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

Miguel Angel Ontiveros-Torres1,2, Leoniel Castellanos-Aguilar1,2, Jonathan Lénnel Gutierrez Murcia1,3, Nayeli Martinez-Zuñiga1, Paola Flores-Rodriguez, Ilana Atenas Figueroa Álvila1, Berenice Dionisio de la Cruz1,2, Jorge Cisneros-Martinez1,4, Charles R. Harrington5, Claude M. Wischik1, Azucena Aguilar-Vazquez, Sofia Díaz-Cintra2, José Luna-Muñoz2*.

1Brain Bank-LaNSE of CINVESTAV-IPN, México, CDMX, México.
2Departamento de Bioingeniería. Instituto Tecnológico de Estudios Superiores de Monterrey. Toluca, México
3Centro cultural Universitario Justos Sierra. Lic. QFB. CDMX, México
4Center for Interdisciplinary Sleep Research, Department of Physiology, Faculty of Medicine and Nutrition, Juarez University of Durango, Durango, México.
5School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK
6Instituto de Neurobiología, Universidad Nacional Autónoma de México. Campus UNAM Juriquilla, Querétaro, México

**Commentary**

The cognitive decline in Alzheimer’s disease (AD) is associated with the accumulation of neurofibriylar 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 (βAPPswe), presenilin-1 (PS1M146V), and TauP301L. Both βAPPswe 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 Alz50, which recognizes a conformational change involving residues 5-15 and 312-

**Figure 1:** Immunostaining with mAb 499 (green) and pAb S199 (red). Mouse hippocampus 3xTg-AD 3 months old (a). The first accumulations of tau phosphorylated at ser-199 and intact tau protein was observed in the cell bodies of the CA1 area adjacent to the subiculum (arrows, b). By 9 months of age, tau expression was abundant in the CA1-CA2 areas (c). At 18 months of age, the expression of both 499 and S199 antibodies colocalized extensively in the neuronal soma and processes. Expression of human protein tau neuronal population invades into the hippocampal area.

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322 of the tau sequence, was detected by 3 months of age in cortical neurons and the CA1 region of the hippocampus. The immunochemoical distribution was cytoplasmic and colocalized with mAb 499 reactivity. Phosphorylation of Ser-199 was detected in the subiculum by 3 months of age. Phosphorylation sites in the C-terminus of tau, such as polyclonal antibody pS400, were detected from 7 months using pAb pS396 and from 18 months and pAb PT231 for 19 months. Phosphorylation at Thr-231, recognized by pAb PT231, was observed at 18 months. mAb AT100, which recognizes phosphorylation at Thr-212 and Ser-214, was observed in the subiculum and CA1 region of the hippocampus from 3 months of age, having a nuclear distribution and, by 9 months of age, this distribution became cytoplasmatic. The expression of proteins antibodies increased with age [6].

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 the periphery and mAb 499 and Alz50 signal colocalization was observed in some neuronal cell bodies (arrowheads; h, i) (image magnifications g). j) Image of an amyloid (Aβ) fibrillar plaque evidenced by the red dye thiazine (k), that is bordered by astroglial cells, which cytoplasm project into the plaque (j and l).

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Figure 2: Immunolocalization of amyloid-β peptide in the brain of the triple transgenic mouse (3xTgAD). a) Accumulation of granule vacuolar amyloid-β peptide [6] in the neuronal soma evidenced by 499 antibody that recognizes the intact tau protein (arrow heads; b, c). d) extracellular deposition of Aβ stained using antibody 6E10 (arrow; e, f). At the periphery, neuronal processes immunoreactive with mAb 499 were observed (small arrows). g) Triple immunostaining with antibody 6E10 (green channel), 499 (red channel) and Alz50 (blue channel). Aβ deposits are seen mainly at the stratum oriens (SO) and stratum reticulata (SR). The pattern of amyloid-β peptide aggregation is diffuse in the periphery and mAb 499 and Alz50 signal colocalization was observed in dendritic tree at SO and SR. The same colocalization was observed in some neuronal cell bodies (arrowheads; h, i) (image magnifications g). j) Image of an amyloid (Aβ) fibrillar plaque evidenced by the red dye thiazine (k), that is bordered by astroglial cells, which cytoplasm project into the plaque (j and l).
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