The molecular implications of a caspase-2-mediated site-specific tau cleavage in tauopathies

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A major focus of current experimental therapies for neurodegenerative diseases is on modulating post-translational modifications (PTMs) of the microtubule-associated protein tau. Tau is a highly soluble, neuronal protein that is comprised of four domains – the N-terminal projection domain, the proline-rich region, the microtubule-binding domain, and the C-terminal tail. As a scaffold protein, tau dynamically interacts with numerous structural and functional biomolecules, such as cytoskeleton and motor proteins, chaperones, enzymes, DNA, RNA, and lipids. Over a dozen types of PTMs, combined with alternative splicing, confer upon tau its enormous structural and functional heterogeneity, which subserves its many (patho-)physiological functions.

Under normal conditions, the modified tau forms are actively involved in regulating a diverse set of processes, including nerve cell differentiation, neuronal morphogenesis and plasticity, neurite polarity, axon outgrowth and elongation, cargo transport along axons, synaptic plasticity, genome stability, and outgrowth of oligodendrocytes (reviewed in Arendt et al. (2016)). Upon encountering cellular stress, the carefully choreographed tau PTMs go awry, leading to the generation of toxic forms, a pathological feature that is present in a group of neurodegenerative disorders known as tauopathies (reviewed in Arendt et al. (2016)). For example, tau phosphorylation and truncation may weaken its binding to microtubules, leading to the accumulation of tau to subcellular compartments (e.g., dendritic spines and nuclei) other than axons, impairing cellular function. In this perspective, we review the impact of a caspase-2-mediated site-specific tau truncation and synaptic dysfunction in tauopathies, and discuss the potential of targeting caspase-2 as a therapeutic strategy against cognitive decline.

Identification of a tau cleavage product that impairs synaptic transmission: Soluble forms of tau impair cognition in tauopathies. Under pathophysiological conditions, tau assumes various structurally distinct forms, among which neurofibrillary tangles (NFTs) are the most extensively studied. NFTs, a pathological hallmark of at least a dozen tauopathies, including Alzheimer’s disease (AD), are comprised of insoluble, intracellular, paired-helical filaments of hyperphosphorylated tau. NFTs have long been believed to drive cognitive decline in AD, because the spread of NFTs in the brain correlates with the severity of cognitive deficits. However, in experimental models, cognitive deficits can occur in the absence of NFTs, and be dissociated from NFTs. In the tau-transgenic Tg4510 mouse line, which expresses the proline-to-leucine mutation at amino acid 301 (P301L) associated with frontotemporal dementia and Parkinsonism linked to chromosome 17, cognitive deficits occur before NFTs emerge, indicating the suppressing transgenic tau expression after NFTs appear ameliorates memory impairment without reducing the NFTs (Santacruz et al., 2005). These findings imply that some NFT subunits are not NFTS, and spurred the search for soluble forms of tau causing deficits in Tg4510 mice.

Δtau314, a soluble, brain-derived tau fragment, is associated with memory impairment. An exhaustive investigation of the correlation between various soluble tau species and cognitive function in Tg4510 mice led to the identification of a ~35-KDa tau fragment, whose levels correlate with the severity of impairment in a spatial reference memory test (Zhao et al., 2016). A comprehensive combination of mass spectrometry revealed this brain-derived tau fragment to be an N-terminally-intact but C-terminally-truncated protein ending at aspartate 314 (D314) (Zhao et al., 2016), hence the name Δtau314. In vitro, cleavage and synaptic dysfunction assays showed that Δtau314 forms Thioflavin T-reactive fibrils less readily and precipitates to a smaller extent than its full-length tau precursor (Zhao et al., 2016), likely due to near-complete truncation and elimination of the paired-helical filaments core that spans amino-acids valine 306 to phenylalanine 378 ( Fitzpatrick et al., 2017).

The protease that catalyzes the cleavage of tau to form Δtau314 is caspase-2. Proteases that cleave after aspartate residues include caspases, matrix metalloproteases, and glycoprotein 8. Based on the residues flanking D314, the strongest candidates for hydrolyzing tau to form Δtau314 are members of the caspase family. An in vitro cleavage assay identified caspase-2 as a primary caspase that cleaves eight caspses expressed in human central nervous system capable of producing Δtau314 (Zhao et al., 2016).

