, and distilled water (1:1:2) for 10 min, washed again with distilled water, dehydrated in graded ethanol solutions, eliminated in xylene, and eventually coverslipped with a resinous mounting medium and mounted onto gelatin-covered glass slides. The sections were dried at RT. The stained sections were observed using the Digital Pathology System (Hamamatsu Photonics, Japan) under 40× magnification. Only

neurons that were completely impregnated and not clouded by adjacent cells and without significantly truncated dendrites were investigated. Three dendrites of three rats were analyzed. To analyze dendritic branching and length, an appropriate concentric circle (34) plastic template partitioned through 10 m equivalent was placed on the cell-centered drawing to acquire data from Sholl analysis that involved counting the number of concentric circle crossings. This template involved the areas 340 m away from cell soma. The overall crossing numbers of dendrite were used for estimating (± 2%) the total dendrite length. We also evaluated the spine density. All notable flanking spines were calculated by an observer blind to grouping (to remove subjective differences as much as possible). The segment length was subsequently determined through a digital tablet and taken as a spine density divisor.

*Western blot*

After obtaining the hippocampus, the tissues were first rinsed with ice-cold saline, subsequently homogenized in RIPA buffer, and then centrifuged for 15 min at 12,000 rpm; all these procedures were performed at 4°C. The supernatant was pooled, and the protein concentration was measured using the BCA assay kit (Beyotime Institute of Biotechnology, Shanghai, China). The protein (40 μg) was placed into lanes and isolated by SDS-PAGE. The target protein phosphorylated-AKT (p-AKT) and (Ser-9)-phosphorylation of GSK-3β (p-Ser-9-GSK3β) were then electrotransferred onto NC membranes. The protein blots were blocked overnight at 4°C and then incubated with p-Akt, Akt, and p-Ser-9-GSK3β antibodies (Santa Cruz, Dallas, USA). All samples were similarly incubated with β-actin antibody (loading reference) (Sigma-Aldrich Corp). Finally, the blots were rinsed with PBST and detected through the ECL and WB system (Amersham Life Science, UK).

*Statistical analysis*

Data were expressed as average ± S.D. All statistical analyses were performed in GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Data of two groups were analyzed using a *t*-test. Then, the other cases were analyzed using ANOVA with Dunnett's and Newman-Keuls multiple comparison tests to evaluate differences. $P < 0.05$ was considered as statistical significance.

The experiment was divided into four groups, respectively, including saline controls; Aβ1-42 alone; TGFβ1 (40 ng/μL) injected into the bilateral hippocampus at day 5 after the Aβ1-42 injection into the rat hippocampus; and the PI3K inhibitor LY294002 (25 μM) was injected into the hippocampus of rats with TGFβ1 to clarify the relationship between TGFβ1 and the PI3K/Akt signaling pathway.

## Results

*TGFβ1 restored the memory loss caused by Aβ1-42 injection via activation of the PI3K/Akt signaling pathway*

In the MWM test, Aβ1-42 injection alone triggered a decrease in the platform crossing number (PCN) (**Figure 1A**), the EL (**Figure 1B**), and the time in the target quadrant compared with saline controls (**Figure 1C**). Administration of TGFβ1 after the Aβ1-42 injection significantly elevated the PCN (**Figure 1A**), the EL (**Figure 1B**), and the time in the target quadrant (**Figure 1C**) compared to those with Aβ1-42 injection alone. However, when the PI3K inhibitor LY294002 was administered with TGFβ1, the PCN (**Figure 1A**), the EL (**Figure 1B**), and the time in the target quadrant (**Figure 1C**) showed no difference compared with Aβ1-42 injection alone. These results show that the protective effect of TGFβ1 against the memory loss caused by Aβ1-42 injection was inhibited by LY294002, an inhibitor of the PI3K/Akt signaling pathway.

*TGFβ1 restored hippocampal SP caused by Aβ1-42 injection via activation of the PI3K/Akt signaling pathway*

In the Golgi staining results, compared with saline controls, Aβ1-42 injection resulted in a decrease in the dendritic branch number (**Figure 2**) and a reduction in dendritic spine density (DSD) (**Figure 2**). TGFβ1 administration significantly increased the dendritic branch number and the DSD, but LY294002, the PI3K inhibitor, inhibited this neuroprotective role of TGFβ1. These findings indicated that TGFβ1 treatment attenuated the synaptic damage in the rat AD model through the PI3K/Akt signaling pathway.

