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TAU OVEREXPRESSION IN TRANSGENIC MICE INDUCES GLYCOGEN SYNTHASE KINASE 3β AND β-CATENIN PHOSPHORYLATION

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Abstract—The abnormal phosphorylations of tau, GSK3β, and β-catenin have been shown to perform a crucial function in the neuropathology of Alzheimer's disease (AD). The primary objective of the current study was to determine the manner in which overexpressed *htau23* interacts and regulates the behavior and phosphorylation characteristics of tau, GSK3β, and β-catenin. In order to accomplish this, transgenic mice expressing neuron-specific enolase (*NSE*)-controlled human wild-type *tau* (*NSE*/*htau23*) were created. Transgenic mice evidenced the following: (i) tendency toward memory impairments at later stages, (ii) dramatic overexpression of the *tau* transgene, coupled with increased tau phosphorylation and paired helical filaments (PHFs), (iii) high levels of GSK3β phosphorylation with advanced age, resulting in increases in the phosphorylations of tau and β-catenin, (iv) an inhibitory effect of lithium on the phosphorylations of tau, GSK3β, and β-catenin, but not in the non-transgenic littermate group. Therefore, the overexpression of *NSE*/*htau23* in the brains of transgenic mice induces abnormal phosphorylations of tau, GSK3β, and β-catenin, which are ultimately linked to neuronal degeneration in cases of AD. These transgenic mice are expected to prove useful for the development of new drugs for the treatment of AD.

Key words: Alzheimer, transgenic, tau, GSK3, Wnt.

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Histopathologically, AD is characterized by a massive accumulation of abnormal fibrous amyloid β-protein (Aβ) and neurofibrillary tangles (NFTs). Aβ is deposited as extracellular senile plaques, which are composed of the 39–43 amino-acid long peptides that are derived from the amyloid precursor protein (APP) as a result of cleavage with β- and γ-secretase. A dysfunction of NFTs consist of paired helical filaments (PHFs) which in turn are composed of phosphorylated and ubiquitinated tau protein, which forms a β-pleated sheet structure.

Tau is phosphorylated by glycogen synthase kinase 3β (GSK3β) both *in vitro* (Lovestone et al., 1996) and *in vivo* (Hong et al., 1997; Munoz-Montana et al., 1997). β-Catenin is also phosphorylated in the N-terminus by GSK3β in transfected cells *in vitro* (Tesco and Tanzi, 1998), and residues 445–576 of β-catenin form complexes with residues 322–450 of presenilin (PS) 1 (Takashima et al., 1998; Murayama et al., 1998; Yu et al., 1998). Moreover, tau and GSK3β are bound directly by an identical region of PS1, the 250–298 residues (Takashima et al., 1998). Interaction of tau with other factors obtained from various types of *in vitro* and animal model experiments has been brought into a mechanistic framework of AD (Brandt and Leschik, 2004).

There are six isoforms of the *tau* gene in the adult human brain, which are expressed from a single gene via the alternative splicing of exons 2, 3, and 10, which are not included in fetal human brains (D'Souza and Schellenberg, 2005). Of the six isoforms, three include exons 10 and 4R [ON4R (*htau24*, 383 amino acids (aa))] with exon 2 [1N4R (*htau34*, 412 aa)] and with exon 2 and exon 3 [2N4R (*htau40*, 441 aa)], and the other half includes 3R [ON3R (*htau23*, 352 aa)] with exon 2 [1N3R (*htau37*, 381 aa)] and with exons 2 and 3 [2N3R (*htau39*, 410 aa)].

