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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. 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. 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 12 h. Control,

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siRNA- and scramble-siRNA-treated cells were collected for protein extraction for Western blotting.

Isolation of the nuclear fraction

The procedures were as described (Pei et al., 1997; Xu et al., 2001). In brief, cells were grown to a density of 5×10^5 cells/mL in a 10-cm petri dish, with the cells first harvested by centrifugation at 500*g*, and then washed 3 times in PBS before being placed in a hypotonic buffer-A resuspension (10 mM KCl, 1.5 mM $MgCl_2$, 10 mM HEPES at pH 7.9, 0.5 mM DTT, 0.75 mM spermidine and 0.15 mM spermine). Cytoplasmic lysate was separated from the cellular components by centrifugation at 12,000*g* for 30 min. The pellet (the nuclear fraction) was then resuspended on ice in a 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) for 30 min, and then centrifuged at 12,000*g* for 30 min. The isolated nuclear fraction was stored at −80 °C until use. For the nuclear proteins, lamin A/C was the internal control using anti-lamin A/C rabbit IgG antibody (1:1000; #2032, Cell Signaling Technology) and a horseradish peroxidase (HRP) conjugated goat anti-rabbit secondary antibody (1:1000; ab6721, Abcam, UK).

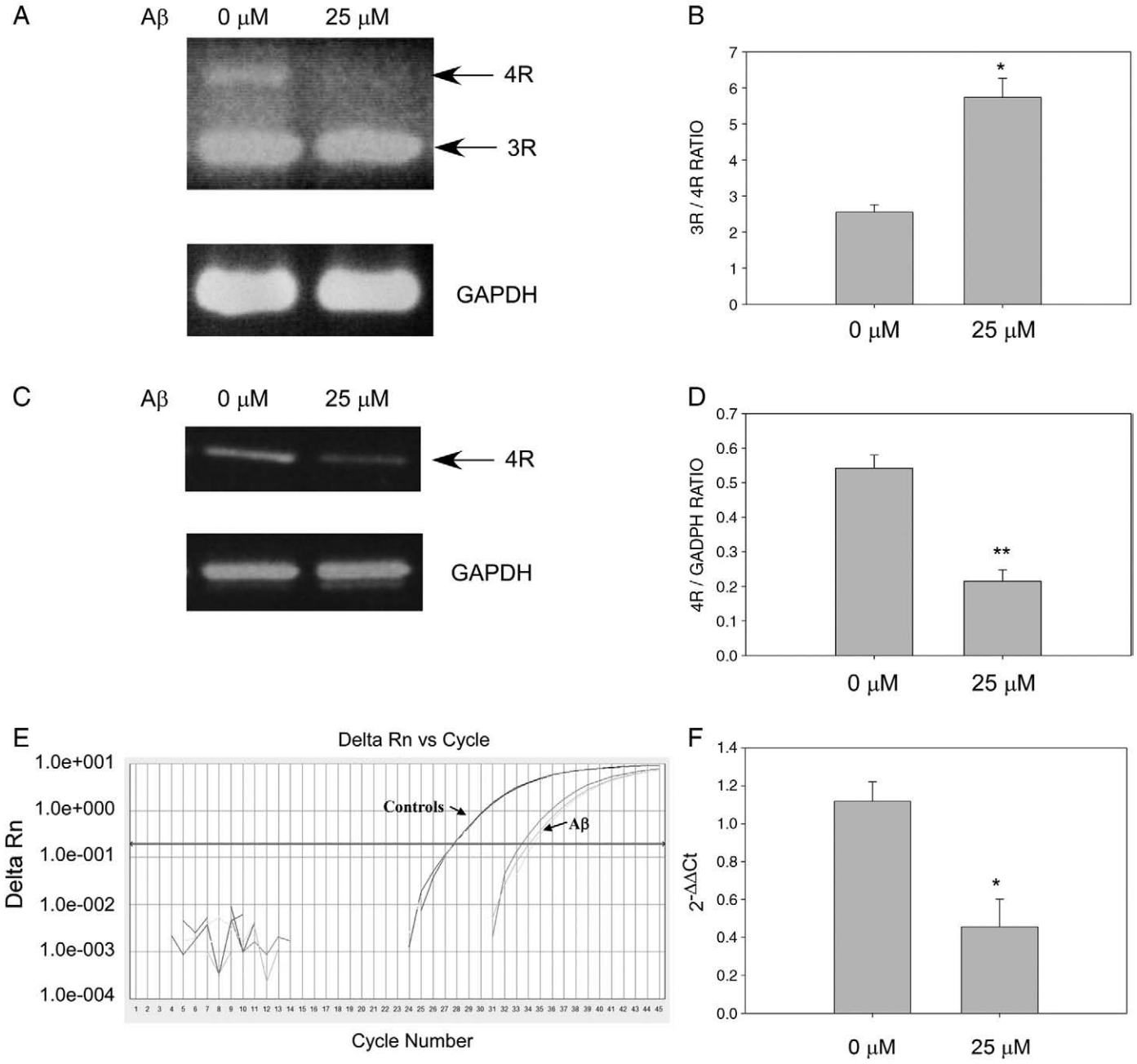

Fig. 1. Aβ alteration of the Tau mRNA 3R/4R ratio. (A) 3R and 4R mRNA expression following Aβ treatment for 12 h was determined by RT-PCR with GAPDH as an internal control. Note Aβ (25 μM) selectively reduced Tau 4R mRNA expression. (B) The bar graph shows compiled data from 3 separate experiments for each paradigm with * denoting $p<0.05$. (C) To confirm results in A and B, a pair of tau 4R-specific primers with forward primer sequence located within exon 10 were used to exclude 3R as a template and to amplify only tau 4R mRNA. The amount of