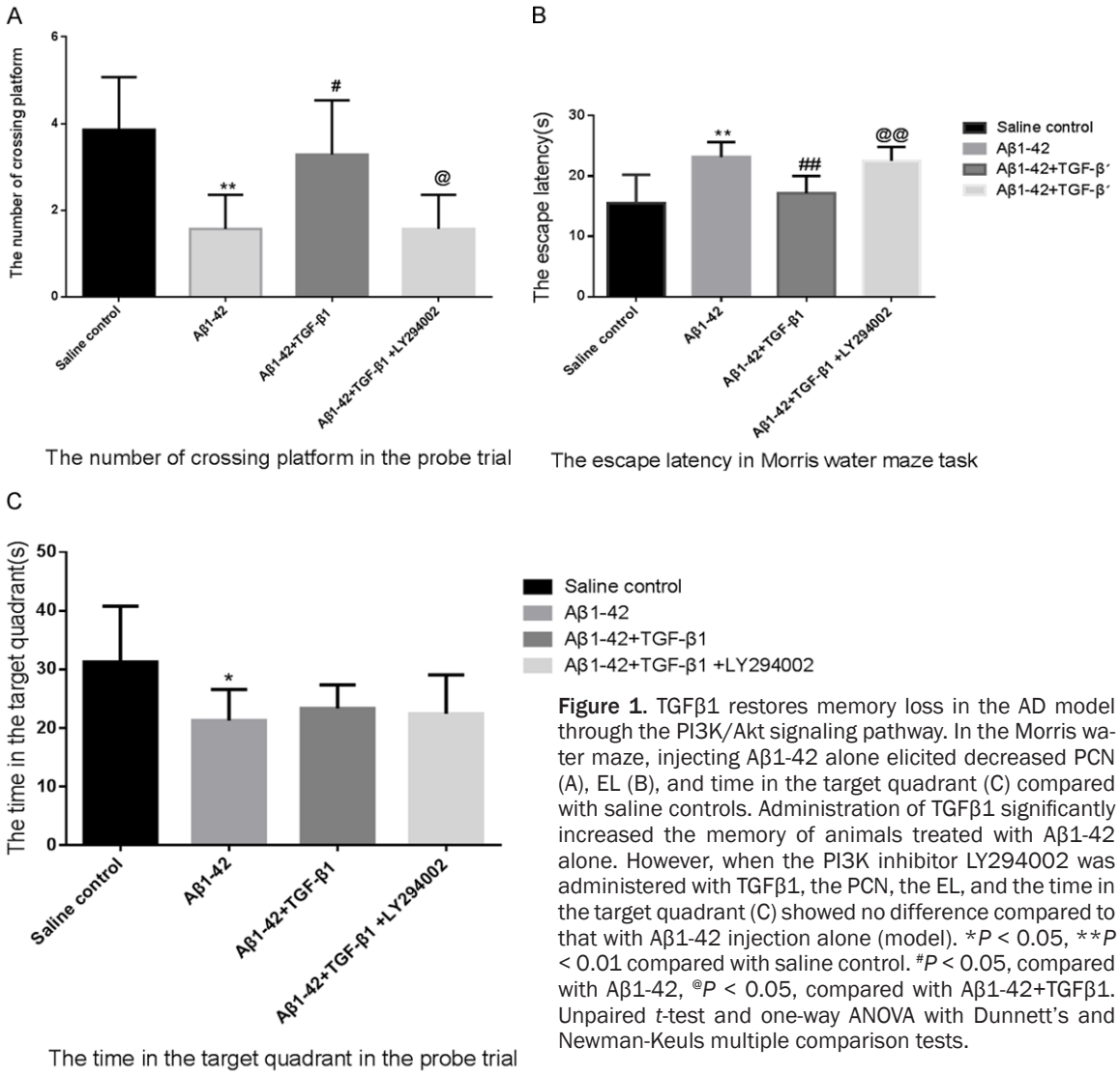


**Figure 1.** TGFβ1 restores memory loss in the AD model through the PI3K/Akt signaling pathway. In the Morris water maze, injecting Aβ1-42 alone elicited decreased PCN (A), EL (B), and time in the target quadrant (C) compared with saline controls. Administration of TGFβ1 significantly increased the memory of animals treated with Aβ1-42 alone. However, when the PI3K inhibitor LY294002 was administered with TGFβ1, the PCN, the EL, and the time in the target quadrant (C) showed no difference compared to that with Aβ1-42 injection alone (model). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with saline control. $^{\#}P < 0.05$, compared with Aβ1-42, $^{@}P < 0.05$, compared with Aβ1-42+TGFβ1. Unpaired *t*-test and one-way ANOVA with Dunnett's and Newman-Keuls multiple comparison tests.

