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Research article

IGF-1 protects against $A\beta_{25-35}$-induced neuronal cell death via inhibition of PUMA expression and Bax activation

Xunyao Hou[a,b,c], Yan Jin[d], Jian Chen[a,b,c], Yan Hong[a,b,c], Dingzhen Luo[a,b,c], Qingqing Yin[a,b,c], Xueping Liu[a,b,c,*]

[a] Department of Senile Neurology, Provincial Hospital Affiliated to Shandong University, 324#, Jing Wu Road, Jinan, 250021, China
[b] Department of Anti-Aging, Provincial Hospital Affiliated to Shandong University, 324#, Jing Wu Road, Jinan, 250021, China
[c] Anti-Aging Monitoring Laboratory, Provincial Hospital Affiliated to Shandong University, 324#, Jing Wu Road, Jinan, 250021, China
[d] Department of Hematology, Provincial Hospital Affiliated to Shandong University, 324#, Jing Wu Road, Jinan, 250021, China

HIGHLIGHTS

- IGF-1 prevents $A\beta_{25-35}$-induced cell death through the activation of PI3K/Akt pathway.
- IGF-1 mediated neuroprotection against $A\beta_{25-35}$ toxicity via inhibition of PUMA expression.
- IGF-1 inhibits $A\beta_{25-35}$-induced PUMA expression via the PI3K/Akt/FOXO3a Pathway.
- IGF-1 mediated neuroprotection against $A\beta_{25-35}$ toxicity via inhibition of Bax activation.

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ABSTRACT

Amyloid-β-peptide (Aβ) is considered to be the toxic species in AD and causes cell death in the affected areas of patient's brain. Insulin-like growth factor 1 (IGF-1) has been reported to attenuate Aβ toxicity in neuronal cells. However, the molecular mechanisms involved in the neuroprotective function of IGF-1 remain largely unknown. In the present study, we for the first time demonstrated that IGF-1 protects against Aβ-induced neurotoxicity via inhibition of PUMA expression and Bax activation. We found that IGF-1 could activate Akt, which in turn inhibited Aβ-induced FOXO3a nuclear translocation and thus decreased the binding ability of FOXO3a to PUMA promoter, leading to decreased PUMA expression. In addition, IGF-1 inhibited the translocation of Bax to the mitochondria induced by Aβ. Notably, addition of wortmannin, a specific inhibitor of PI3K, significantly abolished the neuroprotective effect of IGF-1, suggesting that IGF-1 exerts its anti-apoptotic effect depend on PI3K activity. Our findings may provide new insights into molecular mechanisms mediated by IGF-1 in cell survival against Aβ-induced apoptosis.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by senile plaques, neurofibrillary tangles and neuronal loss which affecting elderly people worldwide [1]. Deposition of amyloid-β-peptide (Aβ) in the brain is a key factor contributing to the pathogenesis of AD [2]. Although there are symptomatic treatments for AD patients, currently there is no effective therapy to prevent this disease.

Insulin-like growth factor-1 (IGF-1), a trophic hormone, is structurally similar to insulin. IGF-1 is shown to have neuroprotective effect in many cell types [3–5]. Accumulating evidences have focused on the central role of the IGF-1 signaling pathway for lifespan regulation in *Caenorhabditis elegans*, *Drosophila*, *yeast*, and mammals [6–8]. IGF-1 decline has been related to age-dependent cognitive impairment and dementia [9]. Consistent with this finding, the pathogenesis of AD is attributed to IGF-1 deficiency [10]. It has also been demonstrated that IGF-1 prevents neuronal cell death in neurodegenerative diseases including Parkinson's disease (PD) [4,11], Huntington's disease (HD) [3], and AD [12]. Although the involvement of IGF-1 signaling pathway in cell survival has been identified in several models, its downstream targets are frequently cell type-specific. Hence, studies on its neuroprotective mechanism

* Corresponding author at: Department of Senile Neurology, Department of Anti-Aging, Anti-Aging Monitoring Laboratory, Provincial Hospital Affiliated to Shandong University, 324#, Jing Wu Road, Jinan, 250021, China.
E-mail address: xuepingliu2002@sina.com (X. Liu).

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against Aβ toxicity could possibly provide a potential therapeutic strategy for AD.

The ability of IGF-1 to promote cell survival has been attributed in part to the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signaling pathway [3,13,14]. Akt is a key mediator of survival signals in response to a range of extra- and intracellular stimuli. Once activated, Akt exerts anti-apoptotic effects by regulating the expression of targeted genes involved in cell death and survival. FOXO3a, a member of the O subfamily of the forkhead transcription factors, is a critical downstream molecule of the PI3K/Akt pathway [15]. In response to IGF-1, FOXO3a is inactivated by PI3K/Akt and excluded from the nucleus to cytosol, thereby preventing its binding ability to the promoters of the target genes.

