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Involvement of endoplasmic reticulum stress in tauopathy

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ABSTRACT

Tauopathy is a pathological condition with an abnormal intracellular accumulation of tau protein in neurons and glias, which is a feature of Alzheimer's disease (AD) as well as frontotemporal lobar degenerations (FTLD). Recent reports showed that tauopathy occupies an important position for pathological process of dementia generally. Previously, we reported that endoplasmic reticulum (ER) stress has an influence on the onset of AD. In addition, some reports on brain autopsy findings suggest that ER stress is associated with AD and tauopathy. However, the mechanism underlying the association between ER stress and tauopathy is still unknown. Here, we show that ER stress, induced by glucose deprivation or chemicals, increases total endogenous tau protein in cultured neurons and primary cultured neurons. Under ER stress, no significant differences were observed in the transcription of tau, and no differences were observed in the translation of tau with or without the 5′-untranslated region (5′UTR) of tau. In contrast, the degradation rate of tau was decreased by 20% under ER stress. ER stress reduced the binding between tau and carboxyl terminus of Hsc70-interacting protein (CHIP), ubiquitin E3 ligase for tau. These results suggest that ER stress increases total tau protein and its mechanism is due to the decrease in the binding between tau and CHIP, which delays the degradation of tau protein through the ubiquitin–proteasome pathway. This mechanism may provide clue to treatment for tauopathy.

1. Introduction

One of the hypotheses of the etiology of AD suggests that neurodegeneration starts from the accumulation of amyloid proteins that leads to the formation of neurofibrillary tangles containing tau protein [1]. This hypothesis, called the "amyloid cascade hypothesis," is currently widely accepted. On the basis of this hypothesis, new treatment strategies targeting amyloid β (Aβ), i.e., amyloid vaccines and γ-secretase inhibitors, have been developed and evaluated; however, anticipated results have yet to be obtained. At present, alternative hypotheses, other than the amyloid cascade hypothesis, are being sought and are gaining increasing interest as the basis for the development of new AD treatments.

In the cause of AD, accumulation of abnormally phosphorylated tau is as equally important as the accumulation of amyloid. In other words, tauopathy is one of the components comprising the etiology of AD. The discovery of frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) revealed that abnormal tau itself is directly linked to neurodegeneration, which has recently attracted considerable interest [2].

The importance of tau in the etiology of AD has been indicated in some studies. When expression of tau was inhibited by crossing amyloid precursor protein-overexpressing mice with tau knock-out mice, the dementia phenotype manifesting as abnormal space perception, abnormal behavior, etc., was not observed, even though the level of amyloid accumulation was unchanged [3]. It suggests that tau is an essential element in the progression of AD. Moreover, a recent new finding on tau accumulation in AD etiology suggested that the tau oligomer level was significantly increased in the cortex of early-stage AD patients [4]. The study of tau transgenic mice (Tg4510 strain) and wild-type mice overexpressing wild-type 4-repeat tau showed that caspase activation occurs before the tangle formation, suggesting that soluble tau may be more toxic than fibrillar tau [5]. These findings imply that soluble tau oligomers may have toxic effects and cause neurodegeneration before forming neurofibrillary tangles, indicating that an increase in tau protein itself may be an important factor in the early stage of AD. Another study shows that aggregated extracellular tau is taken up by cells and induces the intracellular accumulation of tau, and it transfers between co-cultured cells. It suggests that tau aggregates may propagate from the outside to the inside and spread tauopathy [6]. Therefore some researchers recently think tauopathy can be a new target in the development

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of AD treatments. Thus, research on the expression/degradation of tau is important.

Previously, we reported that mutant presenilin-1 inhibits endoplasmic reticulum (ER) stress responses and makes neurons more vulnerable to ER stress. Namely, ER stress is involved in neurodegenerative diseases, including AD [7–10]. Other researchers have reported that ER stress is activated in the hippocampus of AD patients [11]. Recently, activation of ER stress and increased levels of phosphorylated tau were observed in the hippocampus of patients with tauopathy, suggesting that ER stress may be related to tauopaty [12].

These studies suggest that ER stress is associated with AD and tauopathy. However, the pathological mechanism is still largely unknown. Thus, in the present study, the expression and degradation of total tau protein under ER stress were evaluated with the objective of identifying the mechanism of tauopathy.

2. Materials and methods

2.1. Plasmid construction

Human tau gene contained the published 320 nucleotides of the 5′UTR and 1239 nucleotides of the coding region (GenBank accession numbers NM_001123067). -5′UTR: The human tau coding region (1239 bps) was only inserted into pcDNA3.1D/V5-His-TOPO (Invitrogen, Carlsbad, CA, USA).+5′UTR: The human tau 5′UTR (320 bps) was inserted into pcDNA3.1D/V5-His-TOPO (Invitrogen), and the human tau cording region was inserted at the back of the human tau 5′UTR, using Takara BKL Kit (Takara, Shiga, Japan). These were performed according to the manufacturer's instructions.

