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Farnesoid X Receptor (FXR) Aggravates Amyloid- β -Triggered Apoptosis by Modulating the cAMP-Response Element-Binding Protein (CREB)/Brain-Derived Neurotrophic Factor (BDNF) Pathway In Vitro

Chen, Qingfa ; Ma, Hongling ; Guo, Xuewen ; Liu, Jia ; Gui, Ting ; Gai, Zhibo

Abstract: **BACKGROUND:** Alzheimer's disease (AD), which results in cognitive deficits, usually occurs in older people and is mainly caused by amyloid beta (A β) deposits and neurofibrillary tangles. The bile acid receptor, farnesoid X receptor (FXR), has been extensively studied in cardiovascular diseases and digestive diseases. However, the role of FXR in AD is not yet understood. The purpose of the present study was to investigate the mechanism of FXR function in AD. **MATERIAL AND METHODS:** Lentivirus infection, flow cytometry, real-time PCR, and western blotting were used to detect the gain or loss of FXR in cell apoptosis induced by A β . Co-immunoprecipitation was used to analyze the molecular partners involved in A β -induced apoptosis. **RESULTS:** We found that the mRNA and protein expression of FXR was enhanced in Ab-triggered neuronal apoptosis in differentiated SH-SY5Y cells and in mouse hippocampal neurons. Overexpression of FXR aggravated A β -triggered neuronal apoptosis in differentiated SH-SY5Y cells, and this effect was further increased by treatment with the FXR agonist 6ECDCA. Molecular mechanism analysis by co-immunoprecipitation and immunoblotting revealed that FXR interacted with the cAMP-response element-binding protein (CREB), leading to decreased CREB and brain-derived neurotrophic factor (BDNF) protein levels. Low expression of FXR mostly reversed the A β -triggered neuronal apoptosis effect and prevented the reduction in CREB and BDNF. **CONCLUSIONS:** These data suggest that FXR regulates A β -induced neuronal apoptosis, which may be dependent on the CREB/BDNF signaling pathway in vitro.

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Alzheimer's disease (AD), which results in cognitive deficits, usually occurs in older people and is mainly caused by amyloid beta ($A\beta$) deposits and neurofibrillary tangles. The bile acid receptor, farnesoid X receptor (FXR), has been extensively studied in cardiovascular diseases and digestive diseases. However, the role of FXR in AD is not yet understood. The purpose of the present study was to investigate the mechanism of FXR function in AD.



Material/Methods: Lentivirus infection, flow cytometry, real-time PCR, and western blotting were used to detect the gain or loss of FXR in cell apoptosis induced by $A\beta$. Co-immunoprecipitation was used to analyze the molecular partners involved in $A\beta$ -induced apoptosis.

Results: We found that the mRNA and protein expression of FXR was enhanced in $A\beta$ -triggered neuronal apoptosis in differentiated SH-SY5Y cells and in mouse hippocampal neurons. Overexpression of FXR aggravated $A\beta$ -triggered neuronal apoptosis in differentiated SH-SY5Y cells, and this effect was further increased by treatment with the FXR agonist 6ECDCA. Molecular mechanism analysis by co-immunoprecipitation and immunoblotting revealed that FXR interacted with the cAMP-response element-binding protein (CREB), leading to decreased CREB and brain-derived neurotrophic factor (BDNF) protein levels. Low expression of FXR mostly reversed the $A\beta$ -triggered neuronal apoptosis effect and prevented the reduction in CREB and BDNF.

Conclusions: These data suggest that FXR regulates $A\beta$ -induced neuronal apoptosis, which may be dependent on the CREB/BDNF signaling pathway *in vitro*.

MeSH Keywords: **Alzheimer Disease • Apoptosis • Brain-Derived Neurotrophic Factor • Cyclic AMP Response Element-Binding Protein • Farnesol**

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Background

Alzheimer's disease (AD), a chronic neurodegenerative disorder, is the most frequent cause of dementia [1–3]. The incidence of AD is rising worldwide with increasing age [4]. The primary pathological changes in AD are intracellular neurofibrillary tangles induced by tau phosphorylation and intercellular senile plaque accumulation induced by oligomerization of the β -amyloid (A β) protein [5,6].

The toxic effects of A β can lead to dysfunction in neurotrophic factor expression. In particular, brain-derived neurotrophic factor (BDNF) downregulation by A β may lead to synaptic loss and neurodegeneration [7]. A β -induced BDNF dysfunction is mediated by the cAMP-response element-binding protein (CREB), an important transcriptional element necessary for memory and for the survival of neurons [8]. CREB expression was decreased in post-mortem hippocampus of AD patients, in mouse hippocampal neurons, and in A β -triggered hippocampal neurons and SH-SY5Y cells [9]. The farnesoid X receptor (FXR), a nuclear receptor widely expressed in the liver and intestine, plays an important role in the synthesis, secretion, and transport of bile acid [10]. The function of CREB can be suppressed by activated FXR [11].

FXR was proved to be present and function in the brain [12]. We speculated that FXR functions in the progression of AD by regulating brain lipid levels. In the present study, FXR function in AD was analyzed using an A β 1–42-treated neuronal cell model. FXR expression was elevated in differentiated SH-SY5Y cells and in mouse hippocampal neurons treated with A β 1–42. FXR potentiated A β 1–42-triggered cell apoptosis in parallel with decreased expression of CREB. In addition, the CREB/BDNF signaling pathway was inhibited by FXR overexpression and promoted by FXR knockdown. From these data, we speculate that FXR possess an A β -induced cell apoptosis-enhancing property by modulating the transcription of CREB and downstream BDNF expression.

Material and Methods

Cell culture

Human SH-SY5Y cells were purchased from the American Type Culture Collection and were cultured in a 10-cm dish containing DMEM/F12 medium, 10% FBS, and 1% penicillin/streptomycin (P/S). The maintenance of cells was carried out in a CO₂ incubator, and one-half of the medium was changed each day. For cell differentiation, 2×10^5 cells were seeded per well in 6-well plates (Corning) in DMEM/F12, 10% FBS, and 1% P/S for 24 h. The medium was then changed to DMEM/F12, 1% N-2 supplements, 1% P/S, and 10 μ M all-*trans* retinoic acid.

After 7-day incubation with RA, A β 1–42 (1 μ M) was added and the cells were cultured for another 24 h.

Mouse hippocampal neurons derived from neonatal C57BL/6 mice were cultured as described previously [13]. Briefly, brains were removed and the hippocampus was dissected from each animal, and tissues were dissociated by mild mechanical trituration. Cells were plated onto poly-D-lysine-coated 6-well plates in Neurobasal medium containing 2% B27 and 1% P/S in a CO₂ incubator at 37°C, and one-half of the medium was changed each day.

