Neurobiology of Aging xxx (2015) 1–14

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Thrombospondin-1 prevents amyloid beta–mediated synaptic pathology in Alzheimer's disease

Sung Min Son [a], Dong Woo Nam [a], Moon-Yong Cha [a], Kyung Ho Kim [a], Jayoung Byun [a], Hoon Ryu [b,c], Inhee Mook-Jung [a,*]

[a] Department of Biochemistry and Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea
[b] Center for Neuromedicine, Brain Science Institute, KIST, Seoul, Korea
[c] Department of Neurology, Boston University, Boston, MA, USA

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 7 September 2015
Accepted 7 September 2015

Keywords:
TSP-1
Neuron-astrocyte crosstalk
Synaptic dysfunction
Aβ
Alzheimer's disease (AD)

ABSTRACT

Alzheimer's disease (AD) is characterized by impaired cognitive function and memory loss, which are often the result of synaptic pathology. Thrombospondin (TSP) is an astrocyte-secreted protein, well known for its function as a modulator of synaptogenesis and neurogenesis. Here, we investigated the effects of TSP-1 on AD pathogenesis. We found that the level of TSP-1 expression was decreased in AD brains. When we treated astrocytes with amyloid beta (Aβ), secreted TSP-1 was decreased in autophagy-dependent manner. In addition, treatment with Aβ induced synaptic pathology, such as decreased dendritic spine density and reduced synaptic activity. These effects were prevented by coincubation of TSP-1 with Aβ, which acts through the TSP-1 receptor alpha-2-delta-1 in neurons. Finally, intrasubicular injection with TSP-1 into AD model mouse brains mitigated the Aβ-mediated reduction of synaptic proteins and related signaling pathways. These results indicate that TSP-1 is a potential therapeutic target in AD pathogenesis.

1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease among the elderly and is characterized by senile plaques, neurofibrillary tangles, loss of neurons and synapses, astrogliosis, and inflammation (Selkoe, 2004; Walsh and Selkoe, 2004). Along with those clinical features, massive synaptic loss occurs in the early clinical stages of AD, and this loss is strongly correlated with the cognitive defects shown in AD patients (DeKosky and Scheff, 1990; Terry et al., 1991). A number of studies indicate that senile plaques are associated with neurodegeneration in AD, and amyloid beta (Aβ), a major extracellular component of senile plaques, is one of the key factors in the pathogenesis of AD (Yankner, 1996).

Astrocytes are the most abundant cell type in the central nervous system (CNS), and recent studies indicate these cells act as important regulators of neuronal development, metabolism, and synaptic transmission and plasticity (Haydon, 2001; Volterra and Meldolesi, 2005). Astrocytes are often found in close proximity to excitatory neuronal synapses (known as the "tripartite synapse") (Araque et al., 1999). Through this close contact with neurons, astrocytes can modulate synaptic functions by releasing neuromodulatory substances such as growth factors, cytokines, proteases, and so forth (Dowell et al., 2009; Keene et al., 2009). Emerging evidence has demonstrated that thrombospondins (TSPs) expressed by immature and reactive astrocytes are responsible for excitatory CNS synaptogenesis, or synapse formation (Chen et al., 2000; Christopherson et al., 2005).

TSPs, critical astrocyte-secreted proteins, are large oligomeric extracellular matrix proteins that have been previously shown to play important roles in cell migration, angiogenesis, and synaptogenesis (Chen et al., 2000; Christopherson et al., 2005). The TSP family consists of 2 subfamilies, A and B, according to their organization and domain structure: A includes the trimeric TSP-1 and TSP-2, whereas B includes the pentameric TSP-3, TSP-4, and TSP-5 (Lawler, 2002). Expression of all TSPs has been found in the brain, and studies using purified retinal ganglion cell cultures indicate that TSPs, especially TSP-1 and TSP-2, promote the formation of new excitatory (glutamatergic) synapses (Christopherson et al., 2005; Crawford et al., 2012). TSPs mediate these functions via interactions with their various neuronal receptors, including integrin, neuroligin, cluster of differentiation 47 (CD47) and/or integrin-associated protein, and alpha-2-delta-1 (α2δ1) (Eroglu et al., 2009; Graf et al., 2004;

