Neuroscience Letters 661 (2017) 63–70

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

The cytoplasmic nuclear shuttling of Beclin 1 in neurons with Alzheimer's disease-like injury

Li Wang[a], Xiao-Bin Xu[a], Wen-Wen You[a], Xiao-Xia Lin[a], Cheng-Tan Li[a], Hao-Ran Qian[b], Li-Hui Zhang[a,*], Yi Yang[a,*]

[a] Department of Pharmacology, Hangzhou Key Laboratory of Medical Neurobiology, School of Medicine, Hangzhou Normal University, Hangzhou 310036, China
[b] Sir Run Run Shaw Hospital, Medical College of Zhejiang University, Hangzhou 310016, China

ARTICLE INFO

Keywords:
Alzheimer's disease
Beclin 1
Cortical neuron
PC12 cells
Nuclei

ABSTRACT

The abnormal expression of the autophagy-related protein Beclin 1 has been implicated in Alzheimer's disease (AD) brains, whereas the precise involvement of Caspase-mediated Beclin 1 cleavage in AD neurons has not yet been fully clarified. In this study, we investigated the distribution of Beclin 1 fragments in neurons with AD-like injury. Our results demonstrated that Beclin 1 was expressed in neurons but not in astrocytes in both neuron-glia co-cultures and in cortical tissue slices. The full length and C-terminal fragments of human Beclin 1 was mainly expressed in cytoplasm, while the N-terminal fragment of Beclin 1 was predominantly localized in nucleus. Compared to amyloid-β $(Aβ)_{42\text{-}1}$ treatment control, exposure of PC12 cells or cortical neurons to $Aβ_{1\text{-}42}$ resulted in cell injury, with the appearance of neuritic shortening, reduced nuclear diameter in PC12 cells, beading formation and fragmentation in cortical neurons. A partial nuclear translocation of Beclin 1 was detected in cells incubated with $Aβ_{1\text{-}42}$, which could be inhibited by the administration of pan-Caspase inhibitor or Caspase 3 specific inhibitor. Moreover, Beclin 1 mutation at 146/149 sites was resistant to $Aβ_{1\text{-}42}$-induced nuclear translocation. The nuclear translocation of Beclin 1 could also been detected in the brains of 12-month-old APP^{Swe}/$PS1^{dE9}$ transgenic mice. Our findings suggest that after Caspase 3-mediated Beclin 1 cleavage at 146/149 sites, the N-terminal fragments of Beclin 1 may partially translocate into nuclei in neurons subjected to AD-like injury.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive decline of recognition and memory. The principle neuropathologic features of this disease include the deposition of amyloid-β (Aβ) peptide and neurofibrillary tangles in brain [1], which result in the neuritic atrophy and neuronal death. Recent evidence suggests that the failure of autophagy, a major intracellular degradative pathway, contributes to the accumulation of endogenous proteins and may lead to the formation of aggregates and inclusions in AD brain [2]. Therefore, targeting the key regulators of autophagic machinery and therefore interference of autophagic system might represent a promising therapeutic intervention for AD.

Beclin 1, also known as Atg6, regulates the autophagosome formation and plays a crucial role in mediating autophagy initiation [3]. Decreased expression of the full length Beclin 1 has been noted in the human AD brain [4]. Beclin 1 has been identified as a novel substrate of Caspase family. Until recently, several Caspase cleavage sites have been confirmed in Beclin 1, including $DLFD^{124}$ [5], $TDVD^{133}$ [6–9], and $D^{146}QLD^{149}$ [5–10]. Caspase cleavage may leads to the depletion of Beclin 1 in the AD brain [6], resulting in the insufficient autophagic clearance in neuron and ultimately induce neuronal cell injury. Nevertheless, the precise involvement of Caspase-mediated Beclin 1 cleavage in neurons with AD-like injury has not yet been clearly demonstrated.