A β 1–42 and 6ECDCA preparation

A β 1–42 peptide (Sigma-Aldrich) was prepared following previously reported methods [14]. Initially, the A β 1–42 peptide was dissolved in 100% hexafluoro isopropanol to a final concentration of 1 mM and the suspension was aliquoted into sterile microcentrifuge tubes and evaporated under vacuum in a Speed-Vac. Oligomerization of A β 1–42 was performed in DMSO 5 mM, followed by ultrasound for 10 min. Before use, A β 1–42 was diluted to a final concentration of 100 μ M in DMEM at 4°C for 24 h.

6ECDCA was dissolved in 100% ethanol to a final concentration of 1 mM as reported previously [15], and stored at –20°C. The cells were stimulated with 6ECDCA (2 μ M) for 24 h, followed by incubation with or without A β 1–42 (1 μ M) for another 24 h. The effects of forskolin treatment were determined by incubating FXR-overexpressed cells with or without forskolin (10 μ M) for 1 h.

Lentiviral transduction

All lentiviral products with enhanced green fluorescence protein (EGFP)-tags used in this study were supplied by Genechem (Shanghai, China). The titers for lentiviral (LV)-FXR and LV-Control were adjusted to 5×10^8 TU/ml, and the titers for LV-FXR-short hairpin (sh)RNA and LV-Scrambled-shRNA were adjusted to 8×10^8 TU/ml. The sequences for FXR-shRNA and control shRNA were 5'-CCACTTCTTGATGTGCTACAA-3' and 5'-TTCTCCGAACGTGTACAGT-3', respectively. To select effective shRNA sequences targeting FXR, lentivirus encoding trial shRNA sequences were transduced SH-SY5Y cells at a multiplicity of infection (MOI) of 5; after culturing for 3 days, cells were selected for 4 days in medium containing 0.6 μ g/ml puromycin (Sigma-Aldrich). The upregulation and suppression of mRNA expression were analyzed by real-time PCR, and FXR protein levels were detected using immunoblotting.

Table 1. Primer sequences used in this study.

Primer	Sequence (5'-3')
Human <i>FXR</i> -F	GCAGTCTCTGGGACAGAA
Human <i>FXR</i> -R	GCTGGCATAAGCCTGAGTTC
Human <i>GAPDH</i> -F	ACATTGTTGCCATCAACGAC
Human <i>GAPDH</i> -R	ACGCCAGTAGACTCCACGAC
Mouse <i>FXR</i> -F	GGCCTCTGGGTACCACTACA
Mouse <i>FXR</i> -R	AAGAAACATGGCCTCCACTG
Mouse <i>GAPDH</i> -F	CAAGGAGTAAGAAACCCTGGACC
Mouse <i>GAPDH</i> -R	CGAGTTGGGATAGGCCTCT

RNA extraction and real-time PCR

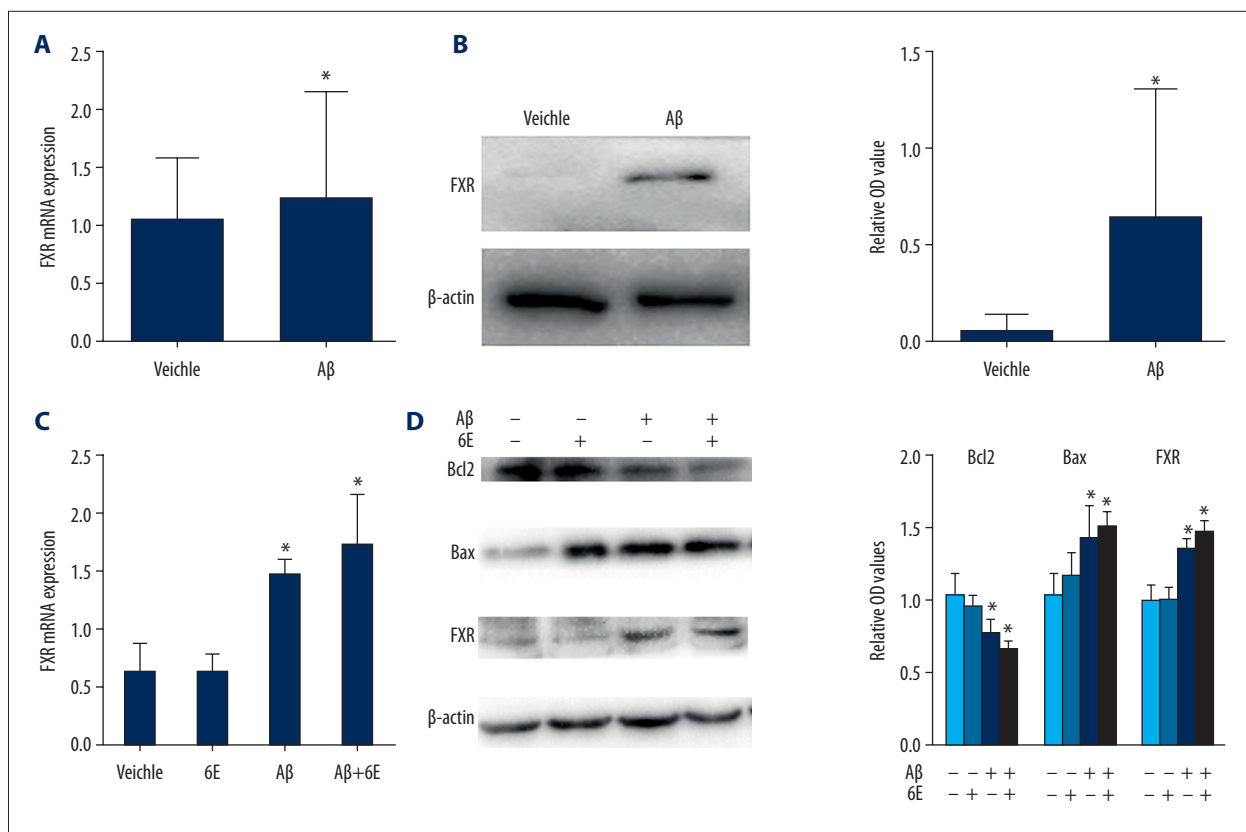
Briefly, total RNA was isolated from SH-SY5Y cells using TRIzol[®] reagent (Thermo Fisher, DE, USA). The PrimeScript[™] RT Master Mix was then used for reverse transcription and SYBR[®] fast qPCR master mix was used for real-time PCR following the kit protocols. The primers used are shown in Table 1. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Co-immunoprecipitation and immunoblotting

Cells were extracted in RIPA buffer, and immunoprecipitation with specific antibodies was performed using protein A agarose. The pellets were washed 3 times. Immunoblotting was then performed with diluted antibodies overnight at 4°C or for 2 h at room temperature using a standard procedure [16,17]. The primary antibodies included: anti-FXR (#sc-25309, 1: 750, Santa Cruz), anti-CREB (#9192, 1: 1000, Cell Signaling Technology), anti-proBDNF (#sc-65514, 1: 750, Santa Cruz), anti-Bax (#ab182733, 1: 500, Abcam), anti-Bcl-2 (#ab32124, 1: 500, Abcam), and anti- β -actin (#A1978, 1: 1000, Sigma-Aldrich). To confirm reproducibility, experiments were performed at least 3 times. Band intensity quantification was carried out using Image J software.