Caspase-2-catalyzed cleavage of tau at D314 leads to synaptic dysfunction: Caspase-2 and Δtau314 are required for tau to accumulate in dendritic spines. While concentrated in axons, small amounts of tau also normally appear in dendritic spines, into which tau accumulates in correlation with the accumulation of tau to subcellular compartments (e.g., dendritic spines and nuclei) other than axons, impairing cellular function. In this perspective, we review the impact of a caspase-2-mediated site-specific tau truncation and synaptic dysfunction in tauopathies, and discuss the potential of targeting caspase-2 as a therapeutic strategy against cognitive decline.

Δtau314 proteins arise from all six tau splice isoforms expressed in the central nervous system (e.g., P301L, Q301L, P301S). Of note, we have recently identified this caspase-2-mediated site-specific tau cleavage event in multiple tauopathies (Tsritsas et al., 2019). Taken together, these results indicate that the accumulation of tau in dendritic spines is regulated by both caspase-2 cleavage within the microtubule-binding domain and phosphorylation in the C-terminal tail of tau.

Phosphorylation in the proline-rich region of tau reduces excitatory post-synaptic neurotransmission. The accumulation of tau P301L within dendritic spines is associated with the internalization of functional glutamatergic a-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptors from the post-synaptic membrane, which causes a reduction in the amplitude of miniature excitatory post-synaptic currents (mEPSCs) (Hooover et al., 2010). However, the accumulation of tau in spines per se is insufficient to induce synaptic dysfunction. In cultured neurons, tau WT that is pseudo-phosphorylated in the C-terminal tail accumulates in dendritic spines, but does not reduce mEPSCs unless at least one of five residues (i.e., S202, T205, T212, T217, and T231) in the proline-rich region is also pseudo-phosphorylated (Teravskis et al., 2019). How tau disrupts postsynaptic anchoring of AMPA receptors and whether synaptic function modulated by Δtau314 are involved remain unclear. One possible scenario is that proline-directed S/T phosphorylation in the proline-rich region enhances the binding of tau to calcineurin, which mediates internalization of AMPA receptors by dephosphorylation of the ΔIluA1 subunit of the receptor (Miller et al., 2014).

Caspase-2-cleavage of tau induces cognitive deficits: Caspase-2-catalyzed cleavage of tau at D314 causes cognitive deficits in tau P301L expressing mice. Memory impairment in Tg4510 is reversed when morpholino antisense oligonucleotides against mRNA of the murine caspase-2 (Casp2) gene are infused into the lateral ventricles (Zhao et al., 2016). Dilation of memory function is accompanied by approximately 35% lower levels of both caspase-2 protein and Δtau314, suggesting that caspase-2 mediates cognitive dysfunction through the processing of tau at D314. Expressing tau P301L in 2–3-month-old mice induces cognitive deficits, but expressing tau P301L D314Δ, which resists cleavage by caspase-2, does not (Zhao et al., 2016), providing additional support for the role of caspase-2 in this site-specific cleavage event in producing cognitive abnormalities.

Of note, we have recently identified this caspase-2-mediated site-specific tau cleavage in a series of mouse lines modeling various types of tauopathies (e.g., frontotemporal dementia, AD, and Huntington’s disease), and found associations with neuropathological and functional phenotypes, such as brain atrophy, premature mortality, and seizures, in addition to impaired cognition (Liu and Asher, manuscript in preparation), supporting its broad impact on the pathogenesis of neurodegenerative disorders.

An unresolved, unexpected observation. It is puzzling that expressing Δtau314 in 2–3-month-old mice causes neither alterations in synaptic transmission nor impairments in spatial reference memory, despite its prominent accumulation in the dendritic spines (Zhao et al., 2016). Although not proven yet, it is possible that additional PTMs are required for Δtau314 per se to impair synaptic function, such as S/T phosphorylation in the proline-rich domain (Teravskis et al., 2019) or acetylation of lysine residues in the second microtubule-binding site (Tracy et al., 2016).

The impact of Δtau314 on dementia in humans: Δtau314 levels are elevated in multiple tauopathies. Δtau314 proteins arise from all six tau splice isoforms expressed in the central nervous system (Liu et al., 2020). Their levels are elevated in the temporal gyrus of individuals with AD
mild cognitive impairment (Zhao et al., 2016; Liu et al., 2020) and Lewy body dementia (Smith et al., 2019), and in the prefrontal cortex and caudate nucleus of individuals with HD (Liu et al., 2019). There is a connection between Δtau314 and cognitive impairment in multiple disorders. Interestingly, levels of Δtau314 predict cognitive impairment in Lewy body dementia as effectively as the stages of Lewy body pathology (Sun et al., 2015). NFTs are characteristic lesions of some forms of tau neuropathology vary markedly between brain regions, future studies on tracking relationships between Δtau314 levels in different brain structures and clinical disease progression will elucidate the role of Δtau314 in the pathogenesis of dementia disorders. Currently, we have not been able to detect Δtau314 reliably and reproducibly in biological fluids (e.g., cerebrospinal fluid and plasma/serum), but are developing better antibodies and protocols to overcome this shortcoming. The ability to measure Δtau314 would be invaluable for assessing Δtau314 as a molecular biomarker of synaptic dysfunction in tauopathies.