2.2. Cell cultures and transfections

SH-SY5Y cells and HEK293 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)/ F12 (Gibco, Grand Island, NY, USA) and in DMEM (Gibco) respectively, supplemented with 10% fetal bovine serum (FBS; JRH Biosciences, Leneza, KS, USA) and 1% penicillin streptomycin (Invitrogen). For glucose-deprivation experiments, cells were incubated in DMEM containing D-glucose (4500 mg/l) (Gibco) or DMEM without glucose (Gibco) supplemented with 10% FBS (JRH Biosciences) and 1% penicillin streptomycin (Invitrogen). Tunicamycin (Sigma–aldrich, St. Louis, MO, USA) or dithiothreitol (DTT) (Sigma–aldrich) were added to the cell media to induce ER stress. MG132 (Calbiochem, San Diego, CA, USA) for proteasome inhibition was added to the cell media. Transient transfections in HEK293 cells were performed using Lipofectamin LTX (Invitrogen) according to the manufacturer's instructions.

2.3. Neuronal cultures

Primary cultured neurons were prepared from E18.5 mice cerebral cortex. The mice were wild type E18.5 mice (PKR-like endoplasmic reticulum kinase (PERK) +/+) and PERK knockout E18.5 mice (PERK −/−). The cells were cultured for 7 days in DMEM (Gibco) supplemented with 10% FBS (JRH Biosciences) and then used for tunicamycin treatment.

2.4. Cell lysis and immunoblotting

Cells were lysed in 1× RIPA buffer (Pierce, Rockford, IL, USA) with protease inhibitor cocktail (Roche, Mannheim, Germany) and phosphatase inhibitor cocktail (Roche). Cleared cell extracts were obtained by centrifugation for 10 min at 31,000×*g* at 4 °C. Protein levels were determined by BCA method (Pierce). Equal amounts of protein were boiled in SDS sample buffer and separated using SDS-polyacrylamide gels (BIO CRAFT, Tokyo, Japan), then transferred to polyvinylidene fluoride (PVDF) transfer membrane (Immobilon-P; Millipore, Billerica, MA, USA). After bloking with 5% ECL Blocking Agent (GE Healthcare, Buckinghamshire, UK), membranes were incubated overnight with primary antibody at 4 °C. After incubation with horseradish peroxidase-conjugated second antibody (Promega, Madison, WI, USA), membranes were developed with enhanced chemiluminescence (ECL; GE Healthcare). Primary antibodies used included anti-Tau-5 (Calbiochem), anti- phosphorylation of eukaryotic translation initiation factor 2 subunit α (eIF2α-P) (Cell Signaling, Danvers, MA, USA), anti-KDEL (for immunoglobulin heavy-chain binding protein (BiP)) (Stressgen, Victoria, BC, Canada), anti-TAR DNA-binding protein 43 kDa (TDP-43) (Proteintech, Chicago, IL, USA), anti-Ubiquitin (Zymed, South San Francisco, CA, USA) and anti-GAPDH (Thermo, Fremont, CA, USA).

2.5. Immnoprecipitation

HEK293 cells were transiently transfected with the plasmid containig the human tau coding region (−5′UTR). After overnight recovery, cells were incubated for 24 h in DMEM with glucose (4500 mg/l) or DMEM without glucose. Cleared cell extracts were immunoprecipitated with Protein G Sepharose (Sigma–aldrich) and 2.5 μg Anti-Tau-5 (Calbiochem). Immunoprecipitates were boiled in 2× SDS sample buffer and separated using SDS-polyacrylamide gels (BIO CRAFT). Primary antibody used for immunoblotting included anti-Tau (DakoCytomation, Denmark), anti-CHIP (Santa Cruz, Santa Cruz, CA, USA).

2.6. Pulse-chase

Before pulse-labeling, SH-SY5Y cells were incubated in methionine/cystine/glutamine-deficient DMEM (Gibco) added with cystine (Sigma–aldrich) and glutamine (Invitrogen) for 40 min. Cells were pulse-labeled with 4 ml of the same media supplemented with 0.28 mCi ^{35}S-methionine/cystine (Perkin Elmer Japan, Kanagawa, Japan) for 20 min. Cells were washed once and chased with either DMEM with glucose (4500 mg/l) (Gibco) or DMEM without glucose (Gibco). At 24 h after labeling, cells were lysed in 1× RIPA with protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche) and immunoprecipitated with Protein G Sepharose (Sigma–aldrich) and 2 μg anti-Tau-5 (Calbiochem). Immunoprecipitates were separated using 4–20% SDS-polyacrylamide gels (Invitrogen). Gels were fixed, dried and exposed to films (Kodak, Rochester, NY, USA).

2.7. RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

SH-SY5Y cells were cultured in DMED/F12 (Gibco) with 1 μg/ml tunicamycin for indicated time. Total RNAs were extracted from cells by Trizol (Invitrogen) according to the manufacture's recommendations. For RT-PCR, 2 μg of total RNA from each sample was used for first-strand cDNA synthesis using Prime Script II (Takara). PCR was performed with the cDNA, 0.5 mM downstream primer, 0.5 mM upstream primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 1 unit of Taq polymerase (Takara) for 25 cycles of 1 min at 95 °C, 1 min at 60 °C and 1 min at 72 °C. The PCR products were resolved by agarose gel electrophoresis and visualized by staining with ethidium bromide. The primers used for amplification were as follows: human tau; 5′-TCATGAAGGGCCTAAACCAC-3′ and 5′-CACCCTCCTCAGTCTTCCTG-3′, β-actin; 5′-GTTTGAGACCTTCAACACC-3′ and 5′-GTGGTGGTGAAGCTGTAG-3′, X-box binding protein 1

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