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Death or secretion?

The demise of a plausible assumption about CSF-tau in Alzheimer Disease?

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Abbreviations: AD, Alzheimer's disease; Abeta, beta amyloid peptide; CNS, central nervous system; CSF, cerebrospinal fluid; 4R0N, tau isoform with 4 microtubule binding repeats and no N terminal inserts; Exon 2,3 – tau lacking N terminal inserts; MTBR, microtubule binding repeat; CJD, Creutzfeldt Jacob disease

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Our recent identification of an exosomal route for tau protein secretion[1] marks a key similarity between tau and other aggregation-prone proteins implicated in neurodegenerative disease pathogenesis and is to some extent congruent with the popular idea that tau pathology spreads between neurons via a "prionlike" template-mediated protein misfolding mechanism in AD and other tauopathies. However, the observation that much of the phosphotau in CSF samples from early AD patients is exosomal (and thus likely to have been secreted) calls into question a very widely held and plausible assumption - the idea that the elevated CSF-tau in AD is due to the passive release and accumulation of tau in the CSF as a consequence of widespread neuronal death. Here we examine this issue directly and explore some of the broader implications of this study for our understanding of AD pathogenesis and the prospects for improving its diagnosis and treatment.

While significant progress has been made toward understanding the role played by tau misprocessing in AD pathogenesis, much remains unclear. At the cellular level of analysis, misprocessing events such as oligomerization, hyperphosphorylation and cleavage that lead to tau aggregation via its microtubule binding repeat (MTBR) region have been linked to many aspects of tau mediated toxicity, especially in "tau-only" tauopathies associated with exonic point mutations in *tau*.[2-4] However, tau is now known to have many cellular functions beyond its classic role in stabilizing axonal microtubules via the MTBR,[5,6] including interactions with signal transduction and (now) unconventional secretory pathways, some of which are implicated in AD and non-AD tauopathy pathogenesis.[7] The classic view of a single MTBR-mediated tau toxicity mechanism in AD has been complicated over the past decade by numerous reports of an alternative toxicity mechanism that does not require the tau MTBR[8-11] and which mediates much of the neurotoxicity due to Abeta in AD.[12,13] The existence of separate MTBR+ and MTBR- tau toxicity mechanisms is particularly relevant to our emerging understanding of interneuronal aspects of tauopathy pathogenesis, which until very recently was based solely on neuropathology patterns suggestive of paracellular and trans-synaptic lesion propagation in AD.[14-16] More recently, we and others have characterized tau secretion, uptake and extracellular toxicity in various cell culture models[1,17-20] and have shown that tau can be secreted from and taken up by non-moribund neurons in situ in a cell-autonomous non-transgenic model.[18,21,22] and that tau misprocessing and secretion resembles that seen in other aggregate-associated diseases, including prion diseases.[23-25] These studies emphasize both the likely relevance of tau secretion to tauopathy pathogenesis and the multiplicity of possible tau transfer and toxicity pathways. While recent studies in murine transgenic models suggest the operation of a "prionlike" mechanism of lesion spread via templated protein misfolding,[26-28] the possible involvement of MTBR- and/or receptor-mediated interneuronal toxicity mechanisms indicate that the identification of any one mechanism as responsible

