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Sensitive quantitative assays for tau and phospho-tau in transgenic mouse models

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Abstract

Transgenic mouse models have been an invaluable resource in elucidating the complex roles of β-amyloid and tau in Alzheimer's disease. Although many laboratories rely on qualitative or semiquantitative techniques when investigating tau pathology, we have developed 4 Low-Tau, Sandwich enzyme-linked immunosorbent assays (ELISAs) that quantitatively assess different epitopes of tau relevant to Alzheimer's disease: total tau, pSer-202, pThr-231, and pSer-396/404. In this study, after comparing our assays with commercially available ELISAs, we demonstrate our assay's high specificity and quantitative capabilities using brain homogenates from tau transgenic mice, htau, JNPL3, and tau knockout. All 4 ELISAs show excellent specificity for mouse and human tau, with no reactivity to tau knockout animals. An age-dependent increase of serum tau in both tau transgenic models was also seen. Taken together, these assays are valuable methods to quantify tau and phospho-tau levels in transgenic animals, by examining tau levels in brain and measuring tau as a potential serum biomarker.

Keywords: Alzheimer's disease; Tau-ELISA; Tau transgenic mice; htau; JNPL3; Sera

1. Introduction

Alzheimer's disease (AD), the most common form of neurodegenerative disease, is characterized pathologically by the formation of 2 protein lesions: neuritic plaques composed of β-amyloid and neurofibrillary tangles (NFT) composed of the microtubule-associated protein tau. The connection between these 2 pathologies remains unclear. However, it seems that tau pathology more closely correlates with neuronal loss and severity of dementia (Arriagada et al., 1992; Bancher et al., 1993; Gómez-Isla et al., 1997; Guillozet et al., 2003). NFTs are mainly comprised of aggregated paired helical filament (PHF)-tau (Grundke-Iqbal et al., 1986; Ihara et al., 1986), and are found in other forms of dementia collectively known as tauopathies some of which are due to mutations in the tau gene (Hutton et al., 1998; Poorkaj et al., 2001; Spillantini et al., 1998). With the recent push toward disease-modifying therapies, it is critical to further elucidate the roles of β-amyloid and tau in AD, and, ultimately, observe how potential therapies may affect the underlying mechanisms.

The formation of filamentous tau seems to occur in several stages, from pretangles to intracellular tangles to extracellular tangles, with a sequential phosphorylation pattern occurring as tau pathology develops (Augustinack et al., 2002; Kimura et al., 1996). There is also evidence, in vitro, that certain phospho-tau epitopes require a particular order of phosphorylation events. The AT100 epitope requires first an initial stimulatory phosphorylation at Ser-

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199, Ser-202, and Thr-205 in any order, then at Thr-212, and finally at Ser-214 (Zheng-Fischhöfer et al., 1998). Being able to quantitatively examine relevant site-specific phosphorylation as tau pathology progresses, or in contrast, is affected by disease-modifying therapies, is a key step in moving the field forward. Currently, few quantitative techniques are available for examining the mechanisms of tau hyperphosphorylation in mouse models, and investigators are required to rely on semiquantitative methods such as immunohistochemistry or immunoblot analyses. There are commercially available enzyme-linked immunosorbent assays (ELISAs) for quantitative analysis of tau, for example from Innotest or Invitrogen, that are mainly used in cerebrospinal fluid (CSF) for biomarker detection (Barten et al., 2011; Lachno et al., 2011; Vanderstichele et al., 2006). The high cost of these assays is prohibitive for their use by investigators at many academic institutions. Moreover, the availability of reliable and relevant phospho-tau epitope assays is very limited.

