Journal Club

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Live-Cell Imaging Reveals Tau Isoforms Imbalance Disrupts Traffic of APP Vesicles in Human Neurons

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Review of Lacovich et al.

Tau is a structural microtubule-associated protein (MAP) that is widely expressed in the mammalian nervous system. It stabilizes neuronal microtubules, facilitating their roles in the establishment of cell polarity, neurite outgrowth, and axonal transport (Wang and Mandelkow, 2015). Prominent accumulations of fibrillar aggregates of hyperphosphorylated tau in the CNS are pathological hallmarks for a broad group of neurodegenerative diseases called "tauopathies" (Ballatore et al., 2007).

The human *tau* gene undergoes alternative splicing to produce six isoforms. Alternative splicing of *tau* exon 10 generates tau proteins that contain either three (3R) or four (4R) microtubule binding repeats. In the adult human brain, the 3R and 4R tau isoforms are expressed at a 1:1 ratio. This delicate balance of 3R and 4R tau appears to be critical for neuronal function because deviations from this ratio are characteristic of several tauopathies. For example, tau mutations associated with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) disrupt only exon 10 splicing without altering the primary sequence of tau (Wang and Mandelkow, 2015). However, the functional differences between 3R and 4R tau and the precise mechanism by which imbalances of tau isoforms contribute to diseases remain largely elusive.

In a recent publication in *The Journal of Neuroscience*, Lacovich et al. (2017) extended our understanding of the relationship between the imbalances of tau isoforms and axonal transport of amyloid precursor protein (APP) by means of live-cell imaging in human-derived neurons. This is important because APP is the precursor protein of Aβ, which plays a central role in Alzheimer's disease (AD) pathology. The authors first altered the relative ratio of endogenous 3R and 4R tau in neuronal cultures differentiated from human embryonic stem cells by means of a *trans*-splicing RNA reprogramming strategy that drives the inclusion or exclusion of exon 10 in the endogenous tau transcript.

Lacovich et al. (2017) next evaluated the effects of perturbations of the ratio of tau isoforms on neuronal survival and morphology. They analyzed neuronal structure and polarization in transduced neurons by immunostaining against APP and Tau, which are makers for membrane and cytoskeletal structures. The authors did not observe significant differences in the number of neurons that expressed Tau and APP, the length of axons and dendrites, the number of axonal swellings, or accumulation of tau in transduced neurons compared with controls. Furthermore, the shift in the balance of tau isoforms did not alter the total amount of tau protein or tau phosphorylation. Together, these results indicate that the use of the *trans*-splicing strategy of Lacovich et al. (2017) successfully modulated the relative ratio of 3R and 4R tau isoforms in human-derived neurons without altering tau protein expression and the phosphorylation state or neuronal survival, polarization, and morphology. This provides a useful human cellular model for investigating the effects of an imbalance of human tau isoforms on early neuronal dysfunction.

A large body of *in vitro* and *in vivo* data suggests that tau mutations disrupt axonal transport. For example, overexpression of wild-type 4R tau impedes vesicle and organelle trafficking by disrupting the interaction between microtubules and motor proteins (Seitz et al., 2002; Stamer et al., 2002). Transgenic mice that express missense human mutations in exon 10 of tau (P301S or P301L) also have deficits in axonal transport (Ittner et al., 2008; Bull et al., 2012; Gilley et al., 2012; Rodríguez-Martín et al., 2016). Furthermore, overexpression of 3R tau disrupts axonal transport and synaptic function in *Drosophila* motor neurons (Chee et al., 2005), and the expression of either wild-type or mutant 4R tau causes axonal degeneration in *Caenorhabditis elegans* (Kraemer et al., 2003). To understand how tau dysfunction influences the generation of Aβ in AD, Lacovich et al. (2017) assessed the effects of imbalance in tau isoforms on axonal transport of fluorescently tagged APP in hu-

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man-derived neurons, using live-cell imaging. Increased levels of either 3R or 4R tau isoforms led to significant reductions in the average velocities and the number of moving APP-carrying vesicles in both anterograde and retrograde directions. These results indicated that changes in the ratio of tau isoforms have an effect on the regulation of axonal transport of APP.

To investigate how tau-isoform imbalance affects transport of APP in greater depth, the authors quantified the run length, the number of pauses per trajectory, reversion of directionality, and segmental velocity distributions. Shifting the balance to 3R tau isoforms favored the anterograde movement of APP vesicles, with a significant increase in anterograde run lengths and a reduction in retrograde run lengths. In contrast, shifting the balance to 4R tau isoforms promoted a retrograde bias by means of a significant reduction in anterograde velocity.

