Leaky endosomes push tau over the seed limit

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The inter- and intracellular propagation of aggregated proteins like tau is emerging as a central mechanism behind progression of various neurodegenerative diseases. The steps by which tau aggregates and propagates is currently unclear. Chen et al. now combine a cell-based model of tau aggregation with a CRISPR interference (CRISPRi) genetic screen to identify components of the endosomal sorting complex required for transport (ESCRT) machinery as mediators of intracellular propagation of tau aggregates. These findings reveal a role for endolysosomal integrity in blocking tau propagation.

To learn more about the steps between uptake of tau seeds and subsequent aggregation, Chen et al. use their modified FRET-based tau aggregation assay in a CRISPRi-based screen to identify regulators of tau uptake and propagation. They first confirmed that they could observe a prion-like propagation of tau aggregation within the cell using seed material of recombinant aggregated tau or aggregates purified from AD patient brains. As in previous studies, the authors observed tau uptake into the cell, presumably via endocytosis, followed by release into the cytosol, where propagation of tau aggregation occurs. The authors then undertook a comprehensive genetic screen using CRISPRi to disrupt genes that might be involved in this process. They focused on genes encoding the proteostasis machinery, anticipating that these cellular factors are most likely to regulate seeding and aggregation. The screen identified two hits, both involved in endosome function, CHMP6 and VPS13D, which enhanced tau aggregation. This suggests that the corresponding proteins might serve as key points of regulation.

CHMP6 is a core component of the ESCRT-III complex, which, along with ESCRT-I/II, coordinates the formation of multivesicular bodies (MVBs). These critical organelles regulate protein degradation via routing to the lysosome. The ESCRT-III complex drives a membrane scission event, freeing vesicle cargoes from the endosome into the lumen of the MVB. The identification of CHMP6 as a regulator of tau propagation places this highly conserved and fundamental machinery at the heart of tau pathology. Similarly, VPS13D is involved in membrane integrity and trafficking via its function in nonvesicular transfer of phospholipids. The authors confirmed that CHMP6 and VPS13D are involved in tau propagation using single guide RNAs, observing that independent CHMP6 and VPS13D knockdown accelerated tau aggregation within the cytoplasm. This acceleration was not due to increased uptake of tau. High-content imaging analysis confirmed that knockdown significantly increased tau aggregation within 48 h post-seeding. Aggregates could be seen in CHMP6 knockdown cells within 12 h post-seeding, but not upon VPS13D knockdown, identifying phenotypic differences in the rate of tau aggregation between the two genes. CHMP6 knockdown clearly propagated tau aggregation faster than VPS13D knockdown.

To gain mechanistic insight into this process, Chen et al. next monitored the effect of tau aggregation on the health of the endosomal-lysosomal system. ESCRTIII has recently been implicated in the resealing of damaged endolysosomal membranes. The possible implications of these findings suggest that tau seeds endocytosed into the cell may compromise endolysosomal integrity. The authors observed co-localization of tau fibrils and late endosomal and lysosomal markers RAB7A
and LAMP1, respectively, demonstrating that tau fibrils are rapidly taken up into endosomes to be potentially degraded via the lysosome. However, when CHMP6 is reduced, the co-localization between tau fibrils and endosomal-lysosomal markers was greatly decreased; instead, tau aggregates formed in the cytosol. Chen et al. argue that efficient uptake and degradation of tau fibrils via lysosomes minimize the potential interaction between tau seeds and cytosolic tau. In this scenario, CHMP6 knockdown destabilizes endosomal-lysosomal integrity, and tau fibrils then leak into the cytoplasm (Fig. 1). This proposal was reinforced through knockdown of other components of the ESCRT pathway. Simultaneous genetic reduction of the redundant ESCRT-III subunits CHMP2A and CHMP2B causes tau aggregation in the FRET assay. Additionally, knockdown of Vps4, the ATPase driving disassembly of ESCRT-III, also induces tau aggregation. Although CHMP2A and CHMP2B function as redundant proteins, mutations in CHMP2B are known to cause FTD (10), albeit a form of FTD that is negative for tau aggregation, reinforcing the importance of the ESCRT machinery in maintaining neuronal integrity. The authors also tested the effects of L-leucyl-L-leucine-o-methyl ester, which destabilizes endosomal/lysosomal compartments, and demonstrate that this compound phenocopies CHMP6 knockdown, accelerating the propagation of tau aggregation.

This work systematically addresses how tau seeds spread in a cellular system and places the highly conserved ESCRT recycling pathway in the center of its regulation. In a world of high-profile failed drug trials for AD, this work introduces an alternative therapeutic strategy to that of removing or inhibiting tau. We might now instead design compounds that preserve and promote the integrity of the endolysosomal system.

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Figure 1. Preservation of the endolysosomal machinery prevents the aggregation of tau seeds. Recombinant tau seeds or tau purified from AD patient brain are taken up into the cell. Tau seeds are normally degraded via lysosomes, with minimal leakage and interaction with cytosolic tau. When the endolysosomal system is compromised (ESCRT-III knockdown), tau seeds leak into the cytosol and form “prion-like” aggregates. Created with BioRender.