Cell apoptosis analysis

The apoptosis of cells was measured with a PE-AnnexinV apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, USA). Briefly, after 3 washes with PBS, SH-SY5Y cells infected with lentivirus were resuspended in 1 \times binding buffer (100 μ l). PE-AnnexinV (5 μ l) and 7-AAD (5 μ l) were then added to the cell suspension and incubated for 15 min at room temperature. The cells were sorted using a FACS Calibur flow cytometer (BD Biosciences). Data from EGFP+ cells were collected and analyzed using FlowJo software.



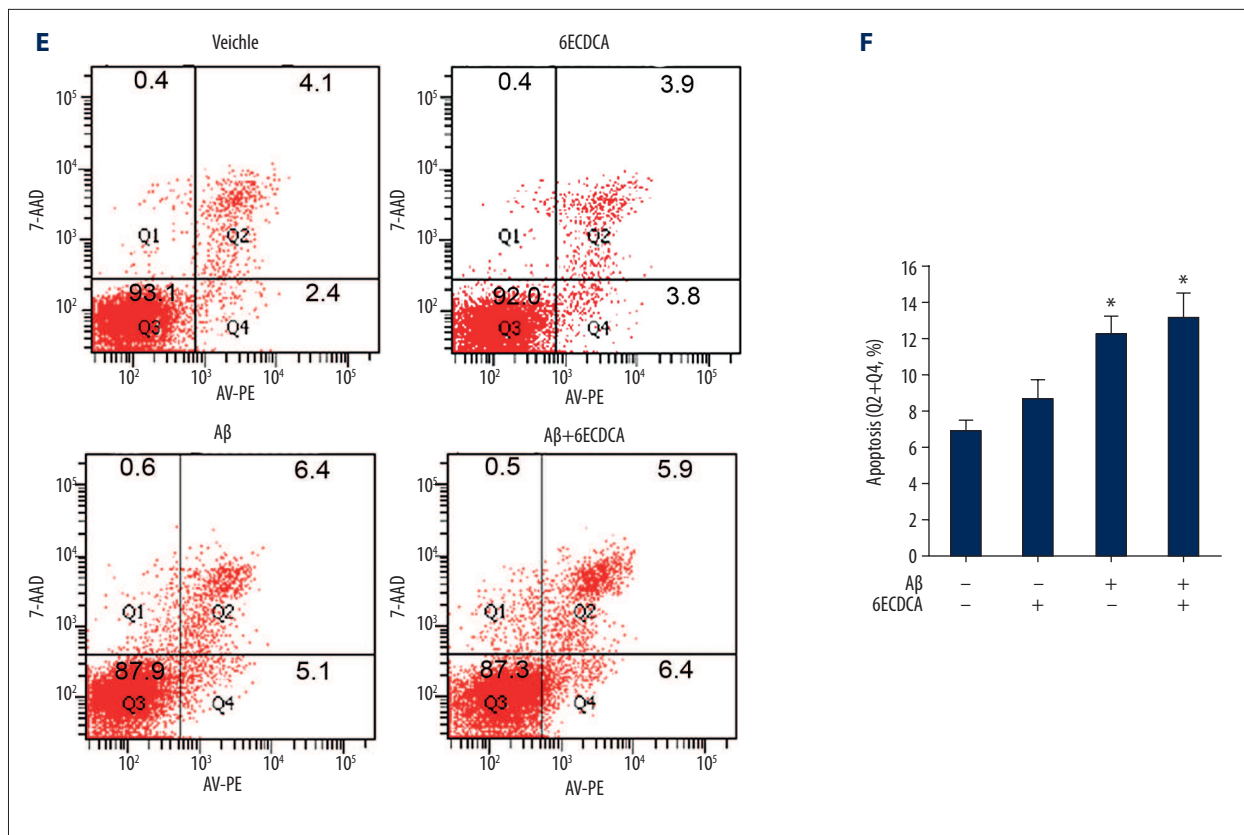


Figure 1. FXR is involved in A β -triggered apoptosis in mouse hippocampal neurons and SH-SY5Y cells. **(A)** Mouse hippocampal neuronal cells were treated with A β 1–42 (1 μ M) for 24 h. Cells were harvested and FXR mRNA was measured by real-time PCR. GAPDH was used to normalize the FXR mRNA expression. * $p < 0.05$ compared with the control group. **(B)** Mouse hippocampal neuronal cells were treated with A β 1–42 (1 μ M) for 24 h. Cells were harvested and protein level of FXR was measured by immunoblotting (**left panel**). Quantification of FXR protein band (**right panel**). Protein expression was normalized to β -actin. * $p < 0.05$ compared with the control group, and data are shown as mean \pm SEM. **(C)** SH-SY5Y cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1–42 (1 μ M) for 24 h. After incubation, cells were harvested and FXR mRNA was measured by real-time PCR. GAPDH was used to normalize FXR mRNA expression. * $p < 0.05$ compared with the control group. **(D)** SH-SY5Y cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1–42 (1 μ M) for another 24 h. After incubation, cells were harvested and protein levels of FXR, Bax, and Bcl-2 were measured by immunoblotting (**left panel**). Quantification of Bax, Bcl-2 and FXR protein bands (**right panel**). Protein expression was normalized to β -actin. * $p < 0.05$ compared with the control group, and data are shown as mean \pm SEM. **(E)** Cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1–42 (1 μ M) for another 24 h. After incubation, cells were harvested and Annexin V-PE+ cells were analyzed by flow cytometry. **(F)** Quantification of Annexin V-PE-stained cells. Experiments were performed using 3 different batches of cells. * $p < 0.05$ compared with the control group, and data are shown as mean \pm SEM. 6E, 6ECDCA.

Statistical analysis

Experiments were performed using 3 different batches of cells and each batch was tested in duplicate. Statistical analysis was performed with GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Two-way analysis of variance followed by Dunnett’s post hoc test for multiple comparisons was used for comparisons, and data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$ were considered statistically significant.