In this present study, we compared the distribution of full length Beclin 1 and its fragments in healthy neurons and neurons underwent AD-like injury in *in vitro* cultured cells as well as model mice. Our findings suggest that the cytoplasmic nuclear shuttling of Beclin 1 is involved in AD-like neuronal injury.

2. Materials and methods

2.1. Reagents

High-glucose Dulbecco's modified eagle medium (DMEM; BI-SH0515) and fetal calf serum (FCS) were purchased from Biological Industries, Israel, and Hangzhou Tianhang Biological Technology Co.,

* Corresponding authors.
E-mail addresses: lhzhang@hznu.edu.cn (L.-H. Zhang), yyang@hznu.edu.cn (Y. Yang).

http://dx.doi.org/10.1016/j.neulet.2017.09.055
Received 17 May 2017; Received in revised form 8 September 2017; Accepted 26 September 2017
Available online 28 September 2017
0304-3940/

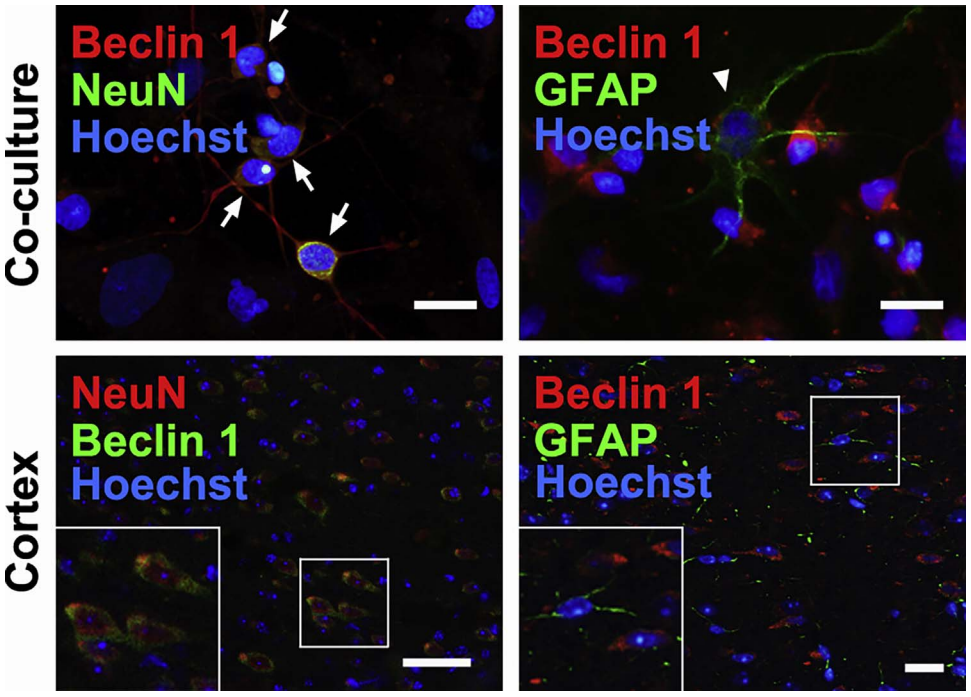

Fig. 1. Cell specificity of Beclin 1 expression. Cortical neurons and glia co-cultures (upper panels) and cortex slices (lower panels) were immunostained with anti-Beclin 1 and NeuN (neuronal biomarker) or GFAP (astrocyte biomarker). Neurons and astrocyte in cultured cells were indicated by arrows and arrowhead, respectively. Areas in white boxes were enlarged. Scale bars, 20 μm.

