doi:10.1017/S143192761005840X
Microsc. Microanal. 16 (Suppl 2), 2010

"Live imaging, immunocytochemistry and electron microscopic studies of the effect of Alzheimer-like phosphorylation on tau"

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Understanding mechanistically the foundation of synaptic withering and loss that precedes cell death in Alzheimer's disease (AD) and other neurodegenerative diseases is pivotal. Accumulation of hyperphosphorylated tau and the disruption of microtubules are correlated with synaptic loss and pathology of AD. One hallmark lesion of neurodegenerative disease in AD is the initial appearance of neurofibrillary tangles inside neurons. These tangles are composed mainly of hyperphosphorylated tau, a microtubule-associated protein (MAP), polymerized into paired helical and straight filaments (PHFs/SFs). Impaired cognitive function and pathology of AD is correlated with this lesion (PHFs/SFs). In *in vitro* assays, hyperphosphorylated tau is disruptive to microtubule assembly, whereas tau, stimulates tubulin assembly and subsequent stabilization of microtubules. Microtubules are disrupted in AD. Microtubules are the "tracks" for axonal transport: disruption of the microtubules leads to compromised axoplasmic flow, dysfunction at the synaptic terminals, and eventually neuronal death.
We have shown that hyperphosphorylation of tau is a pivotal event in the process of neurodegeneration: 1) abolish tau biological activity, 2) inhibits the microtubule assembly, 3) self-polymerizes into tangles of filaments, 4) dephosphorylation restores tau biological properties [1]. Here our objective is to verify our working hypothesis (Fig. 1) of the involvement of tau phosphorylation toxic effect and study the influence of tau phosphorylation at Thr 212, Thr 231, and Ser 262 for tau-tau binding, self-assembly and the effect of its expression on the cells. Pseudophosphorylated tau was generated by site directed mutagenesis. Pseudophosphorylated tau was transiently expressed in PC12 cells, in CHO cells and in Porcine kidney epithelial cells (LLCPK) stably transfected for EB1-GFP (generously provided by Dr. Cassimeris [2]). The effect of expression of mutated taus on self assembly and binding to microtubules was studied by immunocytochemistry and by time-lapse microscopy. Caspase activation and apoptosis were determined by immunocytochemistry after transient transfection. For dynamic studies, we performed time-lapse fluorescent microscopy in two different systems: with fluorescent-tagged tau constructs that we generated and co-transfected in CHO cells with fluorescent-tagged tubulin and in the LLCPK cells transiently transfected with fluorescent tubulin and an inducible system for tau expression. We found that pseudophosphorylated tau aggregates in cells when Thr 212 is mutated to Glu, suggesting that phosphorylation at this site facilitates tau self-assembly. The expression of tau pseudophosphorylated at Thr212, Thr231, and Ser262 triggers caspase 3 activation in as much as 85% of the transfected cells and apoptosis to a lesser degree. These findings suggest that tau phosphorylation at Thr 212 facilitates tau self-aggregation, and that the combination of phosphorylation at Thr212, 231 and Ser262 in the same tau molecule can trigger toxic reaction. Pseudophosphorylated tau, as Alzheimer phosphorylated tau, sequesters normal tau [3].

References

[1] A.D. Alonso, *Current Alzheimer Res.* 5 (2008) 375.
[2] M. Piehl, *Mol. Biol. Of the Cell.* 14 (2003) 916.

[3] This research was supported by the Professional Staff Congress-City University of New York (PSC-CUNY) Research Award 62909-00 40, grants from the National Institute of Health (NIH-NIA) R15AG034524-01, and Alzheimer's Association (Chicago, IL) IIRG-09-133206. The cells provided by Dr. Lynne Cassimeris Lehigh University, Bethlehem, Pennsylvania are gratefully acknowledged.