Results

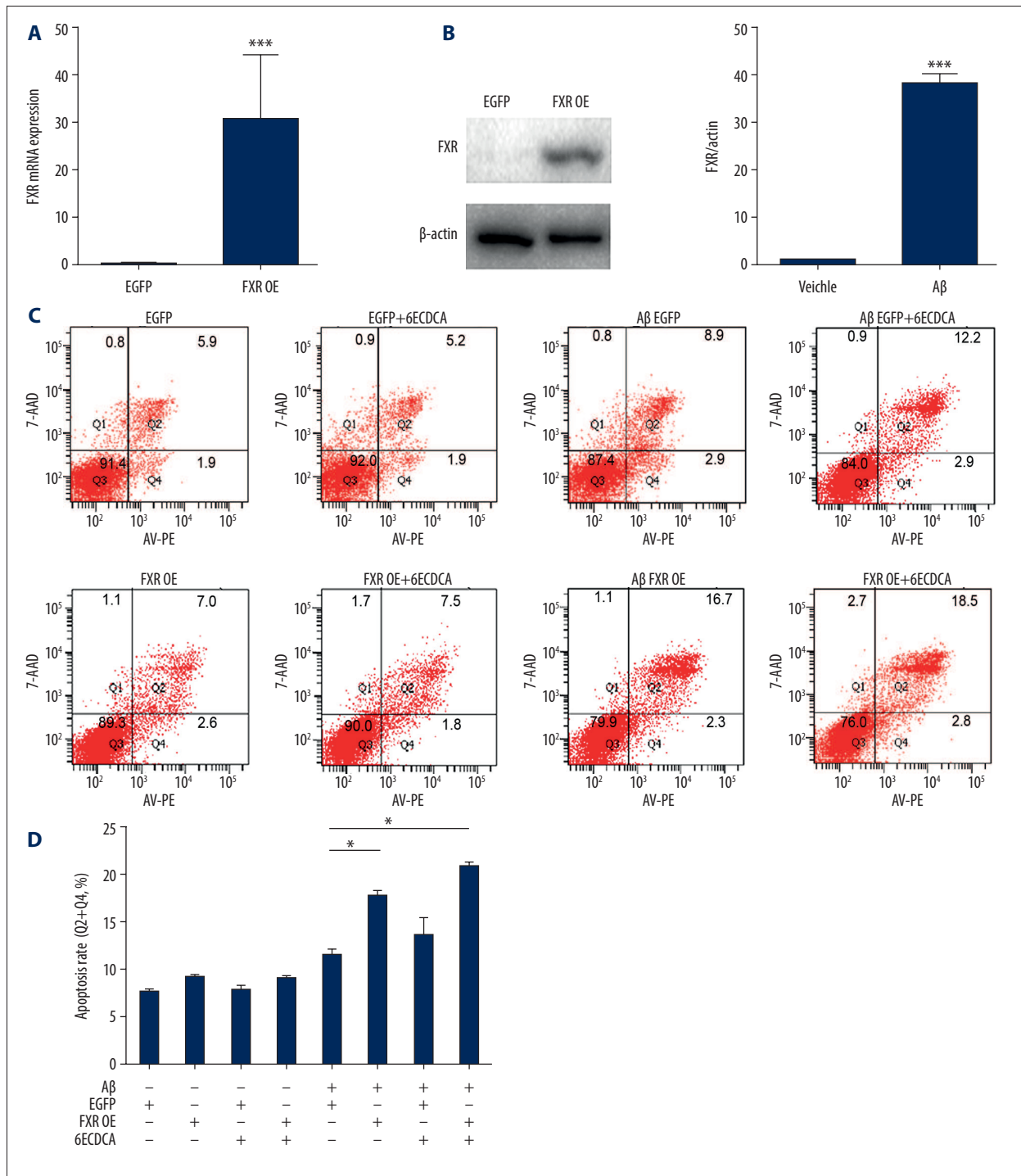
FXR was upregulated in A β 1–42-induced cell apoptosis

Low concentrations of A β 1–42 are directly toxic to neuronal cells [18]. FXR expression in A β 1–42-induced cell apoptosis was evaluated. The mRNA and protein expressions of FXR were significantly enhanced following treatment with A β 1–42 in mouse hippocampal neuronal cells (Figure 1A, 1B) and in SH-SY5Y cells (Figure 1C, 1D). In addition, immunoblotting of markers for A β 1–42-induced apoptosis showed that Bcl-2

protein expression was decreased, while Bax protein levels were significantly increased, indicating that FXR expression is related to A β 1-42-induced apoptosis (Figure 1D).

To determine whether FXR plays a role in A β 1-42-triggered apoptosis in SH-SY5Y cells, we examined the effect of 6ECDCA, an FXR agonist, on A β -triggered apoptosis. 6ECDCA treatment alone did

not induce significant apoptosis compared with the negative control (Figure 1E, 1F). Co-treatment with 6ECDCA resulted in a slight increase in FXR mRNA and protein expression (Figure 1C, 1D); however, this increase was not significantly different between the groups. A β -induced cell apoptosis was also unchanged after 6-ECDCA co-treatment, as shown by flow cytometry and immunoblotting analysis of Bcl2 and Bax (Figure 1D-1F).