*TGFβ1 restored the hippocampal SP and memory loss caused by Aβ1-42 injection by increasing the activation of the PI3K/Akt signaling pathway*

TGFβ1 stimulated the PI3K/Akt signaling pathway in the hippocampus, as evaluated by WB of p-AKT and AKT. We then analyzed p-Ser-9-GSK3β, a kinase that is phosphorylated and inhibited by p-AKT [10, 28]. The results demonstrated that TGFβ1 dramatically enhanced the expression of both p-AKT/AKT (**Figure 3A**) and p-Ser-9-GSK3β (**Figure 3B**) when it was injected into the hippocampus with Aβ1-42 compared with Aβ1-42 injection alone. These data suggested that TGFβ1 restored the SP and memory loss induced by Aβ1-42 injection through the activation of the PI3K/Akt signaling pathway.

## Discussion

AD exhibits gradual cognitive decline. The pathology of AD involves synapse/neuronal dysfunction. Previous research has shown that amyloid beta oligomers induced a reduction in DSD [29]. In the present study, we found that Aβ1-42 injection into the hippocampus of rats induced synaptic damage, including the reduction in DSD and the decrease in the dendritic branch number, which ultimately induced memory loss.

Several studies have reported TGFβ1 plays a pivotal but unequivocal role in AD pathogenesis. Some studies have reported that TGFβ1 exerted a neuroprotective role in the AD brain. For example, the overexpression of TGFβ1 prominently reduced plaque formation and Aβ

