BMP4 overexpression induces upregulation of APP/TAU and memory deficits in Alzheimer's disease

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Abstract

Background: Alzheimer's disease (AD) is a chronic progressive degenerative disease of the nervous system. Its pathogenesis is complex, which is related to abnormal expression of the amyloid β -protein (A β), APP, and TAU protein. Evidence has demonstrated that bone morphogenetic protein 4 (BMP4) is highly expressed in transgenic mouse models of AD, and endogenous level of BMP4 mainly affects hippocampal function.

Methods: To determine whether BMP4 participates in AD development, transgenic mice were constructed with overexpression of BMP4 under control of the neuron-specific enolase (NSE) promoter. We also performed MTT, FACS, Transfection, TUNEL and Western blotting assay to define the role of BMP4 in cells.

Results: We found that middle-aged BMP4 transgenic mice exhibited memory disorder via Morris water maze experiment. Moreover, the hippocampal tissues have high expression of AD related proteins including APP, A β , PSEN-1, TAU, P-TAU (Thr181), and P-TAU (Thr231) proteins. Furthermore, in multiple cell lines, overexpression of BMP4 increased the expression of AD related proteins, whereas downregulation of BMP4 demonstrated opposed effects. Consistently, BMP4 modulation participated in cell apoptosis via regulation of BAX and Bcl-2 expression in cells.

Conclusion: Our findings indicate that BMP4 overexpression might be a potential factor to induce AD.

Background

Misfolding and aggregation of certain proteins are fundamental features of neurodegenerative diseases, like Alzheimer's disease (AD). AD is an age-related, progressive, irreversible neurodegenerative disease that leads to the loss of selected neurons in the basal forebrain, almond nucleus, hippocampus and cortex, as well as the progressive deceleration of cognition and memory [1–4]. It is characterized by an insidious development of hippocampal pathology [5, 6]. The changes in neurogenesis observed during the initial stages and progression of AD demonstrated that modulation of the new productions of neurons at neurogenic sites may exert profound effects on hippocampal function [1]. The pathological trait is touched with the deposition of amyloid β -protein (A β) peptides and abnormal phosphorylation of Tau protein in the cerebral cortex and hippocampal formation [7, 8].

In AD, genetic evidence from mutations of the amyloid precursor protein (APP) and Presenilins strongly suggests role of A β in the pathogenesis of familial [9, 10]. A β mainly has three types: A β 1–40, A β 1–42 and A β 1–43, while A β 42/43 is A β lamellar structure with strong hydrophobicity, easy deposition and neurotoxicity. In normal conditions, 90% of the human have A β 40 and only a small amount of A β 42/43, while in AD patients, the ratio of A β 42/A β 40 in the brain is out of balance and increased, and deposits in the brain forming the core of alzheimer's disease [11]. Tau, a microtubule-related protein, is found in neurons in the brain's frontal temporal hippocampus and olfactory region. It has been shown to play a key role in the pathology of AD and is a potential target for the treatment of AD. Tau is a highly soluble

and phosphorylated unstable substance. The longest tau subtype is about 20% (or 85 amino acid residues) of potential (Ser, Thr, or Tyr) phosphorylation sites. The phosphorylation of Tau protein in normal brain is remained at a low level, while the Tau phosphorylation level in AD patients was 3–4 times higher than that in normal people of the same age [12, 13].

Bone morphogenetic protein 4 (BMP4), a member of the BMP family (BMPs), is part of the transforming growth factor-beta (TGF- β) superfamily. BMPs not only play a physiological role in embryonic hematopoietic and bone tissue, but also regulate the production of neural stem cells in the equine region of the brain [14]. Precise control of the BMP4 signal in extracellular space appears to play a key role in multiple events during development, including neural induction, changes in neurogenesis associated with functional changes in the hippocampus [15]. One study demonstrated that high levels of typical BMP signaling were detected in hippocampal niches. In addition, ligands of the BMP family are involved in the regulation of resting adult hippocampal stem cells and in the control of progenitor cell maturation at multiple stages of the neurogenic lineage [16]. Changes in neurogenesis in the subgranular area of the hippocampal dentate gyrus (DG) have been demonstrated in various animal models of AD and in patients with AD. It usually causes working memory and cognitive impairment in AD. Another study showed that BMP4 has the function of inhibiting the nerve in the small habitat of the hippocampus, with certain behavioral disorders, which is primarily associated with the disease of AD [17].

