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Smuggle tau through a secret(ory) pathway

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Abstract

Secretion of misfolded tau, a microtubule-binding protein enriched in nerve cells, is linked to the progression of tau pathology. However, the molecular mechanisms underlying tau secretion are poorly understood. Recent work by Lee et al. [Biochemical J. (2021) 478: 1471–1484] demonstrated that the transmembrane domains of syntaxin6 and syntaxin8 could be exploited for tau release, setting a stage for testing a novel hypothesis that has profound implications in tauopathies (e.g. Alzheimer's disease, FTDP-17, and CBD/PSP) and other related neurodegenerative diseases. The present commentary highlights the importance and limitations of the study, and discusses opportunities and directions for future investigations.

Commentary

Eukaryotic cells are sovereign entities separated from one another by a selectively permeable plasma membrane. While the plasma membrane allows small and nonpolar molecules to cross freely, macromolecules and particles have to explore more intricate mechanisms to traverse to the opposite side. For instance, enveloped viruses (e.g. HIV-1, Influenza A, and Ebolavirus) utilize virus-encoded fusion proteins to force their way into the cytosol, with entry sites varying from the cell surface to endo/lysosomes [1-3]. Pathogenic bacteria (e.g. *Shigella flexneri* and *Listeria monocytogenes*) and parasitic protozoans (e.g. *Trypanosoma cruzi*) could gain access to the host cytosol by rapidly degrading the host membranes within which they are internalized [4,5]. Some bacterial toxins, plant lectins, and polyomaviruses travel via retrograde transport to the endoplasmic reticulum (ER), where they exploit ER-associated degradation (ERAD) pathway to cross into the cytosol [6-9]. Escaping the cell from the cytosol is also tightly controlled. Classical secretory proteins (e.g. hormones) rely on their signal peptide to enter the lumen of the ER (during translation), from which they are shipped to the Golgi apparatus and then the cell surface by vesicular carriers [10]. Retroviruses, on the other hand, opt to hijack cellular factors to bud from the cell surface, in an elaborate process that closely resembles exosome formation within a type of late endosomes called multivesicular bodies (MVBs) [11]. In fact, viral budding could also take place at MVBs, whose subsequent fusion with the plasma membrane would discharge

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Competing Interests

The author declares that there are no competing interests associated with this manuscript.

the virus along with the exosomes [11]. Notably, exosomes have been shown to load endogenous cargos (e.g. selected proteins and RNA) from the cytosol as well [12]. Recently, the exosome-based secretory pathway has been credited for releasing a-synuclein and tau, both of which are cytosolic, aggregation-prone proteins associated with neurodegenerative disorders [13,14].

In the latest article by Lee et al. [15], yet another fascinating cell exiting strategy was proposed. The authors began their investigation with the intention to better understand the functions of the conserved C-terminal tail region (CTTR) of tau, whose abnormal hyperphosphorylation and aggregation could lead to neurofibrillary tangle (NFT) formation, a trademark of Alzheimer's disease [14]. The authors performed a yeast two-hybrid screen that identified 46 interaction candidates, among which was syntaxin8 (STX8), a SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein required in endosomal fusion [16]. Co-immunoprecipitation experiments indicated that STX8 preferred the phospho-mimicking CTTR to the wildtype, although it could bind either. This raised the possibility that STX8-mediated endosomal trafficking might be involved in the secretion of tau, an event associated with the progression of tau pathology [14]. To address this, the authors examined HEK 293T cells co-transfected with respective expression constructs for tau and STX8, and reported a stunning discovery that STX8 facilitated tau release from co-transfected cells! This observation did not seem to depend on the phosphorylation state of tau, and could not be observed when other CTTR-interacting proteins were co-expressed with tau.

