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Amyloid-β peptide alteration of tau exon-10 splicing via the GSK3β-SC35 pathway

Kun-Lin Chen [a], Rey-Yue Yuan [b], Chaur-Jong Hu [b,c,*], Chung Y. Hsu [d,*]

[a] The Department of Medical Education and Research, Shih Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan
[b] The Department of Neurology and Chi-Chin Huang Topnotch Stroke Research Center, Taipei Medical University, Taipei, Taiwan
[c] Department of Neurology, Taipei Medical University Shuang-Ho Hospital, Taipei County, Taiwan
[d] Graduate Institute of Clinical Medical Sciences, China Medical University, Taichung, Taiwan

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ABSTRACT

Amyloid-beta peptide (Aβ) and Tau protein are the lead constituents in the pathogenesis of Alzheimer's disease (AD). However, their inter-relationship in the disease process remains to be established. Tauopathy refers to a characteristic neurodegenerative process in AD. In tauopathy, Tau accumulates as a consequence of altered pre-mRNA splicing of tau exon 10, resulting in 3R (without exon 10)/4R (with exon 10) imbalance. We studied Aβ effects on tau exon 10 pre-mRNA splicing and relevant signaling events. This is the first demonstration of Aβ alteration of tau exon 10 splicing with an increase in 3R/4R ratio caused by reduced 4R expression. This Aβ action is causally related to its activation of GSK-3β which in turn phosphorylates SC35, an enhancer in tau exon 10 splicing. The establishment of the Aβ-GSK-3β-SC35 cascade broadens insight into development of novel strategies to modulate Aβ action on tau exon 10 splicing for possible prevention of tauopathy.

Introduction

Tau is important for morphogenesis, axonal extension, axonal vesicle trafficking and protein transport in neurons (Chen et al., 1992; Ebneth et al., 1998). Pathological Tau, which is hyper-phosphorylated, is implicated in the pathogenesis of a group of neurodegenerative disorders collectively referred to as "tauopathy". These include AD (Wolozin et al., 1986), fronto-temporal dementia (Spillantini et al., 1998), progressive supranuclear palsy (Pillon et al., 1986), corticobasal degeneration (Uchihara et al., 1994), and Pick's disease (Pollock et al., 1986).

Tauopathy results from aberrant splicing of tau exon 10, causing alteration of the protein isoform ratio in favor of abnormal Tau aggregation (Schweers et al., 1995). Each of the exons 9–12 of tau encodes a 31- to 32-amino acid microtubules (MT)-binding imperfect repeat domain. Exon 10 is an alternatively spliced exon. If exon 10 is excluded, Tau consists of three MT binding repeats (3R); if exon 10 is included, then it carries a fourth MT-binding domain (4R) (D'Souza and Schellenberg, 2000). 4R Tau binds MTs with 3-fold higher affinity, allowing MT assembly to proceed more efficiently than 3R Tau.

Tau in fetal brain is characterized by exclusion of exon 2, exon 3 and exon 10, with only a single isoform (3R) produced (Andreadis, 2005). In an adult brain, balanced splicing of exon 10 maintains approximately equal amounts of 3R and 4R with a 3R/4R ratio close to 1. Alternative splicing of tau exon 10, resulting in a 3R/4R ratio dominated by either 3R or 4R, may play an important role in the pathogenesis of tauopathy. In the parkinsonism-chromosome type-17 fronto-temporal dementia (FTDP-17), mutations are noted within exon 10 and intron 10 sequences, leading to altered exon 10 splicing (Lee et al., 2001). Tauopathy which features 3R predominance includes AD, progressive supranuclear palsy and corticobasal degeneration (Tolnay et al., 2002), while fronto-temporal dementia and Pick's disease with 4R dominance are included in the 4R disease category (Hu et al., 2007).

There are multiple splicing elements on human exon 10 sequences, with the first half of exon 10 containing splicing elements for three splicing enhancers: SC35, polypurine-rich enhancer and A/C-rich enhancer (ACE). SC35, a protease in the HtrA family is regulated by GSK-3β, an upstream kinase (Hernandez et al., 2004).