tau 4R mRNA was reduced after Aβ (25 μM) treatment for 12 h, with GAPDH as an internal control. (D) The bar graph shows compiled data from 3 separate experiments with $^{**}p<0.01$. (E) Real-time PCR for tau exon 10 confirming PCR results. A representative set of real-time PCR survey with triple repeats. (F) The amount of tau 4R mRNA was reduced after Aβ (25 μM) treatment for 12 h. β-actin served as an internal control. $^{*}p<0.05$.

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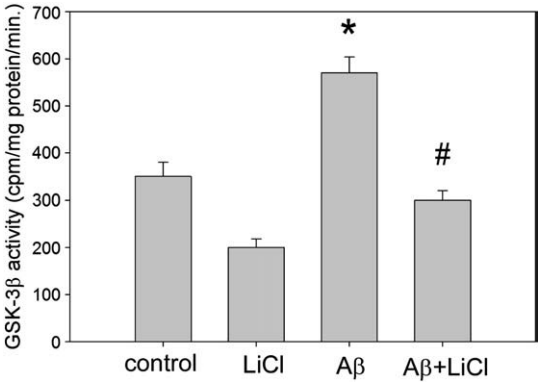

Fig. 2. Aβ activation of GSK-3β. GSK-3β activity was increased following Aβ treatment for 12 h. Note lithium chloride (LiCl) (20 mM), a GSK-3β inhibitor, blocked this Aβ action. * denotes difference from the control and # from Aβ treatment are respectively significant ($p<0.05$).

RNA-immunoprecipitations (RNA-IP)

RNA-IP was conducted as previously described using an affinity antibody of SC35 (Whitfield et al., 2004). Polyribosomes (5–10 μg of polyribosomal RNA) were diluted in 100 μl NP-40 lysis buffer (0.1% Tergitol type NP-40, 50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 50 mM NaF, 1 mM DTT, 10 mM EDTA, 40U RNasin) (Promega, Madison, WI) (Yin et al., 2006). Non-specific interactions between the polyribosomes and protein-A agarose beads were pre-cleared by the addition of 20 μl agarose beads:PBS (1:1) protein-A bead slurry and incubated at 4 °C for 30 min with rotation. Protein-A beads were removed by centrifugation at 1500*g* and the pre-cleared supernatant incubated for one hour at 4 °C with either protein-A-anti-SC35 (1:1000; #S4045, Sigma USA), or IgG. Antibody–protein complexes were recovered by centrifugation at 400 × *g* and washed 3 times with NP-40 lysis buffer. A urea lysis buffer (7 M urea, 2% SDS, 0.35 M NaCl, 10 mM EDTA, 10 mM Tris–HCl, pH 7.5) was added to the pellet (200 μl). RNA was extracted in phenol/chloroform:isoamyl alcohol (24:1, v/v) and then precipitated with ethanol. The RT-PCR based on tau pre-mRNA was as described earlier.

