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Progressive neuronal loss and behavioral impairments of transgenic C57BL/6 inbred mice expressing the carboxy terminus of amyloid precursor protein

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The β-secretase cleaved Aβ-bearing carboxy-terminal fragments (βCTFs) of amyloid precursor protein (APP) in neural cells have been suggested to be cytotoxic. However, the functional significance of βCTFs in vivo remains elusive. We created a transgenic mouse line Tg-βCTF99/B6 expressing the human βCTF99 in the brain of inbred C57BL/6 strain. Tg-βCTF99/B6 mouse brain at 12–16 months showed severely down-regulated calbindin, phospho-CREB, and Bcl-x_L expression and up-regulated phospho-JNK, Bcl-2, and Bax expression. Neuronal cell density in the Tg-βCTF99/B6 cerebral cortex at 16–18 months was lower than that of the non-transgenic control, but not at 5 months. At 11–14 months, Tg-βCTF99/B6 mice displayed cognitive impairments and increased anxiety, which were not observed at 5 months. These results suggest that increased βCTF99 expression is highly detrimental to the aging brain and that it produces a progressive and age-dependent AD-like pathogenesis.

Keywords: AD model; CTF; Neuronal loss; Cognitive deficits; Transgenic animal

Introduction

Proteolytic cleavages of APP generate multiple forms of C-terminal fragments (CTFs): β-amyloid peptide (Aβ) can be produced by sequential action of β- and γ-secretases (Steiner et al., 1999). Aβ-bearing carboxy-terminal fragments (βCTFs) can be produced by β-secretase (Suh and Checler, 2002). Amyloid intracellular domain or AICD (CTF57 and CTF59) can be produced by γ-secretase (Yu et al., 2001a; Suh and Checler, 2002). CTF31 can be produced by caspases (Gervais et al., 1999; Lu et al., 2000). Increased production of β-amyloid peptide (Aβ) has been thought to be involved in the pathogenesis of Alzheimer's disease (AD) (Steiner et al., 1999). Recent studies have postulated that βCTFs or other C-terminal fragments also play a role in AD-related pathogenesis (Suh and Checler, 2002), although the precise roles of these fragments in AD remain yet to be explored.

Presenilin-1 (PS1) may be a key component of γ-secretase complex. PS1 has been considered to be a therapeutic target for the treatment and the delayed expression of AD symptoms (Li et al., 2000; Esler and Wolfe, 2001). The PS1-deficient mice generated by the conventional knockout strategy were embryonic lethal (Wong et al., 1997). However, recent studies with a conditional knockout strategy have circumvented the lethality of PS1-deficient mice and generated adult animals lacking PS1 specifically in the brain (Yu et al., 2001b; Dewachter et al., 2002). The study involving these double transgenic mice carrying both the PS1 conditional mutation and the APP_{V717I} transgene revealed that the elimination of the γ-secretase activity provided by PS1 markedly reduced Aβ production, plaque deposition, and rescued impaired

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hippocampal LTP but that it did not correct the deficits in learning that APP_{V717I} transgenic mice displayed (Dewachter et al., 2002). Although the underlying mechanism has not yet been clearly elucidated, these studies show that the loss of γ-secretase activity in the brain leads to the severe accumulation of βCTF99 and raises the possibility that βCTF99 accumulation might cause cognitive deficits in the absence of plaque deposition in their double transgenic mice. It also raises the question as to whether other biochemical impairments or behavioral alterations present in APP_{V717I} transgene mice can be reverted in their double transgenic mice. Regarding that PS1 has pleiotropic roles in brain cell functions, e.g., Notch (Naruse et al., 1998; Song et al., 1999) and N-cadherin (Marambaud et al., 2003) processing, it needs to be answered whether the observed learning deficits of the conditional PS1 knockout mice used in their studies were produced solely by βCTF99, by other PS1-cleaved products, or by the lack of a PS1-dependent physiology.

More direct evidence for the in vivo role of βCTF99 is to be ascertained from studies with transgenic mice expressing βCTF99 in the brain. To date, nine research groups have independently created transgenic mouse lines expressing various CTF forms of the human APP in the brain. Five of these lines showed either neuronal atrophy (Neve et al., 1996; Oster-Granite et al., 1996; Nalbantoglu et al., 1997; Sato et al., 1997) or impaired learning (Nalbantoglu et al., 1997; Berger-Sweeney et al., 1999; Lalonde et al., 2002a) at age 12–28 months, whereas the other four lines including the recent reported one did not display any obvious neuronal loss or cognitive impairment (Shoji et al., 1990; Sandhu et al., 1991; Araki et al., 1994; Sberna et al., 1998; Li et al., 1999; Rutten et al., 2003). Thus, the developed transgenic mice expressing CTFs showed conflicting results that ranged from no phenotype to AD-like pathogenesis. Accordingly, the in vivo role of βCTF99 remains elusive. All CTF-expressing transgenic lines developed thus far were created from compound genetic backgrounds, such as the C57BL/6–DBA/2 hybrids (Li et al., 1999; Rutten et al., 2003), the C57BL/6–SJL hybrids (Kammesheidt et al., 1992; Fukuchi et al., 1996; Oster-Granite et al., 1996), or the C3H–C57BL/6 hybrids (Nalbantoglu et al., 1997; Sato et al., 1997). It is not clear whether these complex genetic backgrounds contribute to the conflicting results on the in vivo role of βCTF99 or the difference in the design of transgenic cassettes including the choices of promoters and the specific sequences of the transgene itself is involved.