Aβ induces death of neuronal (Estus et al., 1997; Forloni et al., 1993; Sotthibundhu et al., 2008) and non-neuronal cells including oligodendrocytes (Xu et al., 2001; Lee et al., 2004;), astrocytes (Harris et al., 1996; Yang et al., 2004) and cerebral endothelial cells (Sutton et al., 1997;

* Corresponding authors. C.-J. Hu is to be contacted at Department of Neurology, Taipei Medical University Shuang-Ho Hospital, No.291, Jhongjheng Rd., Jhonghe City, Taipei County 235, Taiwan. Fax: +886 2 27372758. C.Y. Hsu, Graduate Institute of Clinical Medical Sciences, China Medical University, No 2 Yuh-Der Road, Taichung, Taiwan. Fax: +886 4 2206 4888.
E-mail addresses: chaurjongh@tmu.edu.tw (C.-J. Hu), hsuc@mail.cmu.edu.tw (C.Y. Hsu).
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Thomas et al., 1996; Yin et al., 2002, 2006). Aβ has been implicated to play a major role in the pathogenesis of AD and cerebral amyloid angiopathy. Tau may act synergistically with Aβ to induce cell death (Alvarez et al., 1999). However, the interaction between Aβ and Tau has not been fully characterized in the pathogenesis of AD or other neurodegenerative diseases. Although both tau and Aβ are each the major component of the hallmark pathological features, neurofibrillary tangle and amyloid plaque respectively, in the AD brain, the interaction between these two key factors in the pathogenesis of AD remains to be fully elucidated. Aβ activates GSK-3β to phosphorylate tau (Takashima et al., 1998). The cytoplasmic domain of amyloid precursor protein with phospho-Thr668 enhances tau accumulation (Shin et al., 2007). In the mouse brain with transgenic overexpression of amyloid precursor protein, Aβ deposition leads to enhanced formation of tau tangles (Lewis et al., 2001). In cerebro-spinal fluid (CSF) of AD patients, an increase in tau protein concentration is frequently accompanied by a decrease in Aβ level (Shaw et al., 2009). In the present study, we report Aβ activation of the GSK-3β-SC35 cascade leading to alteration of tau exon 10 splicing.

Materials and methods

Cell culture

We used SH-SY5Y cells as an in-vitro model. SH-SY5Y cells have been utilized extensively to study molecular mechanisms of neurotoxic actions of Aβ (Levites et al., 2003), Tau (Yu and Fraser, 2001; Duka et al., 2009) and related neurodegenerative processes (Recio-Pinto et al., 1984; Mattsson et al., 1986; Yu and Fraser, 2001; Liu et al., 2005; Giaime et al., 2006). The cells were grown in DMEM (Gibco, NY, USA) containing 10% fetal bovine serum (CSL Ltd. Parkville, Victoria, Australia) and antibiotics in 10-cm dishes and maintained at 37 °C in a humidified atmosphere with 5% CO_2 (Yang et al., 2004).

RNA isolation, RT-PCR and real-time PCR

Total RNA was isolated with an RNAspin Mini Kit (GE Healthcare, Buckinghamshire HP7 9NA, UK), with the total RNA reconstituted in a ratio of DEPC water to wet starting materials (2 ml/g, v/w) for reverse transcription using M-MLV reverse transcriptase (Mbiotech Inc. Korea). PCR primers were (i) 3R and 4R: Forward: 5'-ATG CCA GAC CTG AAG AAT GTC AAG T-3', Reverse: 5'-TTA CTT CCA CCT GGC CAC CTC CT-3' (Recio-Pinto et al.); 4R Exon 10 specific: Forward: 5'-GAA GCT GGA TCT TAG CAA CG-3', Reverse: the same as 3R and 4R reverse primer shown above; (iii) GAPDH: Forward: 5'-GGT CTC CTC TGA CTT CAA CA-3', Reverse: 5'-GTG AGG GTC TCT CTC TTC CT-3'; and SC35 (Chen et al., 2009): Forward: 5'-CTG CGG CAA GGC CTT TCC CA-3', Reverse: 5'-GGT GCG GTA GGT CAG GTT GT-3'. PCR amplification was performed in the presence of HOT START DNA Polymerase (Solis BioDyne OU, Estonia) with samples maintained at 95 °C for 15 min to activate the polymerase, followed by 30–35 cycles at 95 °C for 1 min, 55–58 °C (depending on the primers) for 1 min, 72 °C for 1 min, and a further 72 °C extension for 2 min. PCR products were visualized on a 3% or 2% agarose gel stained with ethidium bromide and quantified using the NIH Image J 1.37 software. For real-time PCR, total RNA was isolated with an RNeasy Kit (QIAGEN). After reverse transcription, real-time PCR was carried out using a TaqMan EZ RT-PCR Core Kit (Applied Biosystems, Foster City, CA) with an ABI-7000 sequence detector according to the manufacturer's protocol. The specific forward primer, reverse primer and TaqMan probe were designed also using the manufacturer's Primer Express software as follows: 5'-GTG CAG ATA ATT AAT AAG AAG CTG GAT CTT-3'(sense), 5'-CCG GGA CGT GTT TGA TAT TAT CCT T-3'(antisense), and 5'-FAM-ACG TCC AGT CCA AGT GTG-FAM-3' (probe). β-actin was an internal reference. The amount of tau exon 10 mRNA was normalized with β-actin to obtain the relative threshold cycle (ΔC_t) and then related to the ΔC_t of the controls to obtain the relative expression level ($\Delta\Delta C_t$) of tau exon 10 (Livak and Schmittgen, 2001; Hsu et al., 2007).