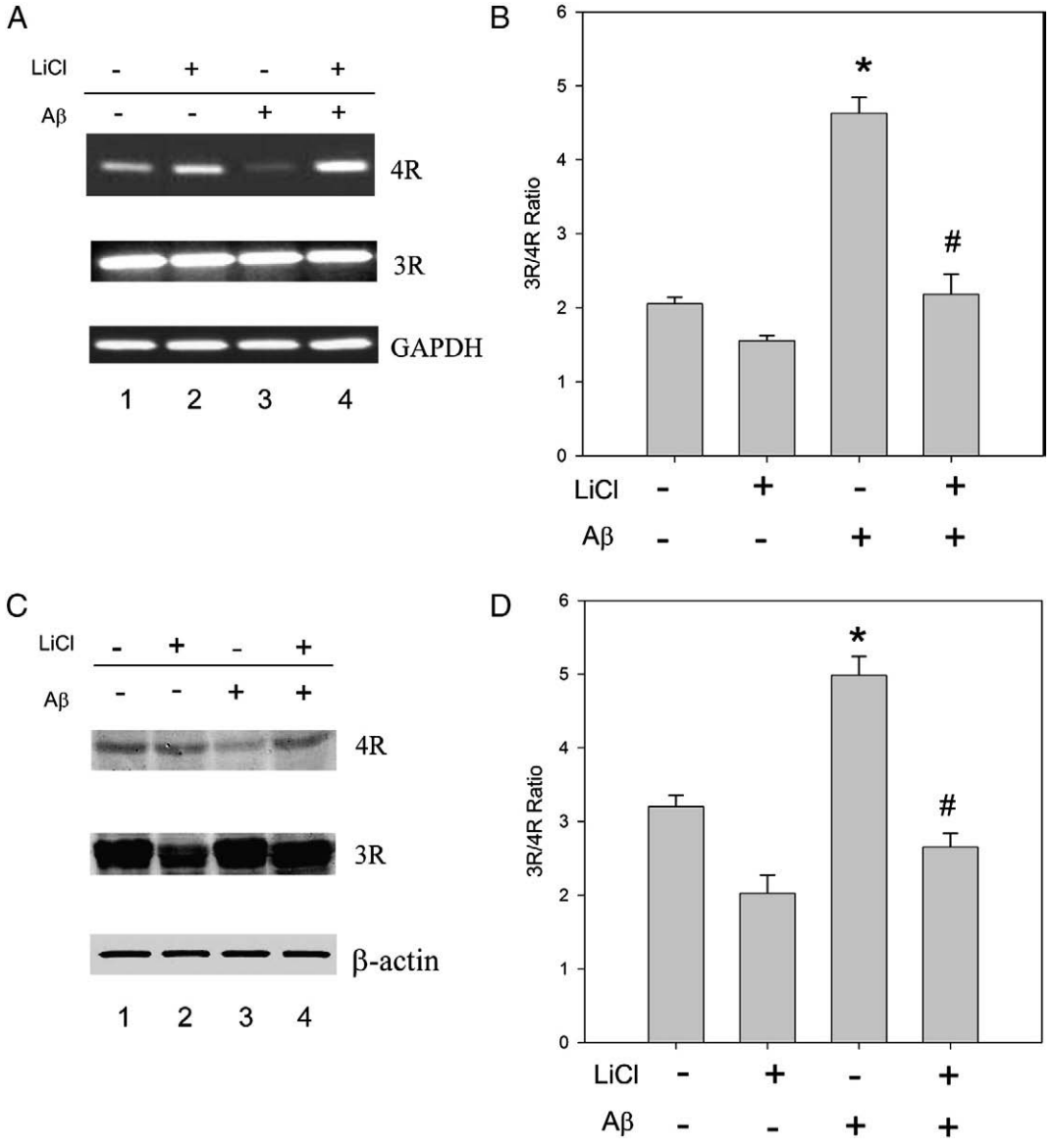

Fig. 3. Reversal of Aβ alteration of the Tau 3R/4R ratio by lithium, a GSK-3β inhibitor. (A) 3R and 4R mRNA expression based on RT-PCR with GADPH as an internal control. Lithium (LiCl) (20 mM), a GSK-3β inhibitor, reversed Aβ alteration of the Tau 3R/4R ratio. (B) The bar graph shows compiled data from 3 separate experiments. (C) Western blot analysis of 3R and 4R protein expression using β-actin as an internal control. (D) The bar graph shows compiled data from 3 separate experiments. * denotes difference from the control, and # from Aβ treatment are respectively significant ($p<0.05$).