* Corresponding author at: Department of Biochemistry and Biomedical Sciences, Seoul National University College of Medicine, 28 Yungun-dong, Jongro-gu, Seoul, 110-799, Korea. Tel.: +82 2 740 8245; fax: +82 2 3672 7352.
E-mail address: inhee@snu.ac.kr (I. Mook-Jung).

0197-4580/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2015.09.005

Ohnishi et al., 2005). Among TSP receptors, α2δ1 is a membrane-spanning protein subunit of the L-type calcium channel complex that is ubiquitous in many tissues and is highly expressed in brain and skeletal muscles (Arikkath and Campbell, 2003; Cole et al., 2005). Several studies indicate that α2δ1 has an important role in regulating calcium channel function (Taylor and Garrido, 2008); however, α2δ1 also has other functions in synaptogenesis independent of calcium channel function (Bauer et al., 2010; Eroglu et al., 2009).

Because astrocytes are important modulators of synaptic activity, dysregulation of astrocytic functions have been linked to diverse neurological disorders, including AD. Buee et al. (1992) showed that TSP-1 expression is significantly decreased in the brains of AD patients. However, the potential mechanism of decreased TSP-1 expression in AD brains has not been studied extensively. In this study, we present in vivo evidence that TSP-1 expression levels are significantly decreased in brains of AD patients and 2 AD mouse models (Tg2576 and Tg6799). Consistent with these in vivo findings, exogenous Aβ attenuates levels of TSP-1 secreted from primary astrocytes and U373MG human astrocytoma without affecting transcription levels. We also show that TSP-1 plays a protective role in Aβ-induced alteration of dendritic spines that appear to be relevant to AD pathogenesis. Cotreatment with TSP-1 prevents Aβ-induced dendritic spine loss and inhibition of functional synaptic activity via the neuronal TSP-1 receptor α2δ1. Finally, intrasubicular injection of TSP-1 into the brains of Tg6799 mice rescued the Aβ-induced reduction of synaptic proteins and related signaling pathways, indicating that TSP-1 is a possible therapeutic agent for treatment of AD.

2. Materials and methods

2.1. Animals and tissues

Twelve-month-old Tg2576 (expressing Aβ precursor protein [APP] Swedish mutation; n = 8) (Hsiao et al., 1996), Tg6799 (n = 5 each for 2, 6, and 9 months old) (Oakley et al., 2006), and non-transgenic littermate controls (B6SJL) were used for the experiments. The Tg6799 mice (formerly JAX stock no. 008730) overexpress both mutant human APP695 with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) familial AD (FAD) mutations, as well as human PS1 harboring 2 FAD mutations (M146L and L286V). All mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and were treated and maintained in Seoul National University's mouse facility. All experiments were approved by the Institute of Laboratory Animal Resources of Seoul National University. Postmortem human brain tissues were obtained from Dr. Hoon Ryu (Boston University, Boston, MA, USA), and their information is contained in Fig. 1A.

2.2. Stereotaxic injection

The mice were anesthetized with a Zoletil and Rompun mixture (1 mL/kg intraperitoneally) and placed in a stereotaxic frame (myNeuroLab, St. Louis, MO, USA). TSP-1 was dissolved in phosphate-buffered saline (PBS). Mice were injected (at rate 0.5 μL/min) with 3 μL of saline or TSP-1 (0.5 μM) in the subiculum (−4.16 mm AP, 3.25 mm ML, and −4.0 mm DV) unilaterally, according to the Bregma landmark adopted from the Paxinos and Watson atlas. Animal treatment and maintenance were approved by the Ethics Review Committee for Animal Experimentation in Seoul National University.