Although these studies indicated that BMP4 might be involved in AD development and progression, it has no direct evidence to reveal the function of BMP4 in AD development. To determine whether BMP4 is a driver to induce AD, transgenic mice were constructed with BMP4 overexpression under control of the neuron-specific enolase (NSE) promoter. We also determined whether BMP4 modulation in multiple cell lines regulated the expression of AD biomarkers, such as APP, A β , PSEN-1, and Tau, leading to regulation of cell apoptosis. Our study will provide the evidence and mechanisms of how BMP4 participates in AD development.

Methods

Animals

C57BL/6 strain mice were bred in the SPF grade animal barrier system in Bengbu Medical College Experimental Animal Center. Wild-type (WT) mice were purchased from the experimental animal center of Yangzhou University (Yangzhou, Jiangsu, China). The neuron specific enolase promoter (NSE)-BMP4 transgenic mice were derived from Northwestern University (Chicago, IL, USA). All animal experiments were conducted in accordance with the rules and regulations of the Animal Ethics Committee of Bengbu Medical College.

Reagents

DNA kit was purchased from Foregene Company (Chengdu, Sichuan, China). MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) was bought from Sigma-Aldrich (St. Louis, MO, USA).

PCR primer synthesis was purchased from Genscript Company (Nanjing, Jiangsu, China). Agarose gel was purchased from Biowest Company (France). TRIzol[®] Reagent and DNA ladder were acquired from TIANGEN (Tianjin, China). SYBR Premix Dimer Eraser and Reverse transcription kit was purchased from Takara (Japan). 40µm cell filters were obtained from Biologix Company (Shanghai, China). Lipofectamine 2000 reagent was obtained from Invitrogen (Waltham, MA, USA). BSA was purchased from Biofroxx Biotechnology (Germany). BCA kit and SDS-PAGE kit were purchased from Beyotime Biotechnology (Shanghai, China). Anti-BMP4 (ab29973) and anti-P-tau231 (ab151559) antibodies were obtained from Abcam (Cambridge, MA, USA). Anti-APP (#P05067), anti-Tau (#P10636-8), anti-P-Tau181 (#P10636-8) antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-BAX (50599-2-Ig), anti-Bcl-2 (26593-1-AP), anti-PSEN1 (16163-1-AP) and anti-Ab42 (25524-1-AP) antibodies were gained from ProteinTech Company (Wuhan, Hubei, China).

Cell culture

Hippocampal neurons (HT22) cell line, neuroblastoma (N2A) cell line and human neuroblastoma (SH-SY5Y) cells were purchased from ATCC Company (Manassas, VA, USA). Cells were grown in DMEM (Dulbecco's modified eagle medium, Gibco Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. The cells were maintained in 5% CO₂ culture incubator at 37°C.

Breeding and screening of BMP4 transgenic mice

Adult NSE-BMP4 mice and WT mice (8 weeks) were placed in cages with male-female ratio 1:1 or 1:2, and vaginal plug was detected 12 hours later. The pregnant female mice were identified and the marked time was 0.5 days of gestation (E0.5). After 21 days, the mother mice finished pregnancy and gave birth to pups. The time was recorded as 3 weeks old, shearing about 3 mm length of rat tail to extract DNA to perform ordinary PCR amplification and DNA gel electrophoresis for validating the BMP4-positive transgenic mice.

Morris water maze assay

Ten transgenic mice with BMP4 overexpression and ten WT mice were randomly selected for Morris water maze experiment at 20 weeks. The Morris water maze pool was divided into four quadrant areas: I, II, III, and IV. The platform was placed in the middle of the third quadrant from the 40 cm of the pool wall, and the water surface was 1 cm above the top of the platform, whichever is unable to see the platform from the water surface. The temperature was maintained at 22°C-24°C. The test time was 60 seconds, and staying on the platform for more than 2 seconds was a success of finding the platform. After 5 days of experimental training, the time required by the mice from entering the water to successful platform search was denoted as the escape incubation period, while the escape incubation period was denoted as 60 seconds when the platform search failed. The average of the results of 4 times of training every day was taken as the total results of the training that day.