These findings imply an intriguing link between the endogenous ratio of tau isoforms and APP trafficking. Increases in either 3R tau or 4R tau increase anterograde and retrograde transport, respectively. This is important because, as discussed below, disrupting APP transport could affect its processing and thus the generation of Aβ.

APP is a trans-membrane protein that is processed by three secretases. In the amyloidogenic pathway, APP is first cleaved by β-secretase (BACE1) to produce soluble APPβ and a C-terminal fragment (βCTF). Subsequent cleavage of βCTF by γ-secretase yields Aβ peptide, which aggregates in extracellular amyloid plaques in AD patients. Although the molecular mechanisms surrounding APP processing have been extensively studied, substantial controversy remains over where cleavage occurs and where Aβ is released.

According to one model, APP is delivered to presynaptic terminals by fast anterograde transport. APP can interact directly or indirectly with the kinesin light chain subunit of the kinesin-1 transport machinery (Scheinfeld et al., 2002). The observation that β- and γ-secretases are contained within the same transport vesicle as APP led to the suggestion that Aβ cleavage occurs in transit (Kamal et al., 2001). According to this hypothesis, the inhibition of anterograde transport of APP by increasing 4R tau could increase dwell time of APP vesicles in transit, which would result in the premature proteolysis of APP and generation of cleavage products, including Aβ. The finding that kinesin light chain-deficient animals, when crossed with APP transgenic mice, displayed axonal pathology, increased amyloid levels and deposits in brain is consistent with this model (Stokin et al., 2005).

Another model proposes that synapses are sites of Aβ release and toxicity. A significant proportion of full-length APP and its C-terminal cleavage products accumulated at presynaptic terminals (Buxbaum et al., 1998). βCTFs, which are generated from axonally transported APP, are cleaved to Aβ peptides that are subsequently released and deposited in the axon terminals (Lazarov et al., 2002). According to this model, increases in the anterograde transport of APP by elevating 3R tau could lead to an accumulation of APP at distal axons, and thus an increase in the generation and release of Aβ at synapses.

Finally, several studies have indicated that nascent APP and BACE1 mature while being trafficked along the constitutive secretory pathway from the ER to the plasma membrane. Unlike many cell surface receptors, most APP is rapidly internalized into early endosomes containing BACE1 and γ-secretase. Indeed, endosomal compartments are acidic, which is optimal for BACE1 activity, and some work indicates that BACE1 interacts with APP predominantly in endosomes under native conditions (Kinoshita et al., 2003). Vesicles containing Aβ are either recycled to the surface, dumping Aβ into the extracellular space, or they fuse with lysosomes, where Aβ is degraded (O'Brien and Wong, 2011). In support of this model, Aβ levels are increased by promoting APP or BACE1 internalization, whereas blocking internalization reduces Aβ production. If this model is correct, impairment of APP anterograde transport relative to retrograde transport by the elevation of 4R tau could reduce the amount of APP being trafficked to the surface and get internalized for processing, thus leading to a reduction in amyloidogenesis. On the other hand, inhibition of APP retrograde transport relative to anterograde transport by the elevation of 3R tau could result in an accumulation of APP in endosomes as they failed to traffic back to the cell soma and fuse to lysosomes for degradation. Reductions in lysosomal degradation could increase the amount of Aβ being secreted extracellularly. The findings by Lacovich et al. (2017) thus provide an important link between tau dysfunction and abnormal endocytic response in AD.

In conclusion, many mouse models of tauopathies have been generated to explore the mechanism of tau dysfunction in neurodegenerative disorders, and many of these models accurately model key aspects of human disorders (Noble et al., 2010). However, the critical differences between the mouse and human genome may make it difficult to analyze the role of tau isoform balance because humans express a mixture of 3R and 4R tau, whereas adult mice express only 4R tau (Takuma et al., 2003). Moreover, most mouse models of tauopathies are transgenic lines that overexpress wild-type tau isoforms or tau isoforms with disease-associated mutations. This raises the possibility that tau expression at nonphysiological levels in such models might itself influence pathogenesis in ways that differ from tau dysfunction in humans, even if the endpoints are similar (Gilley et al., 2011). Lacovich et al. (2017) addressed these issues using a novel *trans*-splicing RNA reprogramming approach in human-derived neurons. They have created a useful human cellular model to dissect the functional consequences of human tau isoform imbalance without altering total tau protein or impairing other tau-dependent functions. Furthermore, their findings that changes in the endogenous 3R:4R tau ratio significantly disrupt the axonal transport of APP in human neurons significantly advance our understanding of the possible pathological links between tau isoform imbalances and APP metabolism. Their data raise new perspectives on the modulation of abnormal tau metabolism as a potential therapeutic intervention for human tauopathies, such as AD.

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