Immunohistochemical Comparison of Antibodies Against Tau in Brain Sections from Human AD and Normal, and Mouse Wild-Type and an AD Model

*C. SEGOVIA, R. C. SWITZER, III, A. P. OSMAND

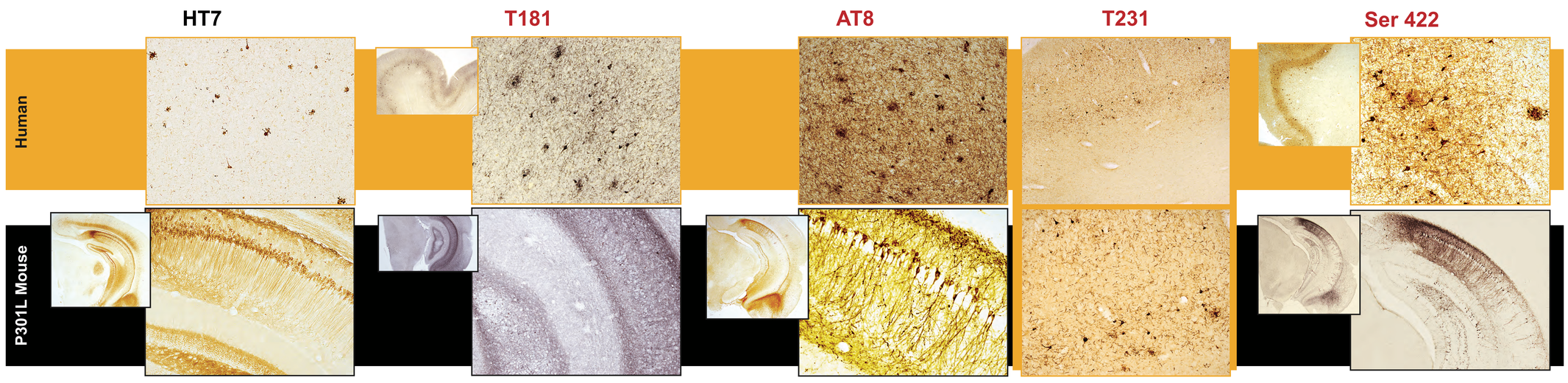
NeuroScience Associates, Knoxville, TN; BCMB, Univ. of Tennessee, Knoxville, TN

Abstract

The paired helical filaments (PHFs) of Alzheimer's Disease (AD) consist mainly of the microtubule-associated protein tau. Tau protein promotes assembly and stabilizes microtubules, which contributes to the proper function of neurons. Phosphorylation alters the structure of tau protein and can affect its role as a stabilizer of microtubules. Numerous phosphorylation sites have been identified in tau protein in AD brains. Antibodies have been generated against different phosphorylated sites of the tau protein as well as against unphosphorylated sites. In this study we compared the Immunohistochemistry staining results of several antibodies against phosphorylated epitopes as well as non-phosphorylated in mouse (WT and P301L) and human (No AD pathology and AD pathology) in free floating sections. We found that antibodies against phosphorylated epitopes (pThr231, pSer396, pSer422, pThr181 & pSer202 & Thr205 (AT8) stained features of AD pathology in the human AD and for the AD-like features in the P301L mouse model of AD except for pThr231 (a.k.a. AT180). There was no staining in normal human or mouse wild type (WT) brain sections with antibodies against non-phosphorylated tau (HT7, Tau 5, Tau 46, Tau 39E10).

Materials and Methods

We used sections from an 85 year old AD patient, and from a mouse model for AD (P301L) kindly provided by Dr. Kimberly Scearce-Levie from Genentech. Sections were processed according to MultiBrain® Technology. For all antibodies tried, the sections were stained free floating, incubated in primary antibody overnight at room temperature, and developed with the Vecta ABC and Ni/DAB, or DAB, accordingly. A broad range of concentrations were applied for each antibody to optimize the dilution. The sections were mounted, air dried, dehydrated and cover-slipped before visualizing.