Transfection

BMP4 siRNA was purchased from Genepharma (Shanghai, China). BMP4 plasmids were purchased from Youbio (Changsha, Hunan, China). N2A, HT22, and SH-SY5Y cells were grown in 6-well plates and then transfected with BMP4 overexpressed plasmid, and empty vector using Lipofectamine 3000. These cells were also transfected with BMP4 siRNA and negative control using Lipofectamine 2000 according to the manufacturer's instructions.

Reverse-transcribed polymerase chain reaction

The level of BMP4 in forebrain tissues was evaluated by reverse transcribed polymerase chain reaction (RT-PCR). Total RNA was first extracted using Trizol reagent. BMP4 was measured as described before. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference.

Western blot analysis

The hippocampal tissues of transgenic mice and wild-type mice aged at 4, 12, 20 and 40 weeks were extracted and cleaved to protein with RIPA lysate with 1% phosphatase inhibitor. N2A, HT22 and SH-SY5Y cells were harvested after transfection, and total proteins from cells were extracted. Protein samples were boiled and electrophoresed on 10% polyacrylmidegels and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% BSA in TBST (Tris-buffered saline with Tween) at room temperature for 2 h, and then they were incubated with primary antibodies overnight at 4°C. After throughout washing with TBST, the membranes were incubated with HRP-conjugated goat anti-rabbit or mouse anti-rabbit secondary antibody for 1 h at room temperature. Finally membranes were added the chemiluminescence reagent and exposed in the darkroom. Densitometric quantification of the protein blots was done using ImageJ software.

Cell viability assay

N2A, HT22 and SH-SY5Y cells were cultured in 96-well plates for overnight incubation, and then cells were transfection with BMP4 overexpressed plasmid or BMP4 siRNA for 72 h. Cell proliferation was assessed by MTT assay [18].

Apoptosis assay

N2A, HT22, and SH-SY5Y cells (5×10^5 cells/well) were seeded in a 6-well plate overnight and then transfection with BMP4 overexpressed plasmid or BMP4 siRNA. After 24h, cells apoptosis was assessed by FITC-Annexin/propidium iodide (PI) staining and flow cytometry. Cells were treated with trypsinization and washed with PBS, then resuspended in 500 μ L binding buffer containing 1 μ L FITC-conjugated anti-Annexin V antibody and 5 μ L PI. Fluorescence-activated cell sorting (FACS) was performed to analyze the apoptotic rate. FCAS data were analyzed with Flowjo software.

Tissue processing

After mice were killed, the scalp of the mice was cut open, and the head was exposed. Then, the skull of the mice was removed with straight tweezers to expose the cerebral cortex, and the cerebral cortex was carefully removed with straight tweezers to expose the hippocampal tissue. The hippocampal tissue was separated from the cerebral cortex and the surrounding brain tissue, and the hippocampal tissue was taken out. Then sniped with scissors and placed in homogenizer to break and added the corresponding amount of RIPA to obtain hippocampal tissue protein

TUNEL assay for measuring cellular apoptosis in mouse brain.

TdT-mediated dUTP-biotin nick end labeling (TUNEL) assays were performed by using the TUNEL cell apoptosis detection kit via green FITC-labeled fluorescence detection approach (catalog: KGA7071, KeyGEN BioTECH Company). Mouse cardiac perfusion was performed to make paraffin slides for mouse hippocampal tissue. After dewaxing the paraffin sections of mouse hippocampal tissue, the protease K working solution with mass concentration of 100 μ l (20 μ g/mL) was dripped and washed with PBS for 3 times at room temperature for 30 min.. Then 100 μ L DNase I was incubated at 37°C for 30 min and then washed with PBS. After drying the slide, 50 μ L TUNEL reaction mixture (50 μ L TdT+450 μ L fluorescein labeled d UTP solution) was added to the specimens and incubated at 37°C for 60 min and then washed by PBS. The DAPI was added dropwise to protect the specimens for 10 min, and the specimen was stained and washed with PBS. The excess DAPI was washed off, and the liquid on the slide was blotted with absorbent paper, and the collected image was observed under a fluorescence microscope with a sealing sheet containing an anti-fluorescence quenching agent. After the nuclei were stained with DAPI, blue fluorescence was displayed, and the apoptotic cells were stained with the TUNEL reaction mixture to show the green fluorescence.