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Statistical analyses

The data are expressed as mean ± SD derived from triplicates of at least three separate experiments. Difference between two experimental groups was based on the two-tailed Student's *t*-test, with a *p*-value less than 0.05 considered significant.

Results

$A\beta_{25-35}$ suppression of tau 4R RNA isoform level

We first determined whether Aβ alters tau exon 10 splicing, based upon the notion that tauopathy encompasses a group of neurodegenerative disorders that also implicate Aβ. In preliminary studies, $A\beta_{25-35}$ and $A\beta_{1-42}$ were of equal potency (data not shown) (Lee et al., 2004; Hsu et al., 2007). Subsequent studies were then carried out using $A\beta_{25-35}$ only (referred to as Aβ hereafter). Exposure of SH-SY5Y cells to Aβ at a concentration (25 μM) used in other in vitro studies (Lee et al., 2004; Hsu et al., 2007;) for 12 h resulted in a reduction in the content of tau 4R, but not 3R, mRNA (Fig. 1A, B). This effect of Aβ was dose-dependent (data not shown).

In order to confirm the selective effect of Aβ on tau 4R mRNA expression, we employed a pair of 4R-specific primers to assess the 4R pre-mRNA splicing, with the forward primer sequence located within exon 10. Aβ treatment reduced specifically 4R mRNA expression (Fig. 1C, D), supporting the preferential effect of Aβ noted above (Fig. 1A, B). To the best of our knowledge, this is the first demonstration that Aβ alters tau exon 10 splicing, resulting in an increase in the 3R/4R ratio.

To confirm the PCR results, real-time PCR was conducted. The ΔC_t of the samples treated with Aβ increased after 12 h and the $\Delta\Delta C_t$ was significantly higher than 0, suggesting the amount of tau 4R mRNA was reduced after Aβ treatment for 12 h (Fig. 1E, F).

Aβ Activation of GSK-3β

Among a number of signaling events in Aβ-mediated neurodegenerative processes, activation of GSK-3β has been shown to be a critical step (Hoshi et al., 2003). A key role of GSK-3β in the pathogenesis of AD has been proposed (Hooper et al., 2008). Interestingly, GSK-3β has also been shown to alter tau exon 10 splicing (Hernandez et al., 2004). Together, these findings raise the possibility that GSK-3β activation may be causally related to Aβ alteration of tau exon 10 splicing. We sought to confirm this contention by studying whether GSK-3β is activated under the experimental paradigm in which Aβ alters tau exon 10 splicing. As shown in Fig. 2, under the same condition in which Aβ reduced the tau 4R content in SH-SY5Y cells (Fig. 1) an increase in GSK-3β activity was noted.

Reversal of Aβ effect on tau 4R expression by inhibition of GSK-3β with lithium or GSK-3β siRNA

To establish the causal role of GSK-3β in mediating Aβ alteration of tau exon 10 splicing, we studied the effect of lithium, a GSK-3β inhibitor (Lovestone et al., 1994; Klein and Melton, 1996; Munoz-Montano et al., 1997). As shown in Fig. 2, lithium blocked the increase in GSK-3β activity induced by Aβ. This inhibitory effect of lithium on Aβ activation of GSK-3β was associated with reversal of Aβ suppression of tau 4R expression at both the mRNA and protein levels (Fig. 3A–D).

To study more specifically the causal role of GSK-3β, we applied siRNA of GSK-3β to knock down *GSK* expression. As shown in Fig. 4A and B, *GSK* knockdown blocked Aβ reduction in tau 4R expression, similar to that caused by the lithium effect.