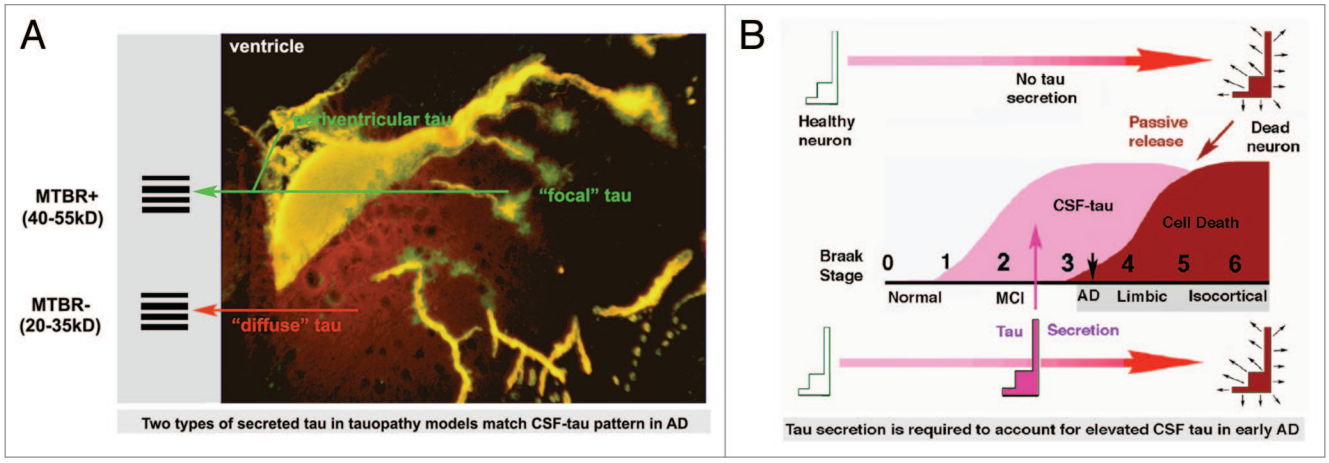

Figure 1. Elevated CSF levels of total tau and phosphotau in early AD are better explained by secretion of misprocessed tau from neurons and glia rather than a consequence of massive neuronal death. **(A)** CSF-tau in AD consists largely of 1) N-terminal fragments between 20–35kD apparent molecular weight with a variable admixture of higher MW species that appear to represent near full length tau. These match the secreted tau species seen in both in situ and cell culture models of tau secretion. The image at right shows an identified neuron (ABC) in the lamprey brain expressing 4R0N human tau with the P301L tauopathy mutation after 20 d of expression immunolabeled with tau12 (N-terminal mAb—red channel) and the GFP tag (green channel). This image illustrates the multiple possible secretion routes for tau that ultimately accumulates in the CSF. The "diffuse" tau described in the lamprey model consists largely of N-terminal fragments that lack the MTBR, whereas the "focal" route requires the presence of the MTBR. Both secretion routes in the lamprey model either introduce tau to the interior surfaces of the IVth ventricle (periventricular tau) or cross it entirely. **(B)** The respective time courses of neuronal death and CSF-tau elevation in AD are inconsistent with postmortem passive leakage of tau into the CSF, with the highest levels of CSF-tau occurring well before the onset of widespread cerebral occurrence of neurofibrillary degeneration in the so-called isocortical stages (Braak 5-6) of AD, and failing to increase with disease severity.

for lesion propagation in tauopathies is premature, particularly AD.[9,29-31] Thus, while we now have much more evidence that tau protein transfer between neurons is important to tau lesion propagation in tauopathies, the central mechanistic features of such transfer remain to be worked out. This is also true for the mechanisms by which both N-terminal (MTBR-) and near full length (MTBR+) tau species are generated in both the extracellular space and CSF.[32-37]

Is Neuron Death a Necessary Preliminary to the Generation of CSF tau in AD?

One of the more important effects of our recent study may be to call in to question a widely held assumption about the significance of CSF-tau in AD pathogenesis—that elevated CSF-tau levels typically seen in AD are caused by passive release of tau from dead neurons.[1] Even in the earliest accounts of elevated CSF-tau in AD in the mid 1990s, this assumption was made without supporting citations or discussion of alternative mechanisms.[38,39] The death-based origin for CSF-tau was generally accepted at that time because it was plausible, consistent with the known progression of neurofibrillary degeneration to extracellular "ghost tangles" and was unopposed by other explanations.[40,41] Also, the lack of tauopathy models and of accurate data on the timecourse of neuron death in AD made acquiring direct evidence for alternative CSF-tau biogenesis mechanisms impractical.[6,42] Since then, this assumption has been repeatedly (if casually) asserted in the medical literature despite the lack of evidence for a causal link between antecedent neuronal cell death and elevated CSF-tau levels in AD.

The continued plausibility of the death-induced hypothesis of CSF-tau in AD appears to be based on analogy with episodic conditions (head trauma, stroke, severe seizures) in which the time course of CSF or blood tau levels can be measured relative to a single generative event. In each of these conditions, CSF and/or serum tau levels undergo a large transient rise that is directly correlated with both event severity and with direct measures of neuronal loss.[43-45] The analogy with AD-induced neuron loss is strengthened by the presence of excitotoxic features in the neuron loss in all of these conditions, although this does not rule out a role for active secretion, since Ca^{2+} fluxes also play a critical role in most unconventional secretion mechanisms.[46] Another situation where neuron death remains a highly plausible (if still unproven) source for elevated CSF-tau may be prion diseases such as Creutzfeldt Jacob disease (CJD), which typically features much higher CSF-tau levels than does AD together with massive neocortical neuron loss over a shorter timecourse after diagnosis.[47]

In the case of AD, however, indications from studies performed in the past decade have generally been either ambiguous or inconsistent with the "death tau" hypothesis.[48,49] The advent of quantitative ELISA-based studies comparing CSF-tau levels in early and late AD cases (including ours) show that the well-established neuropathological "Braak" sequence of AD development does not anticipate the elevation of CSF-tau levels and thus conflicts with the death origin hypothesis (see **Fig. 1A**).[1,14,32] This is especially notable in early "limbic" stage AD (Braak Stages 3–4), when neurofibrillary pathology is confined to limbic regions of the temporal lobe that represent less than 10% of brain volume, while CSF-tau levels are rising sharply to their maximal levels.[1] Moreover,

CSF-tau levels remain largely stable or may even fall in late stage AD, even as neurofibrillary pathology and neuron loss become widespread in the brain.[1,14,32] In this context, our demonstration that tau, particularly in its phosphorylated form, is associated with a secretion marker in CSF from early AD patients provides an alternative mechanism to neuron death, and thus may prompt a broad reassessment of the origin of CSF tau in AD.

The likelihood that tau secretion is involved in the genesis of CSF tau is fundamentally important to critical questions relating to AD diagnosis and treatment, since it raises broad issues of the timing and distribution of degenerative changes in the brain. For instance, a "death" mechanism of CSF-tau pathogenesis implies that tau misprocessing is fatal to affected neurons earlier in the disease than is suggested by a "secretion" based mechanism. It therefore suggests a more pessimistic outlook than the latter for the development of prospective diagnostics and effective therapeutics for AD. It is a truism that the staying power of powerful and compelling ideas is better linked to their plausibility rather than to the actual evidence supporting them—a well known example being the miasmatic theory of infectious disease.[50] By presenting the first direct evidence for an alternative mechanism, this study may crystallize the existing evidence that antecedent neuron death is not currently a plausible mechanism for CSF-tau biogenesis in AD. This provides a new perspective on AD pathogenesis that opens unexplored avenues to improving both the diagnosis and treatment of this devastating and widespread condition.

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