Statistical analysis

All data were analyzed using GraphPad Prism 6.0 software. The results were expressed as the mean $\pm$ SD. Differences between each group of values and its control group were evaluated by Student's t test. Differences in three or four groups were analyzed by ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant. P value representations are indicated: * $P < 0.05$.

Results

Construction and screening of NSE-BMP4 transgenic mice

We used C57BL/6 strain mice to construct a BMP4 (NSE-BMP4) transgenic model regulated by neurospecific enolase (NSE) promoter. Under the regulation of NSE promoter, the expression level of BMP4 was upregulated. The mouse BMP4 gene sequence includes exon and intron. The primers of BMP4 were designed to cross introns, resulting in a specific amplification band about 277bp (Figure 1A). RT-PCR analysis data revealed expression of the transgene in adult NSE-BMP4 brain of mice at 4 weeks age (Figure 1B). This suggests that we have constructed transgenic mice with overexpression of BMP4 under control of the NSE promoter to determine the function of BMP4 in brain development and progression.

The escape latency of NSE-BMP4 transgenic mice is larger

In order to determine the possibility of NSE-BMP4 transgenic mice suffering from AD, Morris water maze experiment was conducted in middle-aged and elderly mice (older than 30 weeks age). The results showed that there was no significant difference in the escape latency of water maze between NSE-BMP4 transgenic mice and wild mice on the first day, but there was a remarkably difference on the 2nd to 5th day after that (Figure 1C). The total distribution of five days escape incubation period in different groups (Figure 1D) showed that there was a difference between the transgenic group and the wild group. This finding indicates that BMP4 could induce AD in mice.

BMP4 transgenic mice exhibits higher expression of AD related proteins

To determine the mechanism of BMP4-mediated AD, we extracted hippocampal tissue proteins from NSE-BMP4 transgenic mice and wild mice at 1, 3, 6, and 9 months, and compared with the differences of APP family related proteins by Western blotting. We found that the expression of the APP, Ab and the PSEN1 was promoted by BMP4 in transgenic mice at 6 months age (Figure 2A). The results showed that there was no difference in expression of APP family related proteins between the one-month-old NSE-BMP4 transgenic mice and wild-type mice (Figure 3A). There is a decrease in APP protein at 3 months, which means that APP is being sheared into Ab and begin to deposited. Moreover, Western blotting data showed that the increase expression of T-TAU protein, p-Thr181 TAU and p-Thr231 TAU in mice with BMP4 overexpression was significantly higher than that in wild-type mice (Figure 2B). Furthermore, the older the mice were, the more obvious increase of these proteins it was (Figure 2B).

Overexpression of BMP4 promotes the expression of AD related proteins in cell lines

To further define whether BMP4 modulates the expression of AD related proteins, we performed in vitro experiments using HT22, N2A and SH-SY5Y cells. Cells were transfected with BMP4 overexpression plasmid, and cell proteins were extracted after 48 hours. Our Western blotting data showed that overexpression of BMP4 promoted the expression of APP protein, Ab1-42 protein and PSEN1 protein in HT22 cells (Figure 3A), SH-SY5Y cells (Figure 3B), and N2A cells (Supplemental figure 1A). Moreover, overexpression of BMP4 increased the expression of T-TAU protein, p-Thr181 TAU and p-Thr231 TAU proteins in HT22 cells (Figure 3C), SH-SY5Y cells (Figure 3D), and N2A cells (Supplemental figure 1B). Altogether, BMP4 overexpression could govern the expression of AD related proteins.

Downregulation of BMP4 reduces the expression of AD related proteins.