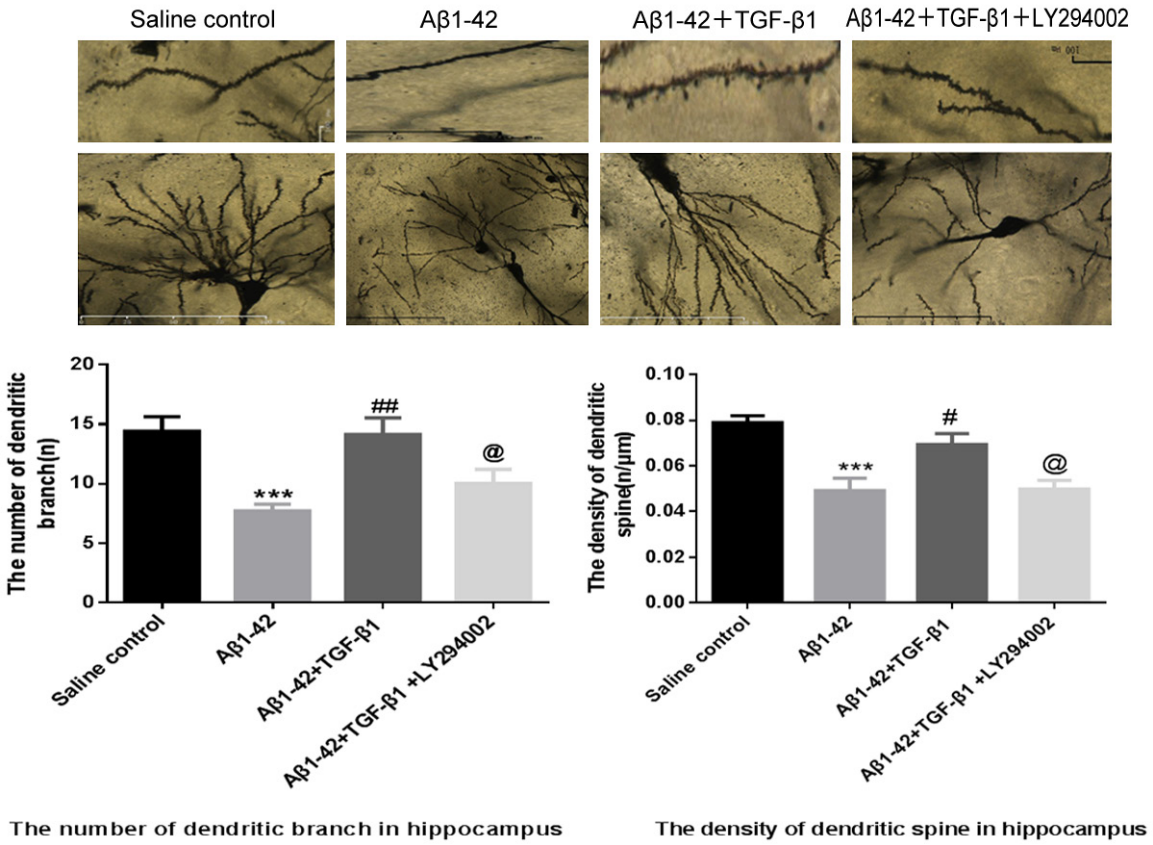


**Figure 2.** TGFβ1 restored the hippocampal synaptic plasticity caused by Aβ1-42 injection via the PI3K/Akt signaling pathway In Golgi staining, compared with saline controls, Aβ1-42 injection decreased the dendritic branch number and reduced the dendritic spine density. TGFβ1 administration significantly increased the dendritic branch number and the dendritic spine density, but LY294002, the PI3K inhibitor, inhibited the neuroprotective role of TGFβ1. $^{***}P < 0.001$ compared with saline control, $^{\#\#}P < 0.01$, compared with Aβ1-42 alone, $^{@}P < 0.05$, compared with Aβ1-42+TGFβ1. Unpaired *t*-test and one-way ANOVA with Dunnett's and Newman-Keuls multiple comparison tests.

accumulation in hAPP mice [8], impairment of TGFβ1 signaling has been demonstrated in the AD brain [30], and a reduction in TGFβ1 signaling increased Aβ deposition and neurodegeneration in transgenic AD mice [31]. In cultivated neurons, estrogen induced the production of TGFβ1 by glial cells [32] or the exogenous application of TGFβ1 was shown to reduce Aβ neurotoxicity [33, 34]. However, some other studies have found that TGFβ1 exerted an adverse role, such as TGFβ1 coexpression in transgenic AD mice accelerated Aβ sedimentation [35], and TGFβ1-overexpressing transgenic mice developed AD-resembling vascular alterations [36]. In addition, vessel-originated TGFβ1 has been confirmed to play a role in inflammatory processes in the AD brain [10, 37]. The present study has confirmed the neuroprotective role of TGFβ1. A previous study showed that TGFβ1 was positively relevant to the dendritic spine number in mice [13]. However, the role of TGFβ1 in the SP of the AD model remains unknown. In the present study, TGFβ1 was administered into ICV and Aβ1-42 was injected into both sides of the hippocampus. The results indicated that TGFβ1 exerted an clear neuroprotective effect after the Aβ1-42 injection by restoring the deterioration of dendritic/synaptic morphology. We then explored the underlying mechanism by which TGFβ1 restored the deterioration of dendritic morphology and memory loss in AD.

The PI3K/Akt pathway, a critical cellular signaling pathway, is directly involved in diverse cellular functions such as metabolism, proliferation, survival, transcription, and protein synthesis [19, 38]. PI3K can be stimulated by diverse factors such as receptor tyrosine kinases and integrins and is inhibited by some onco-