Caspase-2 as a potential therapeutic target for treating dementia: Converging evidence from studies in enzymology, structural biology, physiology, and clinical trials suggests that caspase-2 is a promising target for improving synaptic transmission in neurodegenerative conditions. Caspase-2 has unique enzymatic and structural characteristics (reviewed in Poréba et al. (2013)). For example, caspase-2 is the only caspase with a well-defined S5 substrate. Additionally, a salt bridge between glutamate 217 and arginine 378 that is solely present in caspase-2 regulates substrate/inhibitor recognition. Further, the exclusive presence of a disulfide connection between the two small subunits is the key to maintaining the structure of the hetero-tetrameric, active enzyme. Exploiting some of these features may help in the development of a potent and selective inhibitor of caspase-2.

Caspase-2 knockout (Casp2KO) mice have the same median life-expectancy as wild-type mice, indicating that it is not an indispensable enzyme. However, they exhibit impaired cognitive flexibility, fear memory, synaptic plasticity, and enhanced anxiety, and experience accelerated aging of bone, muscle, and hair pigment cells. The physiological function of caspase-2 is to control the proliferation of numbers through programmed cell death, regulating osteoclast and myoblast differentiation to maintain bone and muscle cell homeostasis, promoting de novo lipogenesis in the liver, and regulating liver polyploidization. A major reason that caspase-2 is an attractive therapeutic target is that its levels and activity are aberrantly upregulated in multiple pathological conditions, including fatty liver diseases, osteoporosis, and various neurodegenerative disorders (Sladky and Villeneuve, 2020). In neurological disorders, caspase-2 mediates neuronal damage, synaptic change, and impairment in cognitive, psychiatric, and motor function caused by several types of stress (e.g., excitotoxicity, increased oxidative stress, exposure to β-amyloid or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, neonatal stroke, retinal ischemia, and transgenic expression of mutant human amyloid precursor protein, huntingtin, or α-synuclein). Caspase-2 may be beneficial in multiple neurological indications, including AD, HD, FTD-17, Parkinson’s disease, stroke, neuroblastoma, and glaucoma (reviewed in Sladky et al. (2017)). Provided that the toxicity of inhibition required to improve symptoms can be achieved without dampening its normal physiological functions.

Indeed, in rats modeling optic neuropathy intravitreal injection of a small interfering RNA (siRNA) results in its local distribution in the retina, lowering caspase-2 mRNA level by ~50%, and protecting retinal photoreceptors from death (Ahmed et al., 2011). Encouragingly, clinical trials (ClinicalTrials.gov identifier: NCT01064505, NCT01965106) featuring intravitreal administration of QPI-1007, a caspase-2 lowering siRNA for treatment of acute non-arteritic optic neuropathy in humans, have demonstrated the safety and efficacy of engaging caspase-2, and the U.S. Food and Drug Administration has granted orphan drug designation to QPI-1007 (http://quarkpharma.com/?page_id=23).

Despite these developments, the potential of caspase-2 as a therapeutic target for cognitive disease intervention remains challenging. Although biologics such as small interfering RNAs are clearly promising, small molecules may prove more difficult to create. There is currently no caspase-2 chemical probe that can be used for target validation in pre-clinical studies; it has not been possible to develop an inhibitor with in vitro potency of >100 nM and >30-fold selectivity relative to other caspases. The chief difficulty is that the caspase-2 binding pocket is similar to the binding pockets of the other caspases, which poses a significant challenge for developing a small molecule that lodges securely inside the binding pocket of caspase-2 but not of the other family members.

Conclusions: Here, we discuss the effects of caspase-2-catalyzed tau cleavage at D314 on synaptic and cognitive dysfunction, the association of Δtau314 - the soluble cleavage product - with dementia, and the advantages and challenges of targeting caspase-2 for treating cognitive decline in neurodegenerative conditions. Our current understanding of the pathophysiological processes leading up caspase-2 activation, the downstream signaling of Δtau314, the diagnostic value of Δtau314, and the most efficient ways to develop of caspase-2 inhibitors is still limited. Future studies focusing on these topics will provide deeper insights into this newly identified cleavage event, and solutions for repairing synaptic transmission caused by the production of Δtau314.

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