To further investigate whether BMP4 governs the expression of AD related proteins, HT22 and N2A cells were transfected with BMP4 small interfering RNA (siRNA) for downregulation of BMP4. We found that BMP4 siRNA decreased the expression of BMP4 in HT22 (Figure 4A) and N2A cells (Supplemental figure 2A). Notably, downregulation of BMP4 weakened the expression of APP, Ab and PSEN1 proteins in HT22 and N2A cells (Figure 4A and Supplemental figure 2A). Furthermore, inhibition of BMP4 decreased the expression of T-TAU protein, p-Thr181 TAU and p-Thr231 TAU proteins in HT22 cells (Figure 4B) and N2A

cells (Supplemental figure 2B). Taken together, BMP4 might control the expression of AD related proteins.

BMP4 overexpression induces BAX expression and reduces Bcl-2 level

To explore whether overexpression of BMP4 induces apoptosis and involves in the development of AD, we extracted hippocampal proteins from old NSE-BMP4 mice and wild mice and measured the expression of Bcl-2 and BAX by Western blotting approach. We found that BMP4 transgenic mice exhibited the increased BAX and downregulation of Bcl-2 at 6 months and 9 months (Figure 5A and 5B). In vitro experiments demonstrated that overexpression of BMP4 increased the expression of BAX and decreased Bcl-2 expression in HT22 and SH-SY5Y cells (Figure 5C) and N2A cells (Supplemental figure 3A). In line with this, downregulation of BMP4 reduced BAX expression and increased Bcl-2 expression level in HT22 cells (Figure 5D) and N2A cells (Supplemental figure 3B). Therefore, BMP4 overexpression induced apoptosis in part via regulation of BAX and Bcl-2 expression.

BMP4 overexpression induces apoptosis in cells

Next, we dissected whether BMP4 overexpression is involved in apoptosis in cell lines. We observed that overexpression of BMP4 induced cell apoptotic death in N2A cells and HT22 cells (Figure 6A and 6B). We also found that BMP4 upregulation inhibited cell viability in N2A and HT22 cells (Figure 6C). Consistently, downregulation of BMP4 inhibited cell apoptosis in N2A cells (Figure 6D) and promoted cell viability in N2A and HT22 cells (Figure 6E).

Apoptosis is increased in DG, CA1 and CA3 of BMP4 transgenic mice

Lastly, we measured the apoptosis in DG region, CA1-3 zones of hippocampus in 9-month old wild type mice and NSE-BMP4 transgenic mice by TUNEL fluorescence assay. We found that CA1, CA3 and DG zones exhibited an increased apoptosis compared with that of wild type mice (Figure 7A-B). The cell arrangement and evacuation of CA1, CA2, CA3 cells in transgenic mice could be observed, and the boundary was not clear. It indicates that NSE-BMP4 mice have the loss of neuronal cells (Figure 7A).

Discussion

In the current study, we explored the role of BMP4 in AD development and progression. We found that middle-aged BMP4 transgenic mice exhibited memory disorder via Morris water maze experiment. Moreover, the hippocampal tissues have high expression of AD related proteins including APP, A β , PSEN-1, TAU, P-TAU (Thr181), and P-TAU (Thr231) proteins. Furthermore, in multiple cell lines, overexpression of BMP4 increased the expression of AD related proteins, whereas downregulation of BMP4 demonstrated opposed effects. Consistently, BMP4 modulation participated in cell apoptosis via regulation of BAX and Bcl-2 expression in cells. Our findings indicate that BMP4 overexpression might be a factor to induce AD.

In recent years, with the gradual improvement of people living standards and the aging of the social population, the number of AD patients is increasing, leading to AD as a global health priority [19]. The main manifestations of AD are progressive memory impairment, cognitive impairment, personality changes and language disorders and other neurological symptoms [20]. The hippocampus is a critical region for learning and memory and is particularly vulnerable to damage in the early stages of AD. Emerging evidence suggests that neurogenesis in the adult hippocampus is an early critical event in the AD process [21]. BMPs perform their biological functions by interacting with membrane binding receptors of the serine/threonine kinase family, including BMP receptors type I (BMPRI) and type II (BMPRII). There are several types of BMPs in the hippocampus, such as BMP2, BMP4, BMP7 and BMP10. This suggests that BMPs or its associated factors could regulate hippocampal plasticity and other important brain functions. One study proved that BMP4 is strongly expressed in the subgranular layer of adult hippocampal dentate gyrus (DG) zone [17]. RNA level of BMP4 was increased in the hippocampus of APP^{swe}/PS1 transgenic mice, linking BMP4 with AD development, but little is known about the mechanism through which BMP4 is involved in the occurrence of AD [17]. In the present study, we used NSE-BMP4 transgenic mouse model to determine whether BMP4 overexpression could trigger AD development. In fact, BMP4 transgenic mice exhibited memory disorder by Morris water maze experiment, indicating that BMP4 overexpression might be involved in AD pathogenesis.