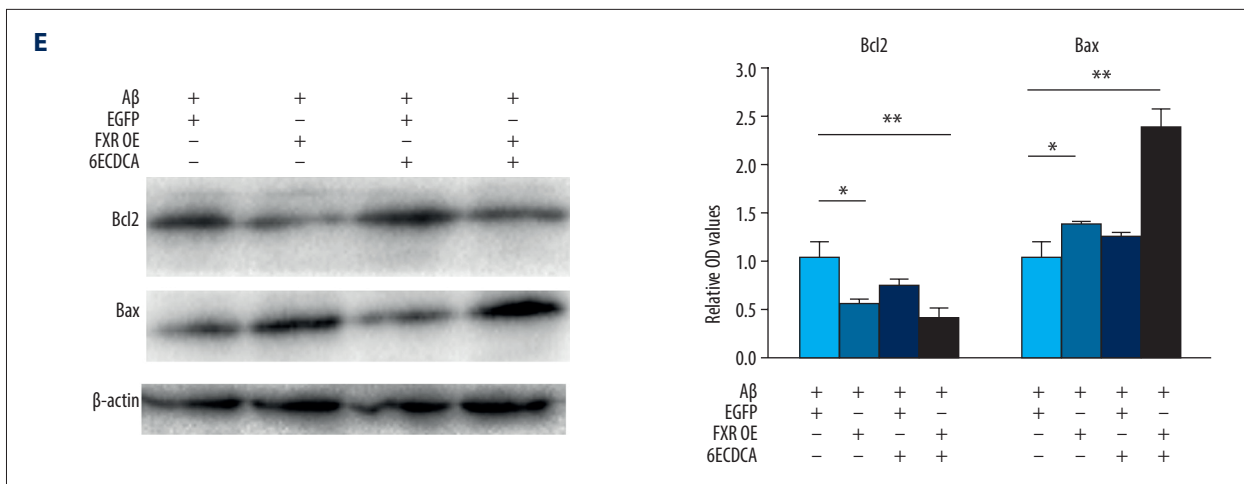


Figure 2. FXR overexpression aggravated A β -triggered cell apoptosis. **(A)** SH-SY5Y cells were infected with overexpressing lentivirus for FXR, and the level of FXR mRNA was assessed by real-time PCR. GAPDH was used to normalize FXR mRNA expression. *** $p < 0.0001$, compared with the control-EGFP group. **(B)** The level of FXR protein was assessed by immunoblotting. Quantification of FXR protein band in SH-SY5Y cells (**right panel**). β -actin was used as an internal control. *** $p < 0.0001$, compared with the control-EGFP group. **(C)** FXR-EGFP cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with or without A β 1-42 (1 μ M) for another 24 h. After incubation, cells were harvested for flow cytometry analysis. **(D)** Annexin V-PE-stained EGFP+cells were regarded as apoptotic cells. * $p < 0.05$, ** $p < 0.01$ compared with the A β -treated control-EGFP cells, and data are shown as mean \pm SEM. **(E)** FXR-EGFP cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1-42 (1 μ M) for another 24 h. After incubation, cells were harvested and protein levels of Bax and Bcl-2 were measured by immunoblotting (**left panel**). Quantification of protein bands is shown in the **right panel**. Experiments were performed using 3 different batches of cells. Protein expression was normalized to β -actin. * $p < 0.05$, ** $p < 0.01$ compared with the A β -treated control-EGFP cells, and data are shown as mean \pm SEM.

FXR overexpression aggravates A β -triggered cell apoptosis

The role of FXR overexpression in A β -induced SH-SY5Y cell apoptosis was determined. FXR overexpression and control lentiviral vectors were generated and transfected into SH-SY5Y cells. Two days after transfection, enhanced FXR mRNA and protein expression were observed in FXR-EGFP cells (Figure 2A, 2B). FXR-EGFP cells were then subjected to A β 1-42 or A β 1-42 plus 6ECDCA and analyzed by flow cytometry (Figure 2C). Compared to the control-EGFP group, a slight increase of apoptosis in the absence of A β 1-42 was observed when FXR was overexpressed (Figure 2D). Figure 2D also demonstrates that 6ECDCA did not significantly enhance A β -induced apoptosis in control-EGFP cells, which was consistent with the results in Figure 1F. However, compared to the A β -treated control-EGFP cells, FXR overexpression induced an increase in early and late (Q2+Q4) apoptosis in the A β 1-42 group. Figure 2E indicates that FXR-EGFP cells showed a decrease in Bcl2 expression and an increase in Bax expression in the A β 1-42 group, suggesting that A β -induced cell apoptosis was enhanced by FXR overexpression. Following 6ECDCA treatment, A β 1-42-induced apoptosis was even higher in the A β 1-42+6ECDCA group, indicating that FXR activation contributes to apoptosis.

FXR overexpression affects A β -induced apoptosis by regulating CREB transcriptional activity and BDNF expression

FXR has been proved to interact with CREB in mouse hepatocytes and in intestinal L cells, thus inhibiting its binding to the DNA binding site [11]. In the present study, the contribution of FXR/CREB to A β -induced apoptosis was determined. The FXR and CREB combination in FXR-EGFP cells was confirmed. The protein interaction between FXR and CREB was detected by immunoblotting with FXR antibody followed by immunoprecipitation with CREB antibody (Figure 3A). The combination of FXR and CREB in A β -treated FXR-EGFP cells was also confirmed and compared with the A β -treated control-EGFP cells. The interaction between FXR and CREB increased in FXR-EGFP cells following A β treatment, and further increased with A β 1-42+6ECDCA co-treatment (Figure 3B).

Compared to the A β -treated control-EGFP cells, the expression level of CREB decreased in FXR-EGFP cells following A β 1-42 treatment and further decreased in the A β 1-42+6ECDCA group (Figure 3C), suggesting that FXR not only interacted with the function and activity of CREB, but also regulated its expression. Physiologically, CREB modulates BDNF, which is very important in A β -induced cell apoptosis. BDNF expression in FXR-EGFP cells was then examined. Figure 3C shows that