Several studies have created transgenic mice expressing the wild-type human *tau* under the control of different promoters. Wild-type transgenic mice expressing the *htau40* and *htau24* cDNA under the control of the human/murine *Thy 1.2* (Gotz et al., 1995; Spittaels et al., 1999; Probst et al., 2000) and the murine *PrP*/*HMG CoA reductase* promoters (Ishihara et al., 1999, 2001; Brien et al., 1999) have been produced. Most models revealed changes associated with the development of neurofibrillary lesions in AD that was sufficient to cause a nerve cell dysfunction. However, wild-type transgenic mice lacked the neuropathological symptoms without the production of NFTs. Moreover, biochemical analysis of tau pathology and behavior in other transgenic mice expressing wild type or mutant tau has not been characterized.

Abnormal phosphorylations of the tau, GSK3β, and β-catenin play a key role in the neuropathology of AD (Grundke-Iqbal et al., 1986). The aim of this study was to determine how the overexpression of *htau23* interacts and regulates the behavior and the phosphorylations of the tau, GSK3β, and β-catenin. This was accomplished by creating transgenic mice expressing neuron-specific enolase

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Abbreviations: aa, amino acid; Aβ, amyloid β-protein; AD, Alzheimer's disease; APP, amyloid precursor protein; EM, electron microscopy; GSK3β, glycogen synthase kinase 3β; NFT, neurofibrillary tangle; Non-tg, non-transgenic littermate; NSE, neuron-specific enolase; PHF, paired helical filament; PS, presenilin; Tg, transgenic littermate.

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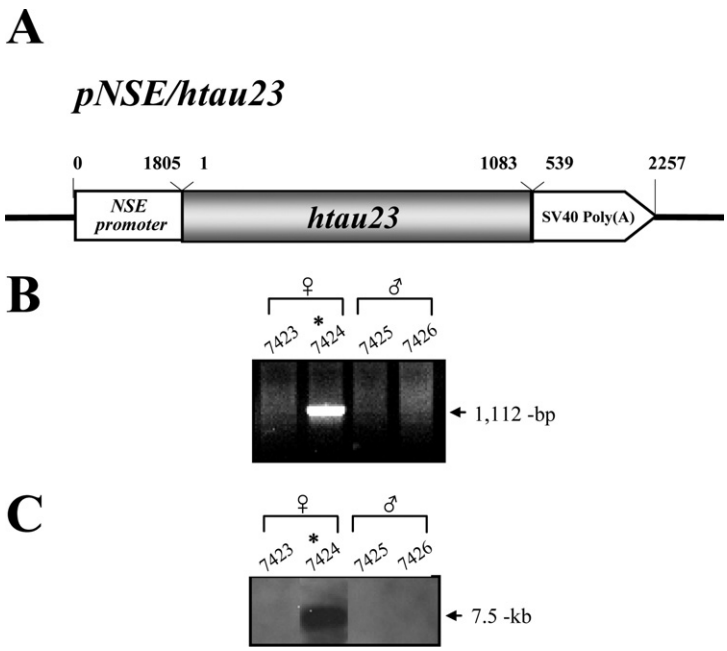

Fig. 1. pNSE/htau23 structure and identification of transgene. (A) Structure of the *htau23* linked to the *NSE* promoter. (B) Identification of the transgene by DNA-PCR. The genomic DNA was isolated from the tail of the founder mouse, and the 1112-bp of product was shown in the transgenic mice carrying the *pNSE*/*htau23* transgene. (C) Identification of the transgene by Southern blot analysis. Approximately 10 μg of the tail DNA from the transgenic mice was subjected to Southern blot hybridization. The genomic DNA digested with *EcoR1* was probed with a ^{32}P-labeled *htau23* fragment to detect the integrated transgene. The fragment sizes (7.5 kb), as calculated by the relative position of the fragment of a known size in 5.2, 6.2, 7.1-kb (lambda DNA digested with *Hind*III/*Hae*III), are indicated at the right of the blot. Specific hybridization signals were visualized by autoradiography for 4 days at −70 °C.

(*NSE*)-controlled human wild-type *tau* (*NSE*/*htau23*), and testing them for studying behavior impairment, tau, GSK3β, and β-catenin phosphorylations.