AD patients are often caused by the mutation of APP gene, and APP dysfunction is prior to TAU dysfunction [22]. Mutations in TAU gene lead to dominant dementia and Parkinson's disease [7], and A β may play its role through TAU in patients with cognitive impairment [23]. Moreover, deposition of TAU in the cortex and hippocampus is conducive to the occurrence and development of AD, although it is not an essential factor for AD development [7]. It has been recognized that A β 1-42, T-TAU and P-Thr181 TAU are auxiliary indicators of AD [24–26]. P-Thr231 TAU appears to be more associated with AD [27, 28]. In line with this, BMP4 transgenic mice have higher expression of AD related proteins, including APP, A β 1-42, T-TAU, P-Thr181 TAU, and P-Thr231 TAU proteins. Consistently, overexpression of BMP4 in cell lines promoted the expression of AD associated proteins. Since BMP4 knockout leads to the lethality of mice [29, 30], we performed the knockdown of BMP4 in multiple neuroma cells. We found that downregulation of BMP4 in cell lines reduced the expression of AD related proteins.

Bcl-2 family proteins, including pro-apoptotic and anti-apoptotic members, are involved in cell apoptosis in AD [31]. In our study, BMP4 transgenic mice have an increased expression of BAX and a reduced Bcl-2 level. Overexpression of BMP4 increased BAX level and inhibited Bcl-2 expression in cell lines, indicating that BMP4 overexpression induces AD in part via regulation of BAX and Bcl-2.

Limitations

This study used the conditional BMP4 transgenic mouse model and found that BMP4 overexpression led to memory disorder in mice. However, there are several limitations of this study. Besides morris water maze experiment, any other experiments could validate AD development in mice with BMP4 overexpression? Do the AD patients have the higher expression of BMP4 in clinic? What are the detailed

molecular mechanisms of BMP4-induced AD development? These questions need to be answered in the future to fully elucidate the role of BMP4 in AD initiation and progression.

Conclusion

In summary, upregulation of BMP4 might increase the expression of AD related proteins and subsequently elevate the BAX/Bcl-2 ratio, leading to cell death and AD development.

Abbreviations

AD

Alzheimer's disease; A β : amyloid β -protein; BMP4: bone morphogenetic protein 4; NSE: neuron-specific enolase; APP: amyloid precursor protein; TGF- β : transforming growth factor-beta; DG: dentate gyrus; TUNEL: TdT-mediated dUTP-biotin nick end labeling; BMPRI: BMP receptors type I.

Declarations

Author Contributions

X.Z. performed the experiments, analyzed the data, and wrote the manuscript. J.L., L.M., H.X., Y.C., W.L. performed the experiments and analyzed the data. J.M. analyzed the data and critically viewed. P.W. wrote the manuscript, and critically viewed the study. Y. L. conceived the work, designed the experiments and critically supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics and Research Committees of Bengbu Medical College, China. All animal experimental procedures were performed by following the China Animal Welfare Guidelines. The study protocol was approved by our Institutional Animal Care and Use Committee at Bengbu Medical College, Bengbu, China.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The escape latency of NSE-BMP4 transgenic mice is larger. A. The primers of BMP4 were illustrated to cross introns. B. RT-PCR analysis was used to measure NSE-BMP4 transgene expression in adult transgenic forebrain. C. Morris water maze experiment was conducted in middle-aged and elderly mice. * $p<0.05$ vs WT. WT: wild type mice. BMP4: BMP4 transgenic mice. D. The total distribution of five days escape incubation period in different groups. * $p<0.05$ vs WT.