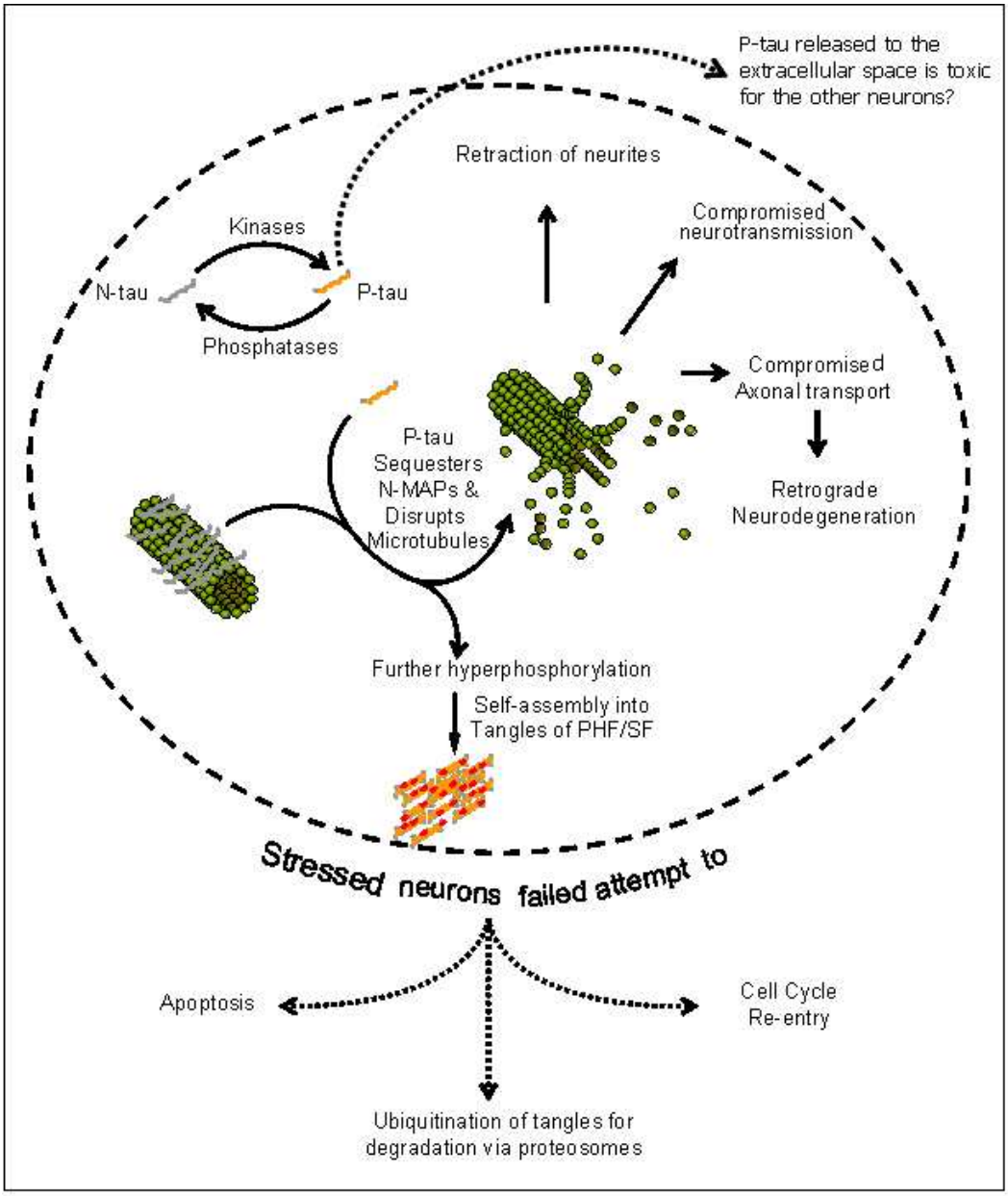

Figure 1. Hypothetical mechanism of tau-induced neurodegeneration.

https://doi.org/10.1017/S143192761005840X Published online by Cambridge University Press