Growing evidences indicate that the BH3-only proteins of the Bcl-2 family are involved in various cell death paradigms, including neurodegeneration [16–18]. In accordant with this notion, recent studies showed that p53 up-regulated modulator of apoptosis (PUMA) plays an essential role in Aβ-induced apoptosis, *in vitro* and *in vivo* [19,20]. Morevover, FOXO3a serves as a transcription factor, leads to the activation of proapoptotic signaling via transactivation of PUMA in Aβ-induced apoptosis [20]. So far, little is known about the relationship between IGF-1 and PUMA expression in Aβ-induced apoptosis. In the present study, we aimed to determine the downstream signaling pathways of IGF-1 that antagonizes Aβ-induced apoptosis in SH-SY5Y cells. We uncovered the possible mechanism and found that the activation of the PI3K/Akt and subsequent reduction of PUMA expression and Bax translocation are important for the survival of SH-SY5Y cells against Aβ neurotoxicity.

2. Material and methods

2.1. Materials

IGF-1 and $A\beta_{25\text{-}35}$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). $A\beta_{25\text{-}35}$ peptides were dissolved in ultrapure deionized distilled water at 1 mM as stock solutions. Before treatment, peptides were preincubated at 37 °C for 5 days to promote aggregation and then diluted with medium to desired concentrations (25 μM) [21]. $A\beta_{1\text{-}42}$ was purchased from Merck Millipore and the peptides were prepared as previously described [20]. Wortmannin was purchased from BIOMOL Research Laboratories, Inc. (Plymouth, PA). Phospho-Akt (Ser473), phospho-Akt (308), Akt, Bax, and phospho-FOXO3a (Ser253) antibodies were purchased from Cell Signaling Technology (Danvers, MA); Anti-PUMA was purchased from Epitomics (Burlingame, CA); Anti-β-actin was purchased from Santa Cruz (La Jolla, CA). DsRed-mito plasmid was obtained from Invitrogen (Carlsbad, CA); GFP-FOXO3a plasmid was a gift from Prof. Wolfgang Link [22].

2.2. Cell culture and transfection

The human neuroblastoma cell line SH-SY5Y was cultured in DMEM (GBICO, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin in 5% CO_2, 95% air at 37 °C in a humidified incubator. The cells were transfected at 60–70% confluence with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) for the overexpression experiments according to the manufacturer's instructions.

2.3. Cell viability assay

SH-SY5Y cells were cultured at a density of 5×10^3 cells/well in 96-well microplates. Cell viability was assessed with CCK-8 (Dojindo Laboratories, Japan) after Aβ oligomers and/or IGF-1 treatment according to the manufacturer's instructions. OD450, the absorbance value at 450 nm, was read with a microplate reader to determine the viability and proliferation of the cells.

2.4. Cell apoptosis assay

Cell apoptosis by Annexin-V/PI staining was performed as described previously [23]. After indicated treatments, apoptotic cell death was determined using the Annexin V, FITC Apoptosis Detection Kit (Dojindo Laboratories, Japan) according to the manufacturer's protocol. Flow cytometry was performed on a BD FACSCanto II flow cytometer (Becton Dickinson).

2.5. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis

RT-PCR was performed as described previously [24]. Puma primer sequence: sense: TTGTGCTGGTGCCCGTTCCA; antisense: AGGCTAGTGGTCACGTTTGGCT. β-actin primer sequence: sense: TCATGTTTGAGACCTTCAA; antisense: GTCTTTGCGGATGTCCACG.

2.6. Western blotting analysis

After indicated treatments, the cells were lysed in RIPA lysis buffer (50 mM Tris, 150 mMNaCl, 1 mM EDTA, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail set I. Equal amounts of protein were loaded on SDS-PAGE, transferred to the PVDF membrane (Millipore). Blocked with 5% nonfat milk in TBST (10 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.4) for 1 h, and the membrane were incubated with indicated primary antibodies, followed by secondary antibodies (Cell Signaling Technology). Detection was performed using the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA).

2.7. Immunofluorescence

Cells were fixed in 3.7%–4% paraformaldehyde and then permeated with 0.1% Triton X-100. Samples were incubated in blocking buffer and then followed by incubation with the indicated primary antibodies. Finally, FITC-conjugated secondary antibodies were added for 2 h at a room temperature. Mitochondria were stained with MitoTracker Red. Slides were mounted and analyzed by confocal microscopy (Carl Zeiss).

2.8. GFP-FOXO3a translocation assay

To monitor nuclear translocation of FOXO3a, SH-SY5Y cells were transiently transfected with pGFP-FOXO3a before treatments. Subcellular GFP localization was assessed by using a 488 nm excitation light from an argon laser and a 500-550 band-pass filter (Carl Zeiss MicroImaging).

2.9. Chromatin immunoprecipitation analysis

CHIP was performed as previously described [20]. Brifely, the DNA was extracted, and the region of FOXO3a binding to the PUMA promoter was amplified using the following primers: sense, GCGCACAGGTGCCTCGGC and antisense, TGGGTGTGGCCGCCCCT.

2.10. Statistical analysis

All data represent at least three independent experiments and are expressed as the mean ± SEM. An unpaired, two-tailed Student's *t* test was employed to determine significant differences