EXPERIMENTAL PROCEDURES

Gene construction

The *pNSE*/*htau23* was constructed by inserting the human wild-type *tau* cDNA (*htau23*) (GenBank accession No. J03778) linked to the *NSE* promoter (Fig. 1A). The *htau23* sequence was amplified by PCR, with a full-length of the RNA as a template, which was isolated from human SK-N-MC cells. The following primers were used for amplification: the sense primer, 5′-GCACT AGTCA GGTGA ACTTT GAACC AGGAT G-3′ (corresponding to nucleotide 7–40 of *htau*), and the antisense primer, 5′-GCACT AGTCT GATCA CAAAC CCTGC TTG-3′ (corresponding to nucleotide 1081–1100 of *htau*). The amplified *htau23* product was cloned into *pGEM-T* (*phtau23-T*; Promega Co., Madison, WI, USA). Sequence analysis (Biotech, Korea) was performed to verify whether or not the cloned wild-type *tau* sequence was identical to *htau23*, and the resulting sequence was aligned with the NCBI sequence database using the BLAST program to identify their corresponding *htau23* gene. The results showed that cloned *htau23* sequence was completely matched to the human wild-type tau sequence. The *NSE*/*htau23* with the prokaryotic sequence eliminated by digestion with *Kpn*I and *Not*I was used for the microinjection. Finally, the *htau23* obtained from the digestion of *phtau23-T* with *Spe*I was then cloned into the *Spe*I site of the *pNSE-splice*. The *pNSE-splice* was constructed by inserting the rat *NSE* sequence into the *pTet-Splice* (Gibco BRL, Rockville, MD, USA), which contained the tetracycline operator sequence (*Tet*) that had been eliminated by digestion with the *Xho*I/*Spe*I enzymes. The rat *NSE* promoter was amplified by PCR, using a sense primer (5′-CGTCG ACTATGGTGG TATGG CTGA-3′) with nucleotides 37–55, and an antisense primer (5′-TCGAG GACTG CAGAC TCAG-3′) with nucleotides 1786–1804 using *pNSE*/*CAT* (Forss-Petter et al., 1990) as a template. The primers were added as a recognition sequence for the *Sal*I and *Spe*I, to the 5′ and 3′ ends of the PCR products, respectively. The *pNSE*/*CAT* was a kind gift from Dr. J Gregor-Sutcliffe at the Research Institute of Scriffs Clinic. The amplified *NSE* product (1777-bp) was inserted into the *pGEM-T*.

Establishment of transgenic mice

The *pNSE*/*htau23* was digested with *Kpn*I and *Not*I to remove the prokaryotic sequences, and diluted to 4 ng/μl. The linear *NSE*/*htau23* fragment was microinjected into the male pronucleus of a fertilized mouse egg. The egg was obtained by mating a female BDF1 mouse with a male BDF1 mouse. The injected eggs were then transferred into the oviducts of the pseudopregnant ICR recipient females on day 1. The founder mice, into which the *NSE*/*htau23* transgene had been inserted, were identified by DNA-PCR and Southern blot analysis of the tail-derived DNA. The transgenic founder mice were then crossed onto the parental strain of the BDF1 background to establish the transgenic lines. Subsequently, the transgenic line was quantitatively crossed for this experiment. The transgenic (Tg) and non-transgenic littermate (Non-tg) groups were handled in an accredited Korea FDA animal facility in accordance with the AAALAC International Animal Care policies (Accredited Unit, Korea Food and Drug Administration; Unit Number 000996). All efforts were made to minimize the number of animals used and their suffering. The mice were housed in cages under a strict light cycle (light on at 06:00 h and off at 18:00 h). In addition, all the mice were given a standard irradiated chow diet (Purina Mills Inc., St. Louis, MO, USA) *ad libitum*. The mice were maintained in a specified pathogen-free state.

Southern blotting

The genomic DNA was prepared from the tails of 4-week-old founder mice, and the transgene was detected by Southern blot