

IMPORTANCE OF CONFOCAL MICROSCOPY IN THE MOLECULAR PATHOLOGY OF NEURODEGENERATIVE DISEASES: ALZHEIMER'S DISEASE.

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The neuropathological hallmarks of Alzheimer's disease (AD, generally referred to as neurofibrillary tangles (NFTs), neuropil threads and distinct types of neuritic plaques are mainly composed by tau protein assembled in the form of abnormal paired helical filaments (PHFs) (1),(2). Several post-translational modifications in the tau molecule like phosphorylation (3), glycosylation (4) and truncation (5),(6) have been considered key mechanisms that promote the pathologic assembly of tau into the PHFs. Recently conformational changes in the normal tau molecule has been described as an early event in AD before the appearance of neurofibrillary changes (7),(8). A Glu³⁹¹ truncation of tau protein associated with NFTs has been proposed to progress according to the evolution of the neurofibrillary pathology (9). On the other hand, apoptotic mechanisms related with caspase activity are suggested to play a role in the early tau processing leading to PHF formation and neuronal loss. Recent evidences have demonstrated that PHF-associated tau carries a site of truncation at Asp421 which is detected by monoclonal antibody (mAb) TauC3 (10). The expression and distribution of abnormal tau protein associated with the neurofibrillary pathology in Alzheimer's disease (AD) has been long studied by our group by using laser scanning confocal microscopy. We perform experiments in AD brain tissue slices combining multiple immunofluorescent probes (fluorescent antibodies to tau protein) and three-dimensional reconstruction of images obtained from pathological structures. Confocal scanning based on the principle of rejection of out-of-focus rays of light allows collection of in-focus optical sections through several microns of the specimens (~ 50 µm) without optical interference from other focal planes which normally introduce blurring in the images. With this x/y and z-sectioning approach stacks of single optical sections from scanned objects can be projected and analyzed as three-dimensional rotations. With this approach we have demonstrated that the conformation change of tau protein detected by Alz-50 antibody is an early modification leading to the formation of PHFs. Alz-50 detects two discontinuous epitopes determined by sites 5-15 and 312-322. The uncommon epitope of the latter antibody appears to depend of the folding of the N-terminus over the repeated domains of tau molecule (11). In this conformation intact tau protein preserving both N- and C- termini is a requirement for the recognition of this antibody (12). Latter in the evolution of the neurofibrillary pathology another conformation change of tau protein is a product of partially truncation of the N- and C- termini. This conformation recognized by the antibody Tau-66 is commonly visualized at intermediate and late stages in the progression of AD (12). More recently we evaluated the early truncation of tau at Asp-421 by using the Tau-C3 antibody and found this truncation precedes that occurring at Glu-391 (13). In AD brain tissue we also visualized high number of neurons presented the nucleus suffering apoptosis coexisting with NFTs which were immunoreactive to mAb Tau-C3. A population of neurons

carrying Tau-C3 immunoreactive NFTs additionally displayed a granular cytoplasmic staining visualized with antibodies to both Caspase-3 and Cytochrome-3 was found in a population of NFTs. The fact that Cytochrome-C it is known to trigger the apoptotic cascade suggests a potential role in the NFT formation. These set of data together strongly supports that apoptosis mechanisms and truncation at Asp-421 may be early molecular steps involved in AD neuropathogenesis. We have proposed a progression of changes occurring in tau protein during the formation and progression of the neurofibrillary pathology in AD.

References

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