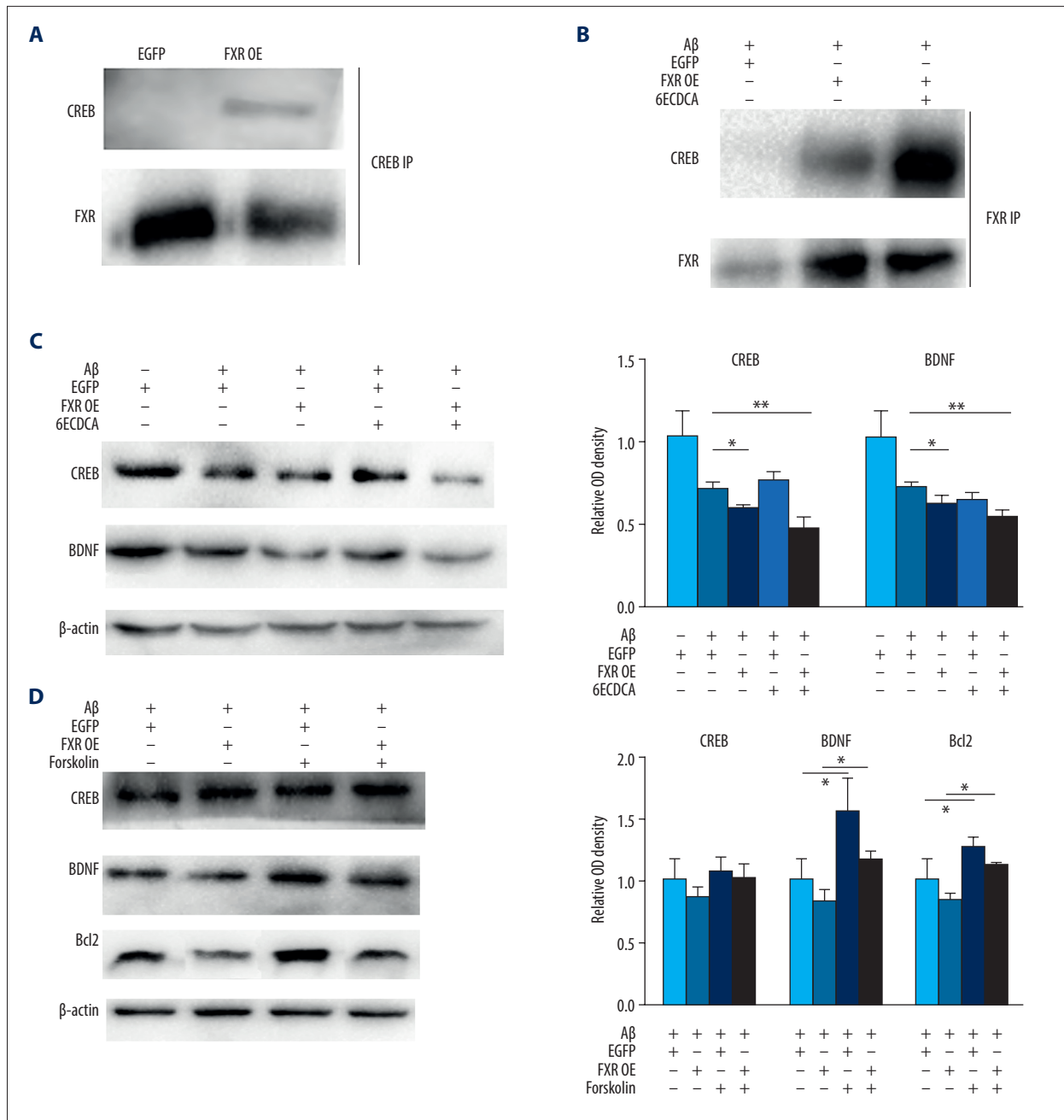
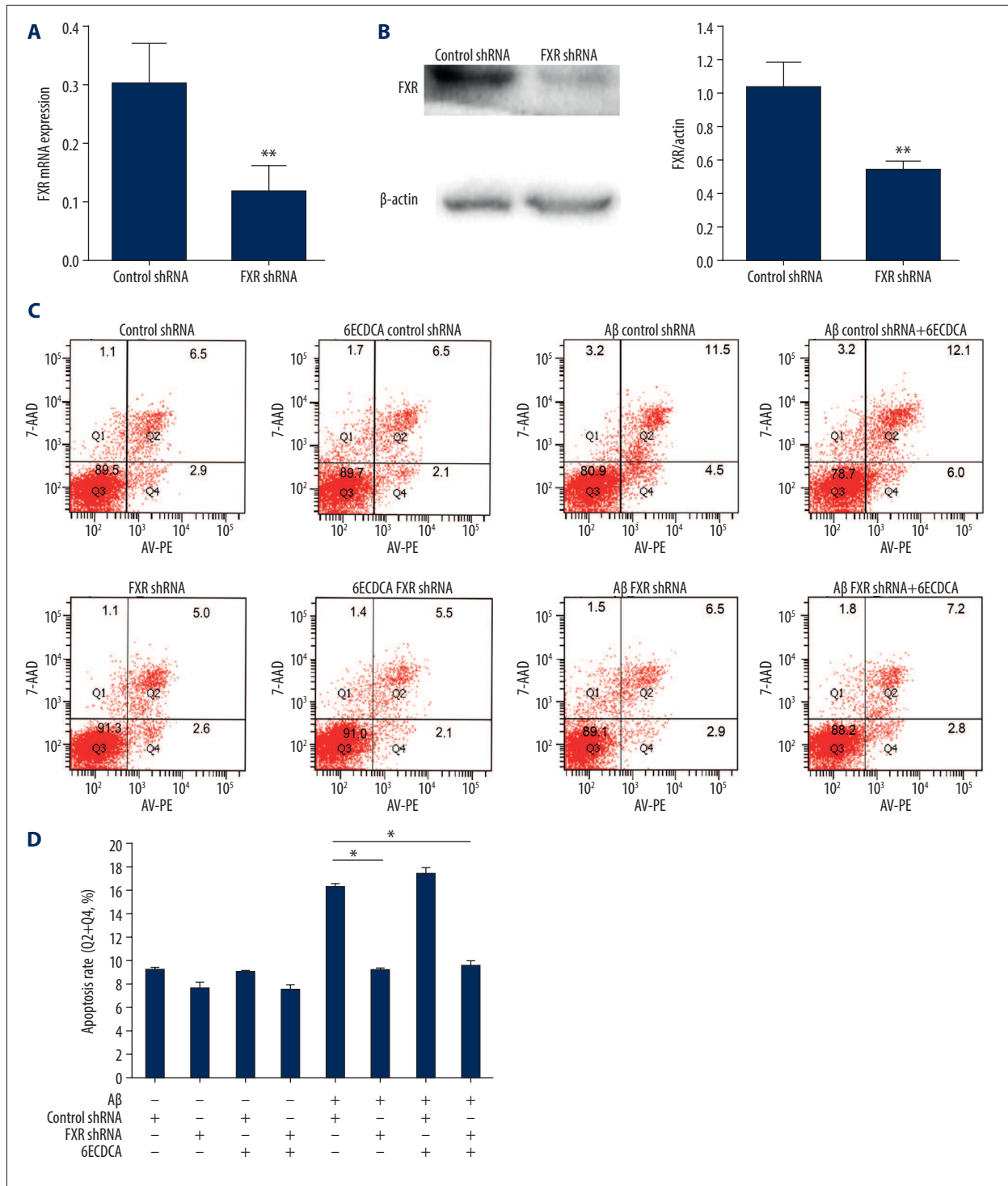


Figure 3. FXR overexpression aggravated A β -induced apoptosis in cells via CREB/BDNF signaling. **(A)** FXR-EGFP cells were harvested and the CREB and FXR interaction was measured by co-immunoprecipitation with the antibodies indicated. **(B)** FXR-EGFP cells were harvested, pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1-42 (1 μ M) for another 24 h. After incubation, cells were harvested and the CREB and FXR interaction was measured by co-immunoprecipitation with the antibodies indicated. **(C)** FXR-EGFP cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1-42 (1 μ M) for another 24 h. After incubation, cells were harvested and protein levels of CREB and BDNF in crude lysates were measured by immunoblotting (left panel). **(D)** FXR-EGFP cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1-42 (1 μ M) for another 24 h. One hour before harvest, cells were treated with or without forskolin (10 μ M) and the protein levels of CREB, BDNF, and Bcl2 in crude lysates were measured by immunoblotting (left panel). Quantification of protein bands is shown in the right panel. Protein expression was normalized to β -actin. Experiments were performed using 3 different batches of cells. * $p < 0.05$, ** $p < 0.01$ compared with the A β -treated control-EGFP cells, and data are shown as mean \pm SEM.

