

## **Tauomics and Kinetics in Human Neurons and Biological Fluids**

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### **Summary**

In this issue of *Neuron*, Sato et al. (2018) combine proteomics and a stable isotope labeling method to examine different forms of tau and their kinetics in human neurons, brain and cerebrospinal fluid, and how these properties relate to clinical state and pathology in Alzheimer's disease.

### **Main text**

In 1986, one year after the sequencing of amyloid  $\beta$  (A $\beta$ ) in plaque cores in Alzheimer's disease (AD) brains, data were published showing that neurofibrillary tangles—the other major neuropathological hallmark of AD, the amount of which correlates with dementia severity—are composed of abnormally phosphorylated and truncated forms of tau (Grundke-Iqbal et al., 1986). Tau is a microtubule-binding axonal protein that promotes microtubule assembly and stability. There are at least 6 different tau isoforms, produced by alternative splicing of the mRNA, and many additional post-translationally modified isoforms and fragments (Figure 1). Abnormal phosphorylation and truncation of tau lead to disassembly of microtubules and impaired axonal transport with compromised neuronal function and tau aggregation into paired helical filaments and neurofibrillary tangles as consequences. These pathological processes are highly active in AD and several other so-called tauopathies, including progressive supranuclear palsy and some forms of frontotemporal dementia.

Apart from executing important intracellular functions, tau is normally secreted from neurons into the brain interstitial fluid. This fluid communicates freely with cerebrospinal fluid (CSF) and, in a more restricted manner that is regulated by the glymphatic clearance system of the brain, with blood, where tau may be enzymatically degraded and cleared from the body. Whether secreted tau performs any biological function is unknown and should be an interesting topic for future research.

Once A $\beta$  and tau had been detected in plaques and tangles, respectively, biomarker tests for the two proteins were developed. For many years, the limitations in analytical sensitivity of available methods precluded reliable quantification in blood, but the proteins were possible to measure in CSF. Different assays were developed and, to cut a long story short, those specifically measuring A $\beta$  ending at amino acid 42 showed reduced levels of the protein in CSF in a manner that correlated inversely with presence of A $\beta$  pathology in the brain, whilst CSF tau concentrations were increased in AD and thought to reflect release of the protein from dying neurons and disintegrating tangles. Tau was regarded as the simple biomarker to measure and interpret, whilst A $\beta$  was sticky, influenced by pre-analytical confounders and difficult to produce standardized assays for. This has turned out to be wrong: A $\beta$ 42 now seems like the easy biomarker to define, measure and standardize, whilst tau with all its isoforms, fragments and complex biology is the challenging one to understand.

The first CSF total tau (T-tau) assay was published in 1993 (Vandermeeren et al., 1993). This assay was a sandwich ELISA in which a monoclonal antibody against the mid-domain of tau was combined with a polyclonal anti-tau antiserum. Two years later, the first assay based on three mid-region monoclonal antibodies that recognize most tau isoforms irrespective of phosphorylation state was published (Blennow et al., 1995). AD patients display clearly increased T-tau concentrations in their CSF, a finding that has been replicated in hundreds of papers using different assays in many different clinical contexts (Olsson et al., 2016). In response to acute brain injury, CSF T-tau concentrations are dynamic; they increase during the first few days following the injury and stay elevated for some weeks until they normalize over 3-4 months, depending on injury severity. This has led to the view that increased CSF T-tau concentration reflects neuronal injury, which in turn may indicate disease intensity. However, in neurodegeneration, there are problems with this interpretation. Why are neurodegenerative diseases other than AD (and Creutzfeldt-Jakob disease) typically normal in regards to CSF T-tau concentration?

The first CSF assay for phosphorylated tau (P-tau), the form of tau that has been thought to represent neurofibrillary tangles, was published in 1995 (Blennow et al., 1995). Since then, P-tau assays for different forms of phosphorylated tau protein have been examined. They correlate well and associate with AD in a similar manner. In general, CSF P-tau concentration correlates with neurofibrillary tangle pathology (Buerger et al., 2006), but not as strongly as the correlation of CSF A $\beta$ 42 with A $\beta$  pathology. Emerging studies show correlations of CSF tau concentration and tau PET in AD dementia, but correlations in pre-dementia stages are absent or weak (Mattsson et al., 2017). A major outstanding research question is why tauopathies other than AD do not show increased CSF P-tau concentration.

It may be that we have misinterpreted the precise meaning of increased T-tau and P-tau concentrations in AD CSF. Maybe the proteins are not at all direct markers of neurodegeneration and tangle pathology, as previously thought. When looking at the cross-disease biomarker literature, another interpretation that makes better sense is that the release of tau from neurons is specifically changed in AD so that AD-affected neurons phosphorylate, truncate and secrete more of both “total” (whatever that is) and phosphorylated tau. Such neurons may be at increased risk of developing tangles and eventually die but this would be downstream of the tau dysmetabolism and release that the currently available assays measure.