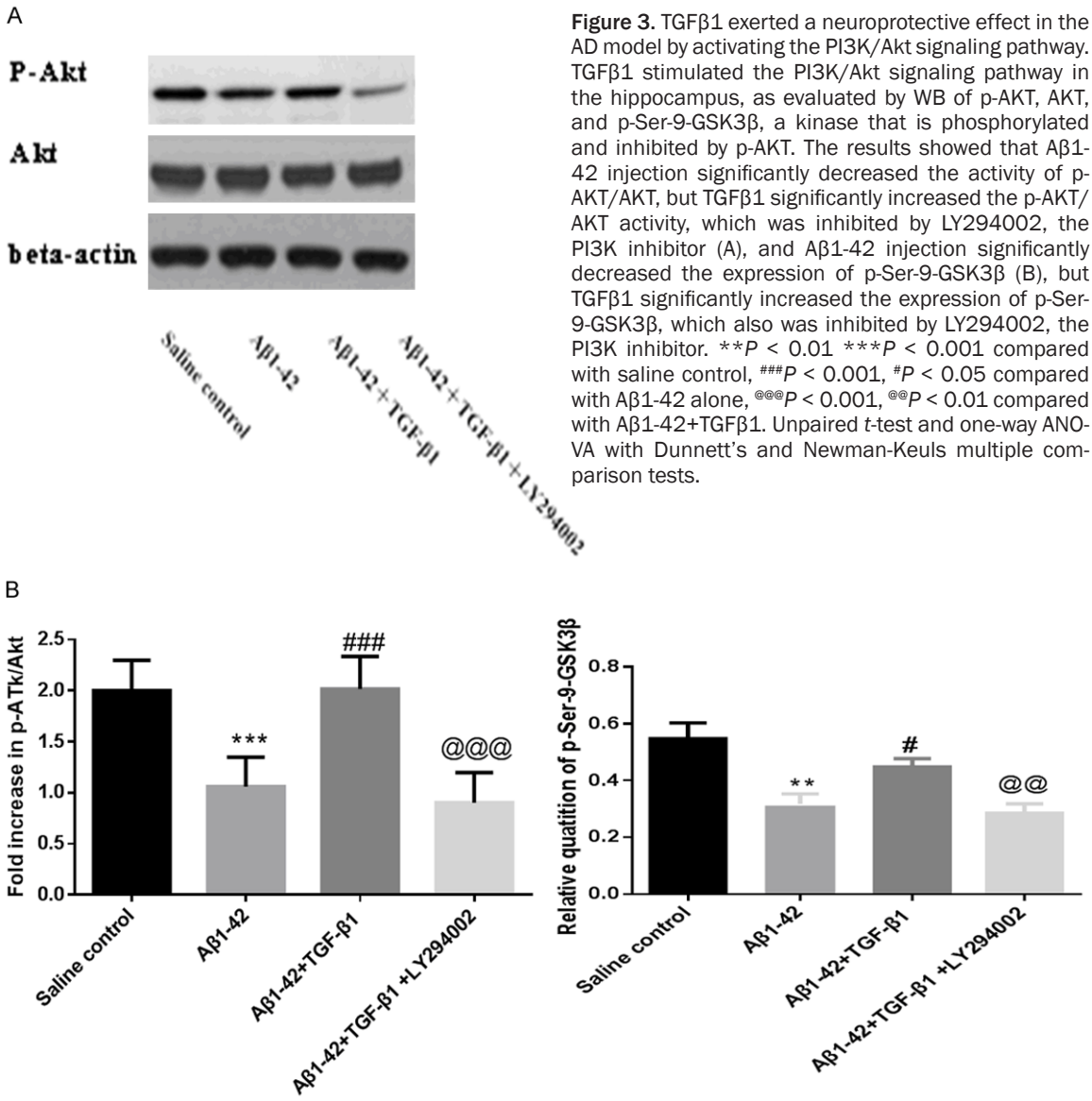


**Figure 3.** TGFβ1 exerted a neuroprotective effect in the AD model by activating the PI3K/Akt signaling pathway. TGFβ1 stimulated the PI3K/Akt signaling pathway in the hippocampus, as evaluated by WB of p-AKT, AKT, and p-Ser-9-GSK3β, a kinase that is phosphorylated and inhibited by p-AKT. The results showed that Aβ1-42 injection significantly decreased the activity of p-AKT/AKT, but TGFβ1 significantly increased the p-AKT/AKT activity, which was inhibited by LY294002, the PI3K inhibitor (A), and Aβ1-42 injection significantly decreased the expression of p-Ser-9-GSK3β (B), but TGFβ1 significantly increased the expression of p-Ser-9-GSK3β, which also was inhibited by LY294002, the PI3K inhibitor. $^{**}P < 0.01$ $^{***}P < 0.001$ compared with saline control, $^{\#\#\#}P < 0.001$, $^{\#}P < 0.05$ compared with Aβ1-42 alone, $^{@@@}P < 0.001$, $^{@@}P < 0.01$ compared with Aβ1-42+TGFβ1. Unpaired *t*-test and one-way ANOVA with Dunnett's and Newman-Keuls multiple comparison tests.

proteins such as tensin homolog and phosphatase [39, 40]. The activation of PI3K promotes Akt phosphorylation and initiation, locates it at the plasma membrane, and then epigenetically and genetically influences its downstream target genes [41-43]. Dysfunction in the PI3K/Akt cascade has been reported to be a radical trigger of neuropsychiatric and developmental diseases with different phenotypes, including autism spectrum disorder, epilepsy, brain impairment, and development of brain malformations [44-47]. Activation of the PI3K/Akt signaling pathway has been shown to contribute to the neuroprotective effects of both estrogens [48] and nicotine [49] against Aβ-induced toxicity. The PI3K/Akt signaling pathway is similarly related to the change in SP. Studies have shown that MST enhanced the PI3K activity at Tyr458/199 and Akt at Ser473 and shielded the cognitive functions and SP in a streptozotocin-triggered sporadic AD model [22]. Other studies have indicated that TGFβ1 played a neuroprotective role in modulating Aβ (25-35) neurotoxicity through PI3K activation [10]. In our study, we also found that TGFβ1 can activate the PI3K/Akt signaling pathway, and to our knowledge, our study is the first to demonstrate that TGFβ1 restored the synaptic damage, including the reduction in DSD and the decrease in the dendritic branch number, and then ultimately restored the memory loss

through the activation of the PI3K/Akt signaling pathway.

## Disclosure of conflict of interest

None.

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