COMMENTARY

β-Arrestin2 arrests the clearance of tau in FTLD

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Frontotemporal dementia (FTD) comprises a spectrum of clinical syndromes associated with several underlying neurodegenerative diseases. The primary brain areas affected in patients with FTD are the frontal and temporal lobes. As such, FTD is also referred to as frontotemporal lobar degeneration (FTLD) (1). In patients younger than 65 y, FTLD is the second most common cause of neurodegenerative dementia after Alzheimer's disease (AD), accounting for ∼10% of cases. Clinically, FTD is characterized by progressive deterioration in behavior, personality, and/or language. FTLD neuropathology is characterized by the pathological aggregation of misfolded proteins in neurons and/or glia. Approximately 45% of FTLD cases are characterized by inclusions of the microtubule-binding protein tau (FTLD-tau) and encompass Pick's disease (PiD), cortical basal degeneration (CBD), progressive supranuclear palsy (PSP), argyrophilic grain disease (AGD), multiple system tauopathy with dementia, neurofibrillary tangle predominant dementia (NFT-dementia), and white matter tauopathy with globular glial inclusions (1, 2). Despite enormous advances in FTLD research over the past 20 y, lack of understanding of the connection between the genetic, phenotypic, and pathological features and the underlying disease mechanisms remains a major gap in the dissection of FTLD pathogenesis.

Tau Physiology and Pathology

Tau is encoded by the microtubule-associated protein tau (*MAPT*) gene, which consists of 16 exons on chromosome 17q21 (3). The adult human brain contains six main tau isoforms, which are generated by alternative splicing of exons 2, 3, and 10. Tau isoforms differ in the number of amino (N)-terminal inserts (0N, 1N, 2N) and contain either three or four highly conserved microtubule-binding repeat domains (3R and 4R tau, respectively). The alternative splicing of exon 10 generates 3R and 4R tau and is associated with distinct tauopathies, which can be classified into three groups based on the tau isoforms found in the aggregates: 4R tauopathies (e.g., PSP, CBD, and AGD), 3R tauopathies (e.g., PiD), and 3R+4R tauopathies (e.g., AD) (4). Together, studies suggest that cell-specific tau isoforms assume distinct physiological roles.

Tau is predominantly expressed in neurons, where it mainly localizes to axonal regions and is involved in microtubule stabilization, maintenance of axonal transport, and regulation of neurite outgrowth (5). Posttranslational modifications, including hyperphosphorylation and glycosylation, and aggregation of tau into paired helical filaments and neurofibrillary tangles (NFTs) are pathological hallmarks of tauopathies. Hyperphosphorylation of tau at specific sites (6) reduces its affinity for tubulin (microtubules) (7), and promotes the accumulation of tau in the somatodendritic compartment (8–11). The progressive hyperphosphorylation and aggregation of tau in AD brains correlate with synapse loss and neurodegeneration and, thus, may be a key driver of cognitive decline. However, the mechanisms underlying tau aggregation and consequent cellular toxicity remain unclear.

β-Arrestin Physiology and Pathology

A small family of multifunctional G-protein–coupled receptor (GPCR) regulatory or adaptor proteins known as the β-arrestins (β-arrestin1 and β-arrestin2) play an almost universal role in facilitating traditional GPCR desensitization and endocytosis (12). Differential regulation of β-arrestins is implicated in type II diabetes and psychiatric disorders (13, 14), where pharmacological manipulation of selective β-arrestin–dependent complexes may provide therapeutic benefits (15). β-Arrestin1 (βarr1) and β-arrestin2 (βarr2) levels are elevated in patients Parkinson's disease with dementia (16). βarr2 levels are elevated in AD patients (17). Interestingly, Woo et al. (18) now demonstrate that βarr2 levels are also elevated in FTLD-tau patients.

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Both βarr1 and βarr2 bind to microtubules. βarr2 displays a stronger affinity for microtubules than βarr1 (19). βarr2 also spatially regulates cofilin, an F-actin severing protein, localization in dendritic spines and plays an important role in dendritic spine and synapse remodeling (20). Recently, Woo et al. (21) demonstrated that cofilin can directly bind to microtubules to displace tau and inhibit tau-induced microtubule assembly. Nevertheless, the involvement of βarr2 in tau-induced microtubule assembly directly or indirectly, through cofilin, remains to be determined.