Discussion

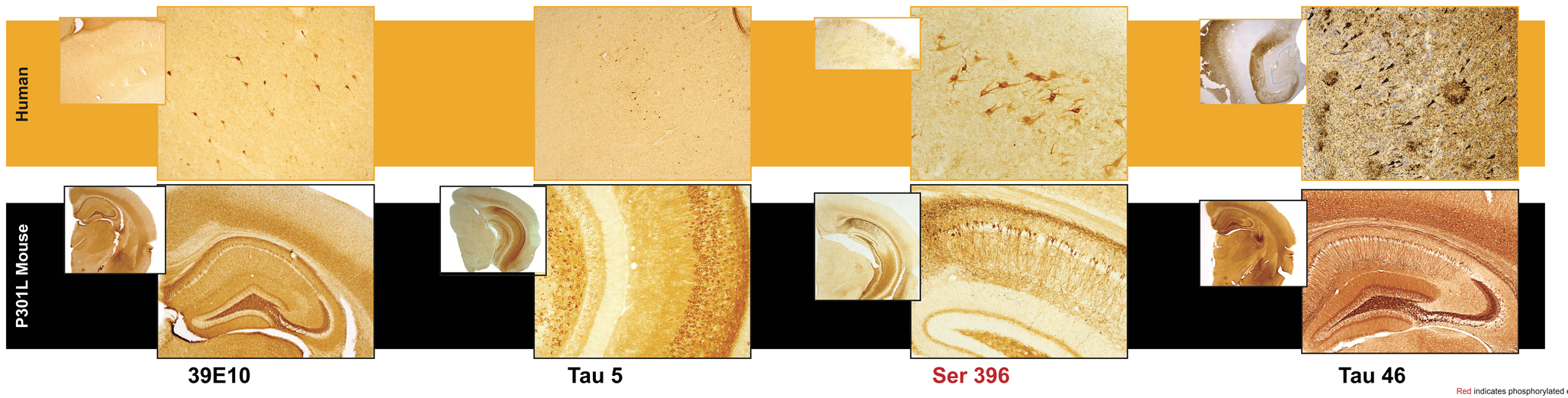
For most antibody vendors, information about validation of antibodies for IHC is scarce and sometimes even nonexistent. Even more perplexing is the lack of validation of antibodies applied to mounted or free floating sections. Furthermore, Tau nomenclature is inconsistent, contains numerous synonyms and lacks any convention for naming tau antibodies.

The brains sections from AD human and from the human "like" AD mouse model P301L show reactivity to all of the antibodies mentioned above with the exception of anti-Tau pThr231 (a different source will be tried). (Figures to the right.)

We found that the brain sections from normal human and wild type mice, did not show reactivity to any of the antibodies tried, whether directed toward phosphorylated epitopes or not, in our MultiBrain free floating sections (figures not shown). This observation is exemplified by the IHC results with the Tau 46 antibody that has an antigenic target somewhere in the C terminal segment spanning amino acid #s 404-441. While this sequence of amino acids is conserved in human and mouse tau and is present in all 6 tau isoforms in human and all 3 in the mouse, no staining was observed in normal human or wild type mice. Robust staining, however, was observed in the human AD and mouse P301L brains sections to show neuritic plaques, neuron cell bodies with neurofibrillary tangles and neuropil threads. (Figures to the right.)

The lack of staining of 'normal' tau has been addressed earlier by Trojanowski et. al. (1989)* where they demonstrated that some epitope unmasking techniques were needed to expose epitopes concealed due to formaldehyde fixation. We speculate that in the AD brain and AD mouse models, where tau is phosphorylated and thereby partially or totally dislodged from mircotubules, that in this state, the nonphosphorylated sites are 'exposed' and accessible to antibodies. To reveal the same epitopes in normal tissue, a means of 'dislodging' tau from the microtubules could allow antibodies against nonphosphorylated segments to reveal normal tau.

*Trojanowski, J.Q. et al, Journal of Histochem. Cytochem, V.37, pp 209-215, 1989



Red indicates phosphorylated epitopes