Carboxy-Terminus Tau Protein Hyperphosphorylation is Associated with Extracellular Deposits of Amyloid-β Fibrillary in a Triple-Transgenic Model of Alzheimer’s Disease

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Commentary

The cognitive decline in Alzheimer’s disease (AD) is associated with the accumulation of neurofibrillary tangles (NFTs) [1] and neuritic plaques (NPs) [2,3]. The NFTs are formed by the accumulation of paired helical filaments (PHFs) in neuronal soma, consisting of tau protein. NPs contain extracellular amyloid-β peptide (Aβ) and dystrophic neurites (DNs) containing PHFs. The development of transgenic animals, has led to a better understanding of the abnormal processing of tau protein and amyloid-β. Oddo et al. in 2003 generated a triple transgenic mouse model (3xTg-AD), in which there is intracellular accumulation of tau protein and extracellular deposition of amyloid-β plaques [4]. The expression and aggregation of human tau protein in the 3xTg-AD mouse (aged between 3 and 28 months) were analyzed using a panel of antibodies directed against different epitopes of phosphorylated tau, amino (N-) and carboxy (C-) terminal domains, using double and triple immunostaining and confocal microscopy. Our results show there is over-expression of human tau protein in the 3xTg-AD mouse from three months of age in the CA1 area of the hippocampus and that the levels increase with age (Figure 1). Phosphorylation of tau protein in the N-terminal domain is associated with mice of a young age. Phosphorylation of tau protein at the C-terminal appears at 14 months, following the first deposits of extracellular, fibrillar Aβ that arises by 11 months. These data suggest that Aβ may generate a toxic environment for the neuron probably promoting tau phosphorylation (Figure 2). Furthermore, the expression of multiple pro-inflammatory cells and cytokines, contributing to an inflammatory microenvironment that may link these events, was also investigated. The 3xTg-AD mouse expresses 3 mutant human genes: β-amyloid precursor protein (βAPPsw), presenilin-1 (PS1M146V), and TauP301L. Both βAPPsw and PS1M146V are found in familiar AD patients and in this model, are responsible for Aβ deposits. Meanwhile, TauP301L is a tau mutation associated with frontotemporal dementia and, in this model, is responsible for hyperphosphorylation of tau protein [5].

To characterize the expression of human tau in the brains of 3xTg-AD transgenic mice at several ages (3, 4, 7, 9, 11, 18, 19, 20, 22, 26 and 28 months), Monoclonal antibody (mAb) 499 was used. Since mAb 499 recognizes a human-specific segment between residues 14-26, not present in murine Tau, it differentiates mutant TauP301L from endogenous murine Tau. mAb 499 showed reactivity exclusively in 3xTg-AD brains as compared with the wild-type mice. Human tau was first detected on the CA1 region of the hippocampus by 3 months of age, with an increased expression with advancing age. Then by 7 months of age, the tau was detected in cortical cells, the hypothalamus, and the amygdala predominantly with a cytoplasmic distribution. To study the different post-translational modifications of human tau in the 3xTg-AD transgenic mice brains, immunostaining with antibodies against specific phosphorylation sites and conformational changes of tau was performed and the findings were as follows: mAb A520, which recognizes a conformational change involving residues 5-15 and 312-

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Accumulation of Aβ in the 3xTg-AD mouse brains was investigated using the antibody 6E10, which recognizes residues 3-8 of Aβ, in conjunction with counterstaining with thiazine red (TR), a fluorescent dye that differentiates between fibrillar and nonfibrillar stages of aggregation of both tau and β-amyloid. The aggregation process of Aβ in this mouse starts as soluble intracellular deposits that precede the fibrillar extracellular deposits, referred to as NPs. Intracellular deposits of Aβ were observed from 6 months of age as vesicle-like structures in fibrillar extracellular deposits, referred to as NPs. Intracellular deposits in this mouse start as soluble intracellular deposits that precede the expression of proteins.

322 of the tau sequence, was detected by 3 months of age in cortical neurons and the CA1 region of the hippocampus. The immunohistochemical distribution was cytoplasmic and colocalized with mAb 499 reactivity. Phosphorylation of Ser-199 was detected in the subiculum by 3 months of age, having a nuclear distribution and, by 9 months of age, this distribution became cytoplasmic and colocalized with mAb 499 reactivity. The expression of proteins

This extracellular Aβ aggregates in triple transgenic mice seem to correlate, at least in a temporal manner, with the change of expression of AT100 antibody from a nuclear to a cytoplasmic distribution and also with the overexpression of GFAP, Iba1 and TNF-a, key players in the inflammatory process. This inflammatory environment in the 3xTg-AD transgenic mouse brain may upregulate several kinases involved in tau protein hyperphosphorylation. Finally, to determine whether there were changes in the expression of some of these kinases, western blots and immunostaining were performed to detect SAPK/JNK and Cdk5. Expression of both SAPK/JNK and Cdk5 was observed by 12 months of age and increased with age followed by a sharp decrease at 26 months, whereas GFAP expression increased at 22 and 26 months. The expression level of Iba1, a molecule that recognizes activated microglia, was found to be increased from 12 months of age.

Thus, extracellular Aβ aggregates in triple transgenic mice seem to correlate, at least in a temporal manner, with the change of expression of AT100 antibody from a nuclear to a cytoplasmic distribution and also with the overexpression of GFAP, Iba1 and TNF-a, key players in the inflammatory process. This inflammatory environment in the 3xTg-AD transgenic mouse brain may upregulate several kinases involved in tau protein hyperphosphorylation. Finally, to determine whether there were changes in the expression of some of these kinases, western blots and immunostaining were performed to detect SAPK/JNK and Cdk5. Expression of both SAPK/JNK and Cdk5 was observed by 12 months of age and increased with age followed by a sharp decrease at 26 months, whereas GFAP expression increased at 22 and 26 months. The expression level of Iba1, a molecule that recognizes activated microglia, was found to be increased from 12 months of age.

All of these results show that, in the 3xTg-AD mouse brain, intracellular aggregation of Aβ may be first to occur but that extracellular aggregates are related, both in time and space, to an inflammatory microenvironment and increased expression of stress kinases potentially involved in tau phosphorylation. This may serve as a link between these two pathological hallmarks. The study also showed that phosphorylation of tau increases with age and that Tau N-terminal phosphorylation precedes phosphorylation of the C-terminal domain, solidifying the 3xTg-AD mouse as a great model for further AD research.

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