2.3. Cell cultures and drug treatment

Human astroglioma U373MG cells (ATCC number: HTB-17) were cultured in Dulbecco's Modified Eagle's medium containing 10% fetal bovine serum and 0.1 mg/mL penicillin and streptomycin (P/S; Sigma-Aldrich, St. Louis, MO, USA) at 37°C under humidified 5% CO_2 air. The U373MG cells (5×10^5 cells) were plated onto 6 well plates. Primary hippocampal neurons were prepared from E18 Sprague-Dawley rat embryos, as described previously with some modifications (Brewer et al., 1993). The neurobasal medium, to which B27 supplement (Invitrogen, Carlsbad, CA, USA), L-glutamine (0.5 mM), and 0.1 mg/mL P/S (Sigma-Aldrich) were added, was changed every 2 days, and experiments were conducted in cultures at 19–21 days in vitro (DIV). Primary astrocytes were prepared from newborn (P1) imprinting control region mice as described previously (Jung et al., 2012). The astrocytes underwent 2 passages for experiments. Cells were treated with 500 ng/mL of human recombinant TSP-1 (R&D systems, Minneapolis, MN, USA) solely, or cotreated with Aβ followed by 24-hour incubation. The small interfering RNAs and short hairpin RNAs were purchased from Bioneer Inc. or SantaCruz Biotechnology (CA, USA) and were transfected into cells using RNAimax or Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The drugs used in this study were as follows: insulin (100 nM), H_2O_2 (50 μM), 3-methyladenine (2 mM), bafilomycin (10 nM), chloroquine (100 μM), TNF-alpha protease inhibitor (100 μM), and rapamycin (100 nM) from Sigma-Aldrich; GM6001 (5 μM) from Calbiochem (San Diego, CA, USA).

2.4. Western blot analysis

Harvested cell pellets and brain lysates were prepared as described (Son et al., 2014). The antibodies for the western blot (WB) analysis were as follows: anti–N-methyl-D-aspartate receptor 2A and anti-NR1 (1:2000; Millipore, Schwalbach, Germany); anti-cAMP response element-binding protein (CREB) and anti-p-CREB (S133) (1:2000, Cell Signaling Technology, Beverly, MA, USA); anti–microtubule-associated protein 1A/1B-light chain 3 (anti-LC3B) (1:2000; MBL; Cell Signaling Technology); anti-α-actinin and anti-α2δ1 (1:1000; SantaCruz); anti-TSP-1, anti-TSP-2, anti-CD47, anti–postsynaptic density protein 95 (anti-PSD-95) and anti-beclin1 (1:2000; Abcam, Cambridge, MA, USA); anti-synaptophysin (anti-SNP), anti-β-actin, anti-α-tubulin, and anti–glyceraldehyde 3-phosphate dehydrogenase (1:4000; Sigma-Aldrich). Immunoreactivity was determined by chemiluminescence (GE Healthcare, Piscataway, NJ, USA). The chemiluminescence signal was detected with a digital image analyzer (LAS-3000; Fuji Film Inc., Tokyo, Japan).

2.5. Aβ preparation

Aβ was prepared as previously described (Cha et al., 2012; Son et al., 2012a). Most of the Aβ forms used were oligomers, but some monomers existed in the mixture. To prepare fibrillar Aβ, 10-mM HCl was added to bring the hexafluoroisopropanol-dissolved Aβ to a final concentration of 100 μM, and the peptide was incubated for 24 hours at 37°C and prepared as described previously (Dahlgren et al., 2002).

2.6. Measurement of TSP-1 release

Extracellular TSP-1 in the culture medium of U373MG cells was measured using trichloroacetic acid (TCA) or TSP-1 enzyme-linked immunosorbent assay (ELISA) kit (DTSP-10, R&D systems) according to the manufacturer's instructions. For analyzing protein in the medium, we performed TCA precipitation as previously described (Son et al., 2012b). Briefly, cell medium was centrifuged at 5000 rpm for 5 minutes to remove cell debris and subjected to TCA precipitation (up to 10%).