Figure 2

BMP4 transgenic mice exhibit higher expression of AD related proteins. **A.** Top panel: Western blotting analysis was used to measure the expression of BMP4, APP, A β 1-42, and PSEN-1 in hippocampus of BMP4 transgenic mice and WT mice. Bottom panel: Quantitative results are illustrated for top panel. * $P < 0.05$ vs WT. WT: wild type mice; BMP4: BMP4 transgenic mice. **B.** Top panel: Western blotting analysis was performed to measure the expression of T-TAU, P-Thr181 TAU, and P-Thr231 TAU proteins in

hippocampus of BMP4 transgenic mice and WT mice. Bottom panel: Quantitative results are illustrated for top panel. *P<0.05 compared with WT.

Figure 3

Overexpression of BMP4 promotes the expression of AD related proteins in cell lines A-B. Left panel: Western blotting analysis was performed to measure the expression of APP, Aβ1-42 and PSEN-1 in HT22 cells (A) and SH-SY5Y cells (B) transfected with BMP4 overexpression plasmid. Right panel: Quantitative

results are illustrated for left panel. *P<0.05 vs pcDNA3.1. C-D. Left panel: Western blotting analysis was performed to measure the expression of T-TAU, P-Thr181 TAU and P-Thr231 TAU in HT22 cells (C) and SH-SY5Y cells (D) transfected with BMP4 overexpression plasmid. Right panel: Quantitative results are illustrated for left panel. *P<0.05 vs pcDNA3.1.

Figure 4

Downregulation of BMP4 reduces the expression of AD related proteins. A. Left panel: Western blotting analysis was performed to measure the expression of APP, Aβ1-42 and PSEN-1 proteins in HT22 cells transfected with BMP4 siRNAs. Right panel: Quantitative results are illustrated for left panel. *P<0.05 vs NC. NC: nonspecific control. B. Left panel: Western blotting analysis was performed to measure the expression of T-TAU, P-Thr181 TAU and P-Thr231 TAU in HT22 cells transfected with BMP4 siRNAs. Right panel: Quantitative results are illustrated for left panel. *P<0.05 vs NC.

Figure 5

BMP4 overexpression induces BAX expression and reduces Bcl-2 level. A. Western blotting analysis was used to measure the expression of BAX and Bcl-2 in hippocampus of BMP4 transgenic mice and WT mice. B. Quantitative results of BAX/Bcl-2 are illustrated for panel A. * $P < 0.05$, compared with WT. WT: wild type mice. TG: BMP4 transgenic mice. C. Left panel: Western blotting analysis was performed to measure the expression of BAX and Bcl-2 in HT22 and SH-SY5Y cells transfected with BMP4

overexpression plasmid. Right panel: Quantitative results are illustrated for left panel. *P<0.05 vs pcDNA3.1. D. Left panel: Western blotting analysis was performed to measure the expression of BAX and Bcl-2 in HT22 cells transfected with BMP4 siRNAs. Right panel: Quantitative results are illustrated for left panel. *P<0.05 vs NC.

Figure 6

BMP4 overexpression induces apoptosis in cells. A. Cell apoptosis was tested via Flow cytometry in N2A and HT22 cells with BMP4 overexpression. B. Quantitative results are illustrated for panel A. *P<0.05, compared with pcDNA3.1. C. MTT assay was performed to measure cell viability in N2A and HT22 cells with BMP4 overexpression. *P<0.05, compared with pcDNA3.1. D. Left panel: Cell apoptosis was tested via Flow cytometry in N2A cells with BMP4 siRNA transfection. Right panel: Quantitative results are illustrated for left panel. *P<0.05, compared with NC. NC: nonspecific control siRNA. siBMP4: BMP4 siRNA. E. MTT assay was used for testing cell viability in N2A and HT22 cells with BMP4 siRNA transfection.

Figure 7

Apoptosis is increased in DG, CA1 and CA3 of BMP4 transgenic mice. A. The apoptosis was measured in DG region, CA1-3 zones of hippocampus in 9-month old wild type mice and NSE-BMP4 transgenic mice by TUNEL fluorescence assay. B: Quantitative results are illustrated for panel A. *P<0.05, compared with WT.

Supplementary Files

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- [Supplementaryfigures.pdf](#)