FXR overexpression significantly decreased BDNF expression following A β 1-42 treatment and was further decreased in the A β 1-42+6ECDCA group.

CREB activation induced BDNF expression and downstream anti-apoptotic protein Bcl2 expression [19]. In the present study,

forskolin, a CREB signaling activator, was used to stimulate FXR-transfected SH-SY5Y cells. Although CREB protein levels did not change significantly, the protein expression levels of both BDNF and Bcl2 were markedly increased after treatment with forskolin, and the inhibitory effects of FXR on CREB as well as BDNF and Bcl2 protein were also partially reversed (Figure 3D).



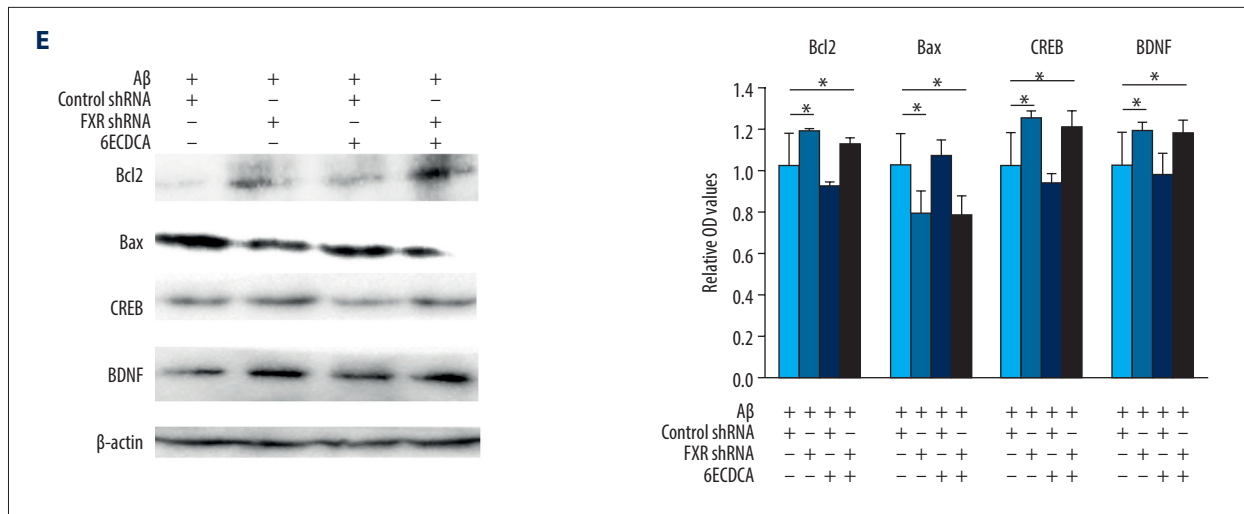


Figure 4. FXR knockdown inhibits A β -triggered cell apoptosis. **(A)** LV-shRNA-FXR was transfected into SH-SY5Y cells to achieve knockdown of FXR, and the level of FXR mRNA was assessed by real-time PCR. FXR mRNA expression was normalized to GAPDH and quantified. ** $p < 0.01$, compared with the control shRNA group. **(B)** The level of FXR protein was assessed by immunoblotting (left panel). Quantification of protein bands is shown in the right panel. Protein expression was normalized to β -actin. ** $p < 0.01$, compared with the control shRNA group. **(C)** LV-shRNA-FXR-transfected SH-SY5Y cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with or without A β 1-42 (1 μ M) for another 24 h. After incubation, cells were harvested and analyzed by flow cytometry. **(D)** Annexin V-PE-stained EGFP+ cells were regarded as apoptotic cells. * $p < 0.05$ compared with the A β -treated control shRNA cells, and data are shown as mean \pm SEM. **(E)** LV-shRNA-FXR-transfected cells were pretreated with or without 6ECDCA (2 μ M) for 24 h and then stimulated with A β 1-42 (1 μ M) for another 24 h. After incubation, cells were harvested and the protein levels of Bcl-2, Bax, CREB, and BDNF were measured by immunoblotting (left panel). Quantification of protein bands is shown in the right panel. Protein expression was normalized to β -actin. Experiments were performed using 3 different batches of cells. * $p < 0.05$ compared with the A β -treated control shRNA cells, data are shown as mean \pm SEM.

These findings indicated that FXR overexpression promotes A β -induced apoptosis via the CREB-BDNF pathway.

FXR knockdown abolishes A β -triggered cell apoptosis

To determine whether the FXR/CREB pathway played a role in A β -induced cell apoptosis, FXR-shRNA lentivirus was transfected into SH-SY5Y cells. Two days after transfection, decreased FXR mRNA and protein levels were observed in SH-SY5Y cells (Figure 4A, 4B). Cells with low expression of FXR were then subjected to A β 1-42 and A β 1-42 plus 6ECDCA treatment and analyzed by flow cytometry (Figure 4D). Compared to control shRNA-transfected cells, FXR knockdown induced a slight decrease of apoptosis in SH-SY5Y cells in the absence of A β 1-42 (Figure 4D). Consistent with the results in Figure 1F, 6ECDCA did not significantly enhance A β -induced apoptosis in scrambled shRNA-transfected cells (Figure 4D). Figure 4D also shows that, compared to the A β -treated scrambled shRNA-transfected cells, FXR knockdown induced a decrease in apoptosis in cells treated with A β and/or 6ECDCA, revealing a stable anti-apoptotic effect.

Figure 4E indicates that, compared to the A β -treated scrambled shRNA-transfected cells, FXR knockdown induced Bcl2

upregulation and Bax downregulation in cells induced with A β and/or 6ECDCA. Low expression of FXR fully reversed the A β -triggered decrease in CREB and BDNF expression in cells treated with A β and/or 6ECDCA (Figure 4E). Collectively, our results show that FXR knockdown induced anti-apoptotic effects in A β -induced SH-SY5Y cells.

Discussion

FXR has been reported to play important roles in the metabolism of bile acids, lipids, and glucose in the pathogenesis of atherosclerosis and in protection of the liver [20]. However, the function of FXR in neural system diseases is not well understood, although its expression in neurons has been demonstrated [12,21,22]. The present study proved that oligomeric A β 1-42 enhanced FXR expression in differentiated SH-SY5Y cells and in mouse primary hippocampal neuronal cells. It was also proved that FXR was involved in A β 1-42-triggered cell apoptosis by interacting with the CREB/BDNF pathway in SH-SY5Y cells, while low expression of FXR inhibited apoptosis in SH-SY5Y cells treated with A β 1-42. This novel mechanism of the FXR/CREB/BDNF axis indicates a potential therapeutic target for AD treatment.