In this study, we have developed 4 different assays suitable for the detection of low levels of mouse and human tau, referred to as Low-Tau, Sandwich ELISA. Taking advantage of newly characterized and previously established tau monoclonal antibodies, we were able to selectively focus on total tau (DA31) and phospho-tau epitopes pertinent in AD including pSer-202 (CP13) (Duff et al., 2000; Lewis et al., 2000), pThr-231 (RZ3) (Vingtdeux et al., 2011), and pSer-396/404 (PHF-1) (Greenberg et al., 1992; Otvos et al., 1994). In order to demonstrate the specific and quantitative qualities of the assays, brain homogenates from 2 different tau transgenic models were used: htau mice, which express all 6 isoforms of the normal human tau protein (Andorfer et al., 2003; Polydoro et al., 2009), and JNPL3 (P301L), which express 0N4R human tau with the P301L mutation (Lewis et al., 2000; Lin et al., 2003a, 2003b). Interestingly, our ELISAs demonstrate enough sensitivity to detect total tau and pSer-202 tau in the serum of these transgenic mice, with an age-dependent increase of tau in serum. We also compared our total tau ELISA (DA31) to commercially available human ELISA kits from Invitrogen (Life Technologies, Grand Island, NY, USA) and Innotest (Innogenetics, Alpharetta, GA, USA) demonstrating its versatility. Hence, these 4 Low-Tau, Sandwich ELISAs are highly sensitive and practical assays to quantify total and phosphorylated tau levels in brain and serum of transgenic mice.

2. Methods

2.1. Production of monoclonal antibodies

Monoclonal tau antibodies DA9 and DA31 were generated as previously described (Davies, 2000). Briefly, 4 intraperitoneal injections of 0.2 mL of purified PHF-tau, at a concentration of 1–2 mg/mL, were administered to a cohort of tau knockout (KO) mice, over an 8-week period. PHF-tau was isolated from severe AD human brain, as previously described (Jicha et al., 1999). These tau KO mice have a targeted disruption by the insertion of green fluorescent protein cDNA into exon 1 of tau (Tucker et al., 2001). The immunized mice were last injected 3 days before the spleen was removed. The spleen cells were fused with myeloma cells (NSO cells) in the presence of polyethylene glycol. The fusion products were plated in 96-well plates in selection medium containing hypoxanthine-aminopterin-thymidine (Invitrogen). Positive clones were identified by assaying the culture media both by PHF-ELISA and by immunoblot. Clones with high specificity for tau were selected, cloned, expanded, and ultimately screened on the epitope mapping ELISA described below.

2.2. Immunoblotting

For initial characterization of the new total tau antibodies, DA9 and DA31, immunoblotting was performed. Recombinant tau, Tau441 (TauA), tau without exons 2, 3, and 10 (TauD), Tau366 (amino acids 1–366), Tau270 (amino acids 1–270), and Tau190 (amino acids 1–190) (constructs are C-terminal deletions) were expressed using pcDNA vectors. Cos7 cells were transfected with a mixture containing 2 μg of cDNA and Lipofectamine 2000 reagent (Invitrogen), in serum-free medium for 6 hours at 37 °C and homogenized after 48 hours. Finally, cells were harvested in Tris-buffered saline (TBS)-based homogenizing buffer: TBS, pH 7.4, containing 1 mM Na_3VO_4, 2 mM ethyleneglycol-O, O'-bis(2-aminoethyl)-N, N, N', N'-tetraacetic acid (EGTA), and 10 mM NaF and Complete Mini-EDTA protease inhibitor cocktail (Roche). Cell homogenates were kept at −80 °C until use. Cellular lysates together with brain homogenates obtained from wild type mice, tau KO mice (Tucker et al., 2001), htau mice (Andorfer et al., 2003), and a PHF-tau preparation were boiled for 5 minutes in Laemmli sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Immunoblotting for RZ3 characterization was run on homogenate samples of healthy control and confirmed AD patients, as well as homogenate from wild type mice, tau KO mice, and htau mice. Brain homogenate preparations are described in a later section. Membranes were probed with human specific total tau CP27 (amino acids 130–170) (Duff et al., 2000; Lewis et al., 2000), DA9, and DA31 at 1:50 dilution, and purified RZ3 was used at a concentration of 2 μg/mL.

2.3. Epitope mapping ELISA

The characterization of 3 new monoclonal tau antibodies, total-tau DA9, total-tau DA31, and RZ3 (pThr-231) was performed as previously described (Espinoza et al., 2008). Briefly, for RZ3 antibody, 96-well plates were coated with NeutrAvidin protein (ThermoScientfic) at a concentration of 2 μg/mL overnight at 4 °C in coating buffer (15 mM KH_2PO_4, 25 mM KH_2PO_4, 0.1 M NaCl, 0.1 M ethylenediaminetetraacetic acid (EDTA) and 7.5 mM NaN_3, pH 7.2).