Western blotting

The procedures have been reported elsewhere (Yin et al., 2006; Hsu et al., 2007). Briefly, proteins were extracted with lysis buffer (20 mM HEPES, pH 7.9, 20% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF, 0.1% NP40). The samples were then adjusted to 1 μg/μl under reducing conditions with a 10 μl/well loading for 10–12% SDS-PAGE, fractionated at 150 V for 90 min. The proteins were subsequently electroblotted onto a BioTrace PVDF membrane (P/N 66543, Pall, Pensacola, FL) followed by incubation in a blocking buffer containing 5% non-fat milk for 1 h at room temperature. The membrane was then incubated overnight at 4 °C with an anti-tau-3R isoform RD3 mouse IgG monoclonal antibody (1:1000, catalog #05-803, Upstate, NY), an anti-tau-4R isoform RD4 mouse IgG monoclonal antibody (1:1000, catalog #05-804, Upstate, NY), an anti-SC35 monoclonal antibody (1:1000, ab11826, Abcam, UK) or a anti-GSK-3β monoclonal antibody (1:250, catalog MAB2506, R&D Systems, Inc.). Horseradish peroxidase (HRP) conjugated goat anti-mouse secondary antibody (1:5000; BioNova, Taiwan) with an ECL chemiluminescent substrate (Amersham, GE Healthcare, Buckinghamshire, UK) was used to visualize the protein-specific bands, with β-actin serving as the internal control using an anti-β-actin antibody (1:1000, #MAB1501, Chemicon).

GSK-3β assays

GSK-3β activity was determined using phospho-GS peptide 2 (no.12-241, Upstate, NY) as the substrate (Pei et al., 1997; Tsujio et al., 2000). An aliquot of 7.5 μg cell proteins was incubated for 30 min at 30 °C with 250 μM peptide substrate and 200 μM γ-^{32}P-ATP (1,500 cpm/pmol ATP) in 30 mM Tris (pH 7.4), 10 mM $MgCl_2$, 10 mM NaF, 1 mM Na_3VO_4, 2 mM EGTA and 10 mM β-mercaptoethanol in a total volume of 25 μl. The reaction was halted by the addition of 25 μl of 300 mM O-phosphoric acid. In order to remove free ^{32}P-ATP, the reaction mixture was applied in duplicate to phosphocellulose filter paper (GF/P30. #1450-523, PerkinElmer, Finland) with the filters washed 3 times with 75 mM O-phosphoric acid and dried for liquid scintillation counting. GSK-3β activity was expressed as pmol phosphate incorporated/mg protein/min.

RNA interference study

In SC35 siRNA knockdown experiments, SH-SY5Y cells were plated at a density of 2.5×10^5 cells per well in 6-well plates, with 200 pmol of duplex RNA (sense strand, AAUCCAGGUCGCGAUCGAAdTdT; Dharmacon) (Gabut et al., 2005) or scramble-siRNA (Dharmacon) mixed with 4 μl of DharmaFECT 1 (Dharmacon) plus 200 μl of serum-free DMEM/F12 medium on the subsequent day. Following RNA duplex–lipid complex formation, the mixture was adjusted to 1 ml with antibiotic-free DMEM/F12 and added to the cells. After siRNA or scramble-siRNA treatment for 24 h, $A\beta_{25-35}$ with or without LiCl, was added to the plates and incubated for 12 h. Control, siRNA- and scramble-siRNA-treated cells were collected for RNA and protein extraction for respective RT-PCR and Western blotting (Yin et al., 2006; Hsu et al., 2007). In GSK-3β siRNA knockdown experiments, SH-SY5Y cells were plated at a density of 2.5×10^5 cells per well in 6-well plates, with 200 pmol of duplex RNA (sc-35527, Santa Cruz) or scramble-siRNA (Dharmacon) mixed with 5 μl of Lipo2000 (Invitrogen) plus 200 μl of serum-free DMEM/F12 medium on the subsequent day. Following RNA duplex–lipid complex formation, the mixture was adjusted to 1 ml with antibiotic-free DMEM/F12 and added to the cells. After siRNA or scramble-siRNA treatment for 24 h, $A\beta_{25-35}$ was added to the plates and incubated for