

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-Torres^{1,2}, Leonel Castellanos-Aguilar^{1,3}, Jonathan Lénnel Gutierrez Murcia^{1,3}, Nayeli Martínez-Zuñiga¹, Paola Flores-Rodríguez⁴, Iliana Atenas Figueroa Ávila^{1,5}, Berenice Dionisio de la Cruz^{1,5}, Jorge Cisneros-Martínez⁴, Charles R. Harrington⁶, Claude M. Wischik⁶, Azucena Aguilar-Vazquez⁷, Sofia Díaz-Cintra⁷, José Luna-Muñoz^{1*}

¹Brain Bank-LaNSE of CINVESTAV-IPN, México, CDMX, México.

²Departamento de Bioingeniería. Instituto Tecnológico de Estudios Superiores de Monterrey. Toluca, México

³CQB. Department of Physiology IPN. México CDMX, México

⁴Center for Interdisciplinary Sleep Research, Department of Physiology, Faculty of Medicine and Nutrition, Juarez University of Durango, Durango, México.

⁵Centro cultural Universitario Justos Sierra. Lic. QFB. CDMX. México

⁶School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK

⁷Instituto 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 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 (A β) 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 A β that arises by 11 months. These data suggest that A β 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 (β APPswe), presenilin-1 (PS1M146V), and TauP301L. Both β APPswe and PS1M146V are found in familial AD patients and in this model, are responsible for A β 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-

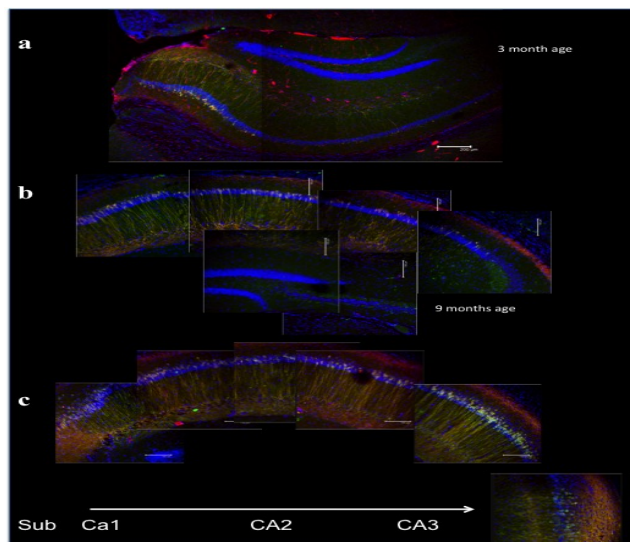


Figure 1: Immunostaining with mAb 499 (green) and pAb S199 (red). Mouse hippocampus 3xTg-AD 3 months old (a). The first accumulations of tau phosphorylated at ser-199 and intact tau protein was observed in the cell bodies of the CA1 area adjacent to the subiculum (arrows, b). By 9 months of age, tau 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.

***Corresponding author:** José Luna-Muñoz, Brain Bank-LaNSE of CINVESTAV-IPN, México, CDMX, México, Tel: +52 57473800; ext:1748; Fax: +52 57473800; E-mail: jluna@cinvestav.mx

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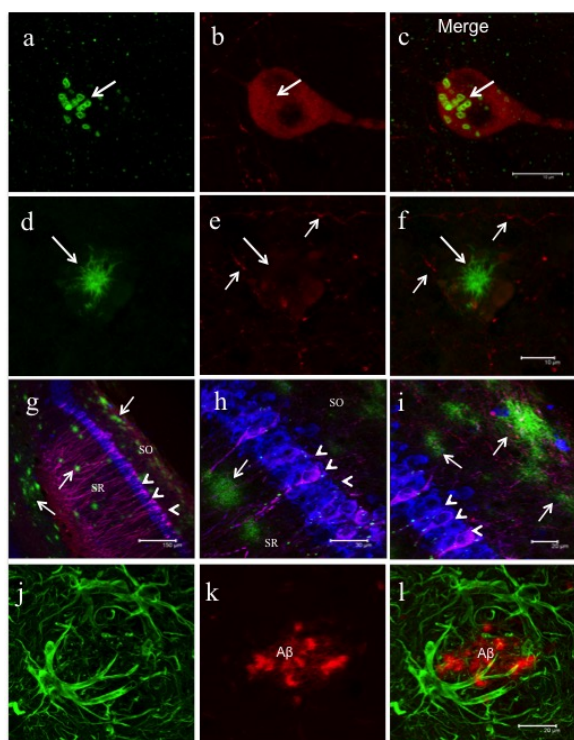


Figure 2: Immunolocalization of amyloid- β peptide in the brain of the triple transgenic mouse (3xTgAD). a) Accumulation of granule vacuolar amyloid- β peptide [6] in the neuronal soma evidenced by 499 antibody that recognizes the intact tau protein (arrow heads; b, c). d) extracellular deposition of A β 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). A β 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 (A β) fibrillar plaque evidenced by the red dye thiazine (k), that is bordered by astroglial cells, which cytoplasm project into the plaque (j and l).

322 of the tau sequence, was detected by 3 months of age in cortical neurons and the CA1 region of the hippocampus. The immunochemical 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 (pAb) pS400, were detected from 7 months using pAb pS396 and from 18 months and pAb pT422 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 cytoplasmic. The expression of proteins antibodies increased with age [6].

Accumulation of A β in the 3xTg-AD mice brains was investigated using the antibody 6E10, which recognizes residues 3-8 of A β , 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 A β in this mouse starts as soluble intracellular deposits that precede the fibrillar extracellular deposits, referred to as NPs. Intracellular deposits of A β were observed from 6 months of age as vesicle-like structures in subiculum and CA1, which increased at 7 and 9 months. After 9 months,

the first extracellular deposits of A β were observed in subiculum from the ages between 18-28 months. A β aggregates could be found both in subiculum and CA1. Furthermore, these extracellular deposits were fibrillar (TR positive). To determine if extracellular A β aggregates may trigger an inflammatory process and microenvironment in the 3xTg-AD mice brains, we used pAb GFAP (Glial Fibrillary Acidic Protein) as an indicator of glial cell (mainly astrocytes) activation, which indicates an inflammatory process is taking place. It was found that GFAP reactivity was increased in hippocampus of 3xTg-AD brains compared to the Wild type (WT) at 11 months. This GFAP expression also increased with age and was colocalized with extracellular A β plaques. Furthermore, the presence of TNF- α , a key cytokine in inflammation was determined in the subiculum and hippocampus of 3xTg-AD transgenic mice brains with anti-TNF- α antibodies through immunofluorescence and western blot. TNF- α expression was first detected by immunostaining at 18 months of age, increased with age, and furthermore showed colocalization with both A β plaques and activated astrocytes. Western blot showed an increased TNF- α expression from 12 months of 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 recognises activated microglia, was found to be increased from 12 months of age.

Thus, extracellular A β 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- α , 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 up to 22 and 26 months. These kinases were also localized in the soma of pyramidal neurons in the CA1 region of the hippocampus.

All of these results show that, in the 3xTg-AD mouse brain, intracellular aggregation of A β 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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