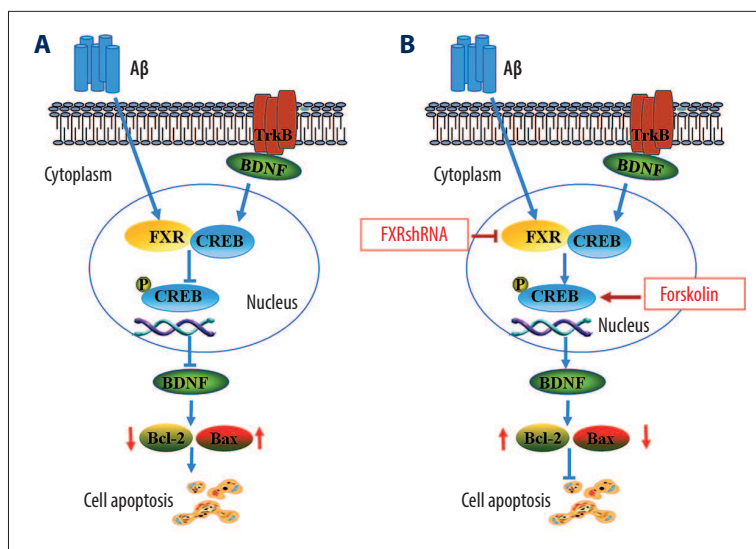


Figure 5. (A, B) Scheme of FXR-mediated A β -triggered neuronal cell apoptosis. Oligomeric A β upregulated FXR, promoted the interaction of FXR and CREB, suppressed CREB activity and subsequent BDNF expression, disturbed mitochondrial function, and then caused cell apoptosis. FXR-shRNA inhibited the interaction of FXR and CREB, and reversed A β -induced cell apoptosis by enhancing CREB and BDNF production and restoring mitochondrial function. The activator of CREB, forskolin, also impeded A β -induced cell apoptosis and mitochondrial dysfunction by enhancing CREB activity and subsequent BDNF production.

In addition to serving as an A β 1–42-treated AD cell model, SH-SY5Y neuroblastoma cells have also been used as an excitotoxic *in vitro* model following exposure to high concentrations of exogenous glutamate. As these cells express both ionotropic and metabotropic receptors, they may be useful in determining excitatory amino acid-induced processes due to the expression of both ionotropic and metabotropic receptors in this cell line [23]. SH-SY5Y cells also serve as a model of serotonergic neuronal cells, as they express monoamine oxidase A [24].

Amyloid plaques, neurofibrillary tangles, and synaptic and neuron loss are the 3 main neuropathological characteristics of AD. AD is believed to be initiated by neural accumulation of A β , which has been reported to activate the apoptosis pathway [5,25]. In addition, elevated levels of A β can inhibit the expression of neurotrophic factors, such as BDNF, which is a synaptic-plasticity regulator involved in neuronal apoptosis, neuron regeneration, and memory and cognitive functions in the pathological cascade of AD [26,27]. As a positive regulator of Bcl-2, CREB plays important roles in A β -induced neuronal apoptosis [19,28]. Therefore, the novel molecules that mediate downregulation of CREB caused by A β could be targets for drug development in the treatment of AD. It is known that Bim and Bax play important roles in matrix metalloproteinase, caspase-3 and Bax activation, and the intrinsic apoptotic pathway. Forkhead box O factors are closely related to the PI3K/Akt pathway [29]. The possible involvement of these apoptotic factors in FXR-mediated A β -induced apoptosis should be considered. FXR was shown to be expressed in the brain, and may regulate the brain lipid levels in neurological diseases [12]. In a previous study, FXR was elevated in rats with chronic unpredictable mild stress-induced depression [21]. In addition, z-guggulsterone, an FXR antagonist, inhibited the inflammatory responses mediated by microglia in the brain [30]. In the present study, we proved that FXR was induced by A β 1–42,

while endogenous FXR expression was low in SH-SY5Y cells, and activated FXR following 6ECDCA treatment did not affect A β -induced cell apoptosis. FXR overexpression has a slight effect on apoptosis in SH-SY5Y cells in the absence of A β 1–42 treatment, while FXR overexpression promoted neuronal apoptosis triggered by A β 1–42 treatment, and 6ECDCA treatment further aggravated A β -triggered apoptosis in cells with FXR overexpression. It is speculated that when overexpressed, FXR potentiates A β -triggered apoptosis, and 6ECDCA stimulation further enhances A β -induced apoptosis in FXR-overexpressed cells. Knockdown of FXR inhibited both basal and A β 1–42-triggered neuronal apoptosis.

Various mechanisms for FXR-mediated regulation of the CREB-BDNF pathway have been reported previously. FXR may reduce the lipid levels in the brain, as it was shown that hyperlipidemic and hypercholesterolemic status decreased BDNF expression [31,32]. Z-guggulsterone attenuated symptoms in a scopolamine-lesioned AD mouse model by augmentation of the CREB-BDNF signal, while the direct interaction of FXR and CREB was not mentioned in this report [22]. It has been reported that oligomeric A β can reduce CREB expression and phosphorylation, and subsequent BDNF expression [7]. In the present study, we found that FXR directly interacted with CREB and suppressed its expression and transcriptional activity, leading to reduced BDNF expression. The A β -stimulated CREB-BDNF pathway and 6ECDCA-activated FXR pathway might crosstalk with each other. As shown in Figure 5, we speculate that oligomeric A β suppresses CREB activity and subsequent BDNF expression, partially through upregulating FXR in A β -induced cell apoptosis. However, FXR overexpression-induced increase in neuronal apoptosis in the A β group might not mean a possible treatment option for AD, as different neuronal proteins are involved in neuropathological changes of AD (amyloid plaques, neurofibrillary tangles, and synaptic and neuronal loss), and

the pathogenic relationships among them are unclear. As hypothesized based on the amyloid cascade, A β deposition may be the initial event in AD, and may initiate the process of neuronal apoptosis, while the Braak staging of neurofibrillary degeneration correlates better with the severity of dementia compared to the density of neuritic plaques. Moreover, neurofibrillary tangles also represent a mechanism of neuronal death independent of apoptosis.

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Conclusions

Collectively, from these results, we speculate that FXR possess an A β -induced cell apoptosis-enhancing property *in vitro* by attenuating CREB and BDNF expression.

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Conflict of interest

None.