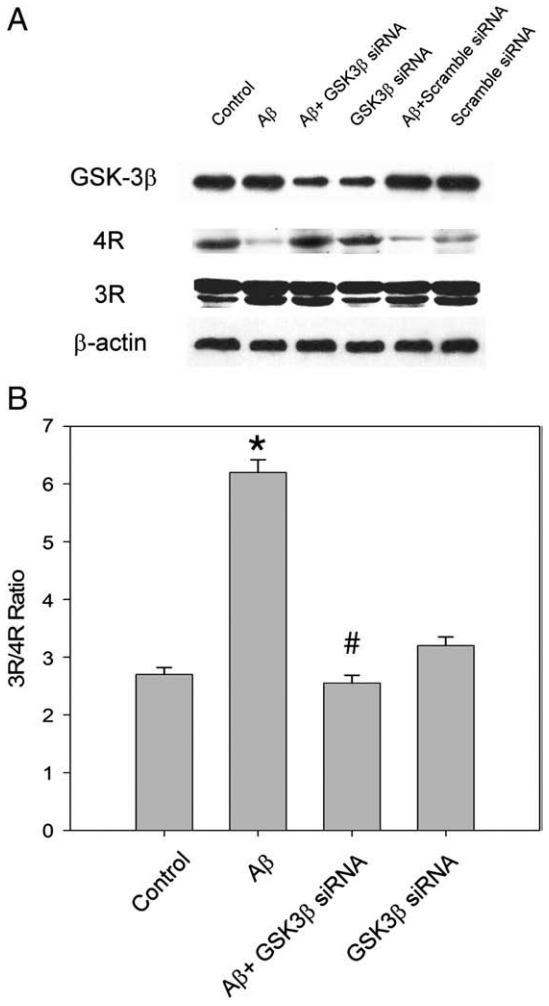

Fig. 4. Reversal of Aβ suppression of 4R expression by GSK-3β knockdown. (A) siRNA-mediated knockdown of GSK-3β protein expression based on immunoblotting using β-actin as an internal control. (B) The bar graph shows compiled data from 3 separate experiments. * denotes difference from the control, and # from Aβ treatment are respectively significant ($p<0.05$). Note a lack of effect of the scramble siRNA.

The GSK-3β–SC35 cascade in Aβ alteration of tau exon 10 splicing

SC35 is one of the 3 enhancers for splicing elements in the tau exon 10 sequence (D'Souza and Schellenberg, 2000). SC35 has also been shown to be one of the substrates for GSK-3β phosphorylation (Hernandez et al., 2004). These findings together raise the possibility that a GSK-3β-SC35 cascade may exist in Aβ alteration of tau exon 10 splicing. To examine whether SC35 is involved in Aβ alteration of tau exon 10 splicing, we applied siRNA of SC35 to study the effect of SC35 knockdown on Aβ suppression of tau 4R mRNA expression. SiRNA knockdown of SC35 mRNA and protein expression was accompanied by a reversal of Aβ effect on tau 4R mRNA expression. A scramble-siRNA, serving as the control, was without effects (Fig. 5A–D).

Tau exon 10 co-precipitated with the SC35–antibody complex

To further confirm that SC35 is involved directly in tau exon 10 splicing, we incubated nuclear extracts and anti-SC35–protein A agarose beads complex in a immunoprecipitation study to explore SC35-tau exon 10 interaction. Tau exon 10 specific splicing element was analyzed by RT-PCR. Results shown in Fig. 6 indicate that Aβ induced tau exon 10 interaction with the SC35 complex. This Aβ effect was also blocked by lithium.