The current study was undertaken to evaluate the role of βCTF99 in the pathophysiology of the aging brain, using a newly generated transgenic mouse line expressing βCTF99 in inbred C57BL/6 mice. We found that the transgenic expression of βCTF99(V717F) is highly toxic to the aging brain and results in the progressive and age-dependent expression of the biochemical markers of AD, in combination with neuronal loss, and impaired psychiatric and cognitive behaviors.

Materials and methods

Generation of βCTF99 transgenic mice

The cloning of the human APP cDNA sequence has been described recently (Lee et al., 2004). The cDNA sequence for βCTF99 carrying the V717F mutation of APP751 was produced by a PCR method using the second half of APP751 cDNA as a template and the primer sets of app-sig-f (5′-CGATTTAGATCTTGACGGGGAAAG-3′), app-sig-r (5′-CGGAATTCTGCATCCGCCCGAGCCGTCCAGGCGGC-3′). Here, V717F in βCTF99(V717F) represents the Indiana mutation described previously (Lee et al., 2004). The resulting product was subcloned into the *Bam*HI/*Eco*RI site of pBluscript II, which was used for another PCR using the primers of app-koz-f (5′-GCTCTAGACCATGCTGCCCGGTTTGGCACTGCTC-3′), app-koz-r (5′-CCCGCGCGGCGGCCGCTTCATTAATG-3′). After digesting with *Eco*RI, the resulting product was fused to the βCTF cDNA to translate the signal peptide and βCTF fusion protein. To increase the translation efficiency, we introduced the Kozak sequence (GACC) in front of the ATG codon of the signal sequence. The V717F mutation of APP751 was introduced by PCR using two primers of app-717-f (5′-GCGACAGTGATCTTCATCACCTTG-3′) and app-1r (5′-GGGGGACTAGTTCTGCATCTGCTC-3′).

The intron B of human β globin gene was amplified by PCR using the primers of hglob-f (5′-GATCCTGAGAACTTCAGG-3′) and hglob-r (5′-TCTTTGCCAAAGTGATGG-3′) and genomic DNA, which was obtained from the human neuroblastoma cell line SH-SY5Y, as a template. The resulting 918 bp fragment was inserted between the PDGF-β promoter and the βCTF99(V717F) cDNA. The SV40 late polyadenylation sequence (247 bp) was prepared from pGK-neo-pA (Lee et al., 2002). The PDGF-β gene promoter (1.2 kb) was a gift from Dr. Tucker Collins (Harvard Medical School, USA). Finally, PDGF-βCTF99(V717F)-pA and PDGF-β-intron-βCTF99(V717F)-pA were constructed. The APP signal sequence and mutation in βCTF99(V717F) were confirmed by DNA sequencing. Each of the purified linearized transgenic constructs was microinjected into the pronuclei of fertilized eggs prepared from inbred C57BL/6 mice (Korea Research Institute of Chemical Technology, Daejon, Korea). The injected eggs were transferred to the oviduct of pseudopregnant female (ICR) mice by following a standard method (Horgan et al., 1994).

The transgenic lines were determined by genomic Southern blot and PCR methods. Blots carrying 15 μg per lane of *Spe*I-digested genomic DNA were hybridized with a ^{32}P-labeled probe prepared from the 350 bp *Spe*I fragment at the C-terminus of APP cDNA (Fig. 1A). The PCR primers used were trapp-fs (5′-GCTTGATATCGAATTCCTGCAGC-3′) and trapp-r1 (5-ATGTATCTTATCATGTCTGGACCG-3′) for the amplification of βCTF99 (-intron), trint-f1 (5′-AATGTATCATGCCTCTTTGCACC-3′), and sv40pA-r1 (5′-GTTCGAGCTCATAATCAGCCATACCACATTTG-3′) for the amplification of βCTF99. The transgenic mice lines were maintained by crossing with C57BL/6 inbred mice. The transgenic mice used in this study were heterozygous with respect to the transgene.

Northern blot analysis

Northern blot analysis was performed as previously described (Lee et al., 2004). To be brief, a membrane blot carrying 30 μg of total RNA was prepared after separating on denaturing agarose gel (1% agarose, 6.2% formaldehyde in 0.5× MOPS) and hybridized with a ^{32}P-labeled probe prepared from the *Spe*I-digested fragment (350 bp) of βCTF99 (Fig. 1B).

Western blot analysis and histological works

Western blot analysis and immunostaining were performed as previously described (Che et al., 2001). For the Western blot analysis, mouse brain tissue was homogenized in 4°C lysis buffer