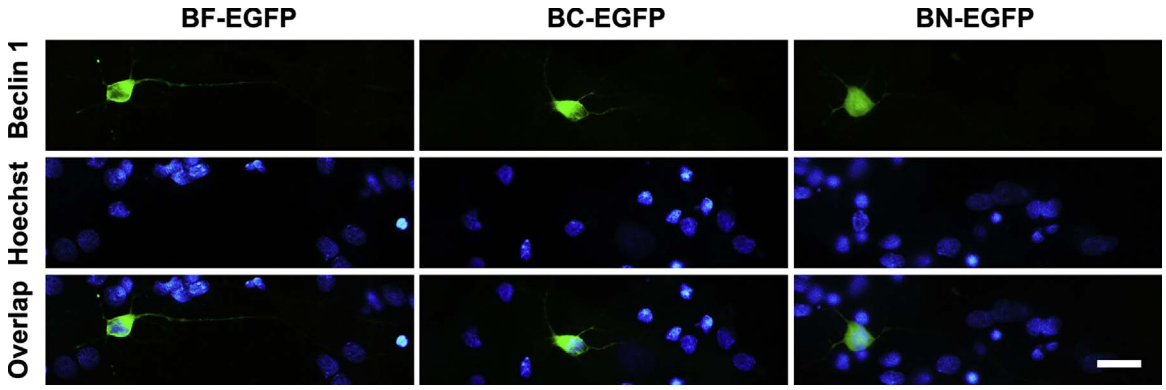

Fig. 2. Intracellular localization of Beclin 1 fragments. EGFP plasmids carrying Beclin 1 full length (BF-EGFP), Beclin 1 C-terminal (BC-EGFP) or N-terminal (BN-EGFP) fragments were transfected into cortical neurons. Twenty-four hours after transfections cells were fixed and nuclei were labeled with Hoechst 33342. The fluorescence was examined under fluorescent microscope. Scale bar, 20 μm.

Ltd., Hangzhou, China, respectively. Opti-MEM (31985), Neurobasal medium (21103), B-27 serum-free supplement (17504-044) and MitoTracker® Green (M7514) were purchased from Thermo Fisher Scientific, Grand Island, New York, USA. Human $A\beta_{1\text{-}42}$ (20276) and $A\beta_{42\text{-}1}$ (27276) were obtained from AnaSpec, Fremont, CA, USA. Poly-D-lysine (P7280) and cytosine β-D-arabinofuranoside (AraC; C1768) was bought from Sigma-Aldrich (Shanghai) Trading Co., Ltd, Shanghai, China. Rabbit polyclonal Beclin 1 antibodies against N-terminal (3613) and C-terminal (3611) Beclin 1 were bought from ProSci Incorporated, Poway, CA, USA. Mouse anti-NeuN antibody (ab104224) and rabbit anti-Aβ antibody (8243) were purchased from Abcam, Cambridge, UK and Cell Signaling Technology, Inc., Boston, MA, US, respectively. Mouse anti-glial fibrillary acidic protein (GFAP) antibody (MA5-15086) was obtained from ThermoFisher Scientific. Pan-caspase inhibitor Z-VAD-FMK (219007) and Caspase 3 inhibitor Ac-DEVD-CHO (235420) were bought from EMD Biosciences, Inc., San Diego, CA, US.

2.2. Ethics statement

Procedures of animal studies were approved by Animal Ethical Committee of Hangzhou Normal University. All efforts were made to minimize the animal suffering.

2.3. Animals

Male and female Sprague Dawley (SD) rats, weighting 200–230 g, were obtained from the Experimental Animal Center of Hangzhou Normal University, Hangzhou, China. New-born rat puppies were used for preparing primary neurons. Twelve-month-old $APP^{Swe}/PS1^{dE9}$ transgenic mice and age-matched wild type (WT) control mice were purchased from Shanghai Biomodel Organism Science & Technology Development Co., Ltd., Shanghai, China.

2.4. Cell culture and co-culture

Highly differentiated PC12 cells (Kunming Cell Bank of the Chinese Academy of Sciences (Kunming, China) were cultured in high-glucose DMEM containing 10% fetal calf serum (FCS). Cells were maintained at 37 °C in a carbon dioxide incubator at humidified atmosphere. For immunocytochemistry and transfection experiments, cells were seeded onto coverglass bottom dishes.

Primary cortical neurons were prepared as previously described