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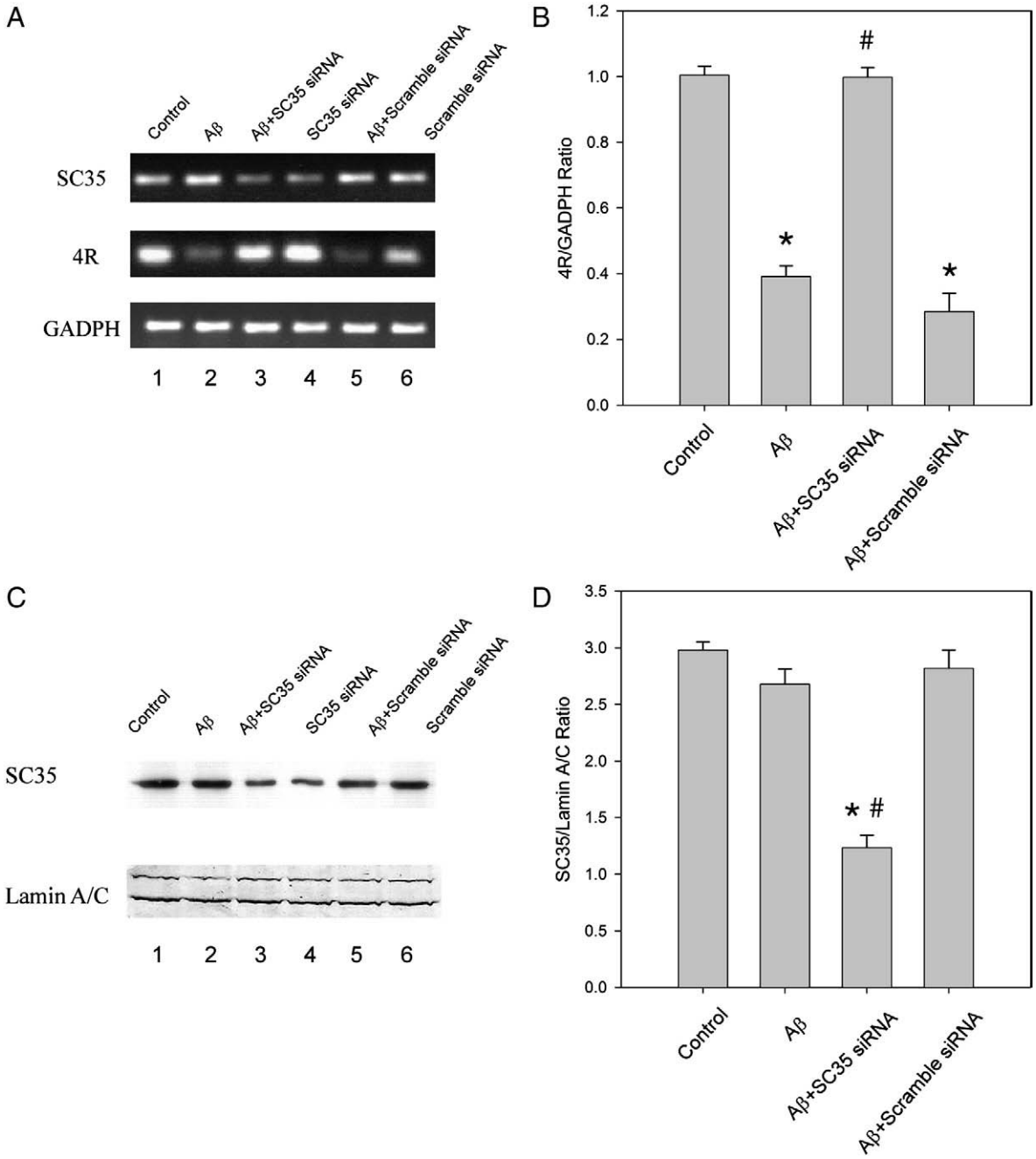

Fig. 5. Reversal of Aβ suppression of 4R expression by SC35 knockdown with siRNA. (A) siRNA (0.1 μM)-mediated knockdown of SC35 mRNA expression based on RT-PCR, using GADPH as an internal control. Note the basal SC35 mRNA level was not affected by Aβ. (B) The bar graph shows compiled data from 3 separate experiments. * denotes difference from the control, and # from Aβ treatment are, respectively, significant ($p<0.05$). No significant difference is noted between the control and Aβ + SC35 siRNA groups or between the Aβ treatment alone and Aβ + Scrambled siRNA groups ($p>0.05$). (C) siRNA-mediated knockdown of SC35 protein expression based on immunoblotting using lamin A/C as an internal control. (D) The bar graph shows compiled data from 3 separate experiments. * denotes difference from the control, and # from Aβ treatment are respectively significant ($p<0.05$). Note a lack of effect of the scramble siRNA.

Discussion

Despite the extensive documentation of the pathogenetic roles of Aβ and Tau protein in AD, the interaction between these key mediators has not been fully explored. In the present study, we noted that Aβ alters tau pre-mRNA splicing by shifting the tau 3R/4R ratio toward 3R dominance. This Aβ effect is ascribed to selective suppression of 4R expression at the RNA and protein levels. This is the first demonstration that Aβ alters tau exon 10 splicing to increase 3R/4R ratio. To study the mechanism of this Aβ action, we sought to establish the GSK-3β-SC35 cascade based on the earlier observations that GSK-3β is activated by Aβ (Takashima et al., 1998; Liu et al., 2002) and GSK-3β phosphorylates SC35 (Hernandez et al., 2004). SC35 is a key enhancer of splicing elements important in the regulation of tau exon 10 splicing (D'Souza and Schellenberg, 2000).