In PNAS, Woo et al. (18) mechanistically address βarr2 regulation of tau accumulation in vitro and in a tau transgenic mouse model—in HeLa-V5-tau cells that stably express tau (0N4R) and in primary cortical neuronal cultures from a tau *P301S* transgenic mouse model. The authors demonstrate that overexpression of βarr2 leads to an increase in the levels and phosphorylation of tau. In contrast, reducing βarr2 expression results in a decrease in tau levels. Interestingly, tau mRNA levels are unaffected. However, tau turnover is reduced following the overexpression of βarr2. The authors go on to show that genetic deletion of *Arrb2*, the gene that encodes βarr2, in tau *P301S* mice, results in a reduction in Sarkosyl-insoluble tau in the absence in a change in soluble tau levels. They further demonstrate that long-term potentiation, which is thought to underlie learning and memory, is not impaired in *P301S;Arrb2*$^{+/-}$ and *P301S;Arrb2*$^{-/-}$ mice relative to *P301S;Arrb2*$^{+/+}$ mice, suggesting that synaptic plasticity is not compromised in the absence of βarr2. From these data, the authors propose that βarr2 and tau exhibit a positive and deleterious feedback loop that elevates the accumulation of insoluble tau.

β-Arrestin Oligomerization and Tau Clearance

β-Arrestins have emerged as adaptors and scaffolds for a growing number of signaling pathways (12) and have been shown to form homo- and hetero-oligomers (22, 23). Inositol hexakisphosphate (IP6), an abundant phosphoinositide involved in the regulation of both GPCR endocytosis and signaling, promotes the homo- and hetero-oligomerization of βarr1 and βarr2 (22–24). βarr2 mutants that do not bind IP6 prevent βarr2 oligomerization (24). However, these mutants retain GPCR regulation and the overall capacity of βarr2 to shuttle between the cytosol and nucleus. Recent structural data also indicate that IP6 acts as a nonreceptor activator of βarr2 (25). Interestingly, Woo et al. (18) demonstrate that βarr2 oligomerization is involved in the regulation of tau turnover. Specifically, expression of βarr2 oligomerization mutants (i.e., ΔIP6N-N-terminal domain mutant [K158A, K161A, and R162A] or ΔIP6C-C-terminal domain mutant [K232A, R234A, K252A, K326A, and K328A]) leads to an increase in tau turnover and a reduction in tau levels in cortical neuronal cultures from tau *P301S* mice and a significant decrease in Sarkosyl-insoluble tau in HeLa-V5-tau cells.

One strategy to treat FTLD-tau is to remove intracellular tau aggregates by activating cellular clearance mechanisms. The autophagy–lysosome pathway (ALP) is the primary system involved in the removal of insoluble, misfolded, aggregated, and long-lived proteins (26, 27). The ALP is affected in tauopathies (28). Specifically, hyperphosphorylated tau colocalizes with light chain 3 (LC3), an autophagosome marker, and p62 (encoded by the *SQSTM1* gene), an autophagy cargo receptor, in CBD and PSP patients (29). Notably, the absence of p62 protein results in childhood-onset neurodegeneration and defects in autophagosome formation (30). Mutations in *SQSTM1* have also been identified that are genetically associated with FTLD (31, 32). In AD patients, p62 specifically colocalizes with NFTs (33). Overall, several studies support the potential therapeutic targeting of p62-mediated selective autophagy in FTLD.

In PNAS, Woo et al. mechanistically address βarr2 regulation of tau accumulation in vitro and in a tau transgenic mouse model.

p62 recruits discrete classes of proteins, e.g., tau, to the autophagosomal membrane (34). Oligomerization of p62 (35) promotes its interaction with LC3B (36) and recruitment to autophagosomes (37). Recently, overexpression of p62 in a tau transgenic mouse model has been shown to lead to a reduction in Sarkosyl-insoluble tau accumulation (38). Accordingly, Woo et al. (18) sought to determine whether p62 is involved in the βarr2-mediated effect on the accumulation of insoluble tau. The authors demonstrate that overexpression of βarr2 reduces p62 dimerization by coimmunoprecipitation experiments and proximity ligation assays in HeLa-V5-tau cells. In contrast, expression of βarr2/ΔIP6N or βarr2/ΔIP6C does not affect p62 dimerization. Further in vitro atomic force microscopy experiments indicate that βarr2 expression reduces the size of p62 particles, i.e., p62 oligomerization. To assess the therapeutic potential of reducing βarr2 oligomerization in vivo, Woo et al. (18) stereotactically injected βarr2/ΔIP6N or βarr2/ΔIP6C into the hippocampus of *P301S* mice at 5 mo of age when the mice exhibit a significant accumulation of tau. The authors demonstrate that expression of βarr2/ΔIP6N or βarr2/ΔIP6C does not affect soluble tau levels. However, Sarkosyl-insoluble tau levels are significantly reduced 2 mo after injection. From these studies, the authors suggest that the development of small-molecule βarr2 oligomerization inhibitors may be a potential therapeutic avenue for intervention in FTLD-tau to enhance tau clearance in the absence of potential effects on GPCR signaling pathways. Indeed, targets or agents that promote the clearance of misfolded, aggregated proteins, such as tau, are attractive therapeutic strategies for intervention in neurodegenerative proteinopathies. It will be important to first gain further insight into the complex interplay between clearance and other pathophysiological processes, e.g., neuroinflammation.

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