This is exactly where the study by Sato and colleagues comes in. The authors start by re-examining the complexity of tau in human brain tissue and CSF using state-of-the-art mass spectrometry techniques. They succeed in detecting tau peptides spanning the full sequence of the protein and conclude that most tau in the brain is indeed full-length, whilst most tau in the CSF lacks the microtubule-binding region and more C-terminal parts. These results are in general agreement with earlier data (Barthelemy et al., 2016; Meredith et al., 2013) but add quantitative rigor. The tau profile in stem cell-derived neurons is found to be similar to brain, whilst cell media resemble CSF, which speaks for the usefulness of these cells—with some caveats—to study tau homeostasis in health and disease.

Employing a stable isotope-labeling kinetics (SILK) method in which cells or research participants are labelled with a stable isotopically labeled tracer,  $^{13}\text{C}_6$ -leucine, that is incorporated into newly synthesized proteins over time, the authors leave the static biomarker measurements behind and quantify production and clearance rates of different tau forms

instead. Tau from protein extracts is captured using antibodies and digested with trypsin, whereafter labeled and unlabeled fragments are measured as reporters to calculate production and clearance rates of different tau forms. A mid-domain fragment of tau serves as the tau kinetics reporter in the human CSF studies, while in the cell experiments, several tau fragments could be monitored.

In stem cell-derived neurons, tau was found to have a slow turnover both inside and outside cells over a few weeks. N- and mid-domain fragments behaved very similarly but there was a 3-day time lag until they appear in the cell media suggesting an active process underlying the secretion. Tau fragments representing full-length or C-terminal tau, however, had shorter half-lives than N-terminal forms with similar results inside and outside cells and no delay in appearance in the cell media. This result may seem surprising but could reflect passive release from dying neurons (a normal occurrence in these types of neuronal cultures), which may be of immediate relevance to biomarker research; current T-tau assays might reflect secretion of tau from AD-affected neurons, whilst an assay that measures full-length tau could be used to monitor cell death and neurodegeneration. Sensitive enough assays of this kind do not yet exist but should be developed. Perhaps such an assay would detect neurodegeneration also in non-AD neurodegenerative diseases, much like neurofilament assays do. There were also interesting phosphorylation-dependent differences in tau kinetics. In general, phosphorylated tau forms, which are known to have lower affinity for microtubules, were turned over faster than their non-phosphorylated counterparts were.

In the human CSF studies, a mid-domain tau reporter fragment was used to monitor tau kinetics. Given the results above, this has some limitations, but monitoring a broader set of tau peptides is simply not possible given current analytical sensitivity limitations. In a set of experiments over months with two slightly different labeling protocols, it could be shown that tau labeling peaked after a few weeks and decreased slowly over 3-4 months with an overall half-life of around 3 weeks. Marrying these results to the cell model data, most of this signal might come from the tau that is actively secreted from neurons. Examining patients who had undergone amyloid-PET, there was a positive correlation of tau production rate (reflecting synthesis and/or release; it is for natural reasons not possible to separate these processes) and A $\beta$  tracer retention in the brain; *i.e.*, more A $\beta$  pathology correlated with more tau release into the CSF. This would fit with a model in which A $\beta$ , or a mediator(s) of A $\beta$  toxicity, induces neuronal tau secretion before frank neurodegeneration is evident, similar to what has been

seen in mouse models. There was also a correlation of tau production rate with tau PET signal. However, it is presently not clear whether this reflects a direct relationship between tangle pathology and tau secretion or if the correlation is indirect; as discussed above A $\beta$ -affected neurons that secrete more tau may be more likely to develop tangles and degenerate in the future. The latter interpretation would fit with data showing that CSF T-tau and P-tau concentrations correlate with tau PET results mainly in the dementia stage of AD, but not that well in mild cognitive impairment and not at all in pre-clinical stages of AD (Mattsson et al., 2017).

The new tau SILK method opens new avenues for a wide range of exciting studies. Obviously, it would be of great interest to examine if there are differences between AD and non-AD tauopathies in terms of tau production and clearance rates. Tau SILK could also prove an important readout in clinical trials of novel disease-modifying therapies against AD. Irrespective of the mechanism of action, a lowering of tau production rate should be expected over a few months in response to successful treatment. Finally, the method enables very exciting basic research experiments. If tangle formation could be reproducibly induced in neuronal cultures, changes in tau fragment kinetics before and after such an induction could be examined. It would be particularly relevant to monitor fragments representing the tau filament core (residues 306-378) and compare their production and clearance rates to those of N-terminal fragments. The tau SILK method is likely to increase our understanding of when and how tau pathology starts to accumulate in neurodegenerative diseases and should also provide information that increases the interpretability of the currently available static CSF and PET tau markers.

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### **Figure legend**

Tau is a complicated and heterogeneous biomarker. (A) There are at least six isoforms of tau as a result of alternative splicing of exons two, three and ten. These isoforms contain three or four microtubule-binding domains (green boxes; the fourth domain is in exon 10; numbers to the right in the panel denote the number of amino acids in each isoform). (B) Numerous threonine and serine phosphorylation sites have been identified in tau. In addition to phosphorylation, tau may also be proteolytically processed and potentially also oxidized and glycosylated, which increases the complexity further.