Under the same experimental condition when Aβ altered the 3R/4R ratio, GSK-3β was also activated. The causal role of GSK-3β in Aβ suppression of tau 4R mRNA expression to increase the 3R/4R ratio is substantiated by the finding that lithium, a GSK-3β inhibitor, or siRNA knockdown of GSK-3β was effective in blocking Aβ alteration of tau exon 10 splicing. SC35, a key factor in tau exon 10 splicing, is downstream in the Aβ-GSK-3β cascade. GSK-3β

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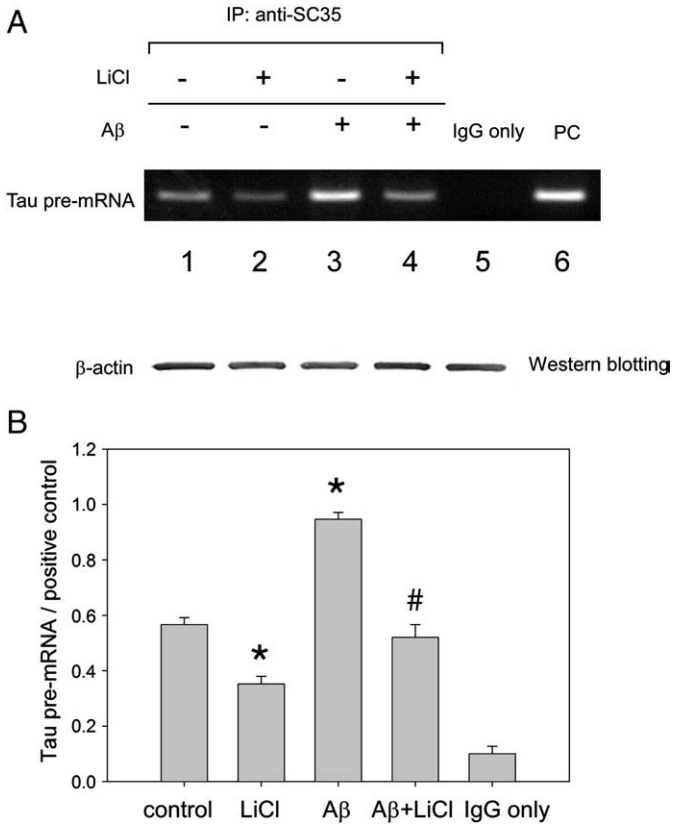

Fig. 6. Aβ enhancement of SC35 binding to tau pre-mRNA exon 10 splicing sequence. (A) To determine whether SC35 binds directly to Tau pre-mRNA, nuclear extracts were incubated with an anti-SC35–protein A agarose bead complex (anti-SC35 serum, 1:1000), with the bound fraction recovered by centrifugation. RNA was separated from the complex by phenol/chloroform extraction for RT-PCR. The amount of tau pre-mRNA bound to SC35 was increased with Aβ treatment (lane 3), but this effect was reversed by lithium, a GSK-3β inhibitor (lane 4); no tau pre-mRNA binding with IgG (1:1000) is noted suggesting the specificity of tau pre-mRNA-SC35 interaction (lane 5), with total RNA serving as a positive control (PC, lane 6). Note in this study, no internal control is feasible for RT-PCR. β-actin (Western blotting) was used as an internal control for protein loading in immunoprecipitation. (B) The bar graph shows compiled data from 3 separate experiments. * denotes difference from the control, and # from Aβ treatment are respectively significant ($p<0.05$).

phosphorylation of SC35 has been shown to alter tau exon 10 splicing (Hernandez et al., 2004). The causal role of SC35 in Aβ activation of GSK-3β to alter tau exon 10 splicing is supported by the finding that siRNA knockdown of SC35 blocked Aβ alteration of tau 4R mRNA expression.

In an immunoprecipitation study, we noted that SC35-tau exon 10 interaction was enhanced following Aβ treatment. Thus, it is logical to consider that GSK-3β action on SC35 is a downstream event following Aβ activation of GSK-3β. Results shown in Fig. 6 confirm the pivotal role of GSK-3β in Aβ enhancement of SC35-tau exon 10 interaction as lithium, a GSK-3β inhibitor, was effective in blocking this Aβ action.

Results from the present studies establish a series of sequential events entailing Aβ activation of GSK-3β which in turn phosphorylates SC35 to alter tau exon 10 splicing, resulting in an increase in the tau 3R/4R ratio. Direct phosphorylation of SC35 by GSK-3β was not examined in the present study but has been reported previously (Hernandez et al., 2004). Together, these findings establish a regulatory pathway of tau exon 10 splicing that may be amenable to pharmacological or genetic modulation for future development of novel strategies directed at disconnecting Aβ-Tau interaction for preventing tauopathy associated with Aβ, especially AD.

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