Because STX8 is closely related to syntaxin6 (STX6), a TGN (trans-Golgi network)/ endosomal SNARE linked genetically to tau-induced PSP (progressive supranuclear palsy) [17,18], the authors temporarily shifted their focus to STX6, and demonstrated that STX6 behaved virtually the same way as STX8. Importantly, overexpression of STX6 or STX8 did not cause any change in cell viability, ruling out the implications of cell death/ lysis in observed tau release. The authors then generated a series of different truncation variants of STX6, and revealed that the transmembrane (TM) domain alone was sufficient to cause extracellular accumulation of tau when the two were co-overexpressed in HEK 293T cells. Fluorescence microscopy indicated that both full-length EGFP-tagged STX6 and EGFP-tagged STX6 TM were localized to perinuclear punctate structures in transfected HEK 293T cells, in line with the previously reported TGN localization of STX6 [19]. However, no TGN markers were used to confirm this. To examine the subcellular localization of tau, the authors performed immunostaining on primary mouse neurons that had been transfected with EGFP-STX6 and V5-tau (anti-V5 was used to mark tau), and showed strong co-localization between tau and STX6 in vesicular structures. This gave rise to the notion that the TM domain of STX6 (and perhaps that of STX8) might be responsible for smuggling tau into secretory carriers for exocytosis (Figure 1). It is necessary to point out here that whether tau co-localizes with either STX6 TM or STX8 in neuronal cells has not been demonstrated.

To test the hypothesis that TM domains from STX6 and STX8 might translocate tau into vesicular/endosomal compartments, the authors conducted an *In vitro* tau protection assay. First, they reconstituted the TM domain from STX6 or STX8 into liposomes (size

undetermined). Full-length recombinant tau was then added to the proteoliposomes to a final concentration of 90 μM. Following 24 h incubation at 37°C, trypsin was added to the samples to briefly digest tau proteins that remained accessible. Western blot analysis showed recombinant tau was protected from trypsinization in the presence of reconstituted vesicles bearing STX6 TM or STX8 TM, whereas no protection was observed when the TM domain from RAMP1 (receptor activity modifying protein 1) was used. Accordingly, a mass-spectrometry-based *in vitro* TM interaction assay showed virtually complete binding between recombinant tau and STX6 TM or STX8 TM, but not RAMP1 TM. Taken together, there is substantial evidence suggesting that the TM domains of STX6 and STX8 could recruit tau to facilitate its entry into the secretory network of cells.

If proven to be true in future studies (see discussions below), this would not be the first time that a membrane protein was found to mediate protein transport across lipid bilayers. Sec61, a multispan membrane protein, has long been recognized as the translocon to transfer nascent secretory proteins into the ER and misfolded proteins out of the ER [20]. Likewise, the TM domains of SNAREs can act more than just a membrane anchor. The TM domains of v-SNAREs (localized to transport vesicles; in contrast with t-SNAREs that are localized to the target membrane) are known to undergo homodimerization [21] and have been shown to control fusion pore expansion [22,23]. Neuronal t-SNAREs (including syntaxin1 and SNAP25) form clusters in artificial membranes, neuroendocrine cells, and at the neuromuscular junction [20], where they modulate lipid organization and membrane curvature at the site of fusion [24]. Remarkably, systematic mutagenesis studies have provided strong evidence that the TM domains of syntaxin1 molecules form a proteinaceous pore to initiate the merger of the apposing membranes [25]. Therefore, it is not inconceivable that either STX6 or STX8 could be exploited to channel tau proteins into the secretory pathway under pathological conditions.

However, serious questions arise from this hypothesis as well as the supporting data generated by Lee and colleagues. First of all, is TM domain-mediated membrane crossing reversible? While there might be additional players in neuronal cells to help ensure unidirectional translocation of tau (i.e. allowing tau to enter the lumen but not the other way around), the orientation of TM domain in reconstituted lipid bilayers was not controlled and likely to be random. So what mechanisms were in place in the tau protection assay to enrich the luminal concentration of tau beyond what could be achieved through diffusion? A related question is: What is unique about the TM domains of STX6 and STX8? The recent finding that overexpression of VAMP8 — a v-SNARE on late endosomes — could also lead to tau secretion from neuronal cells [26] seems to imply that TM domain-mediated tau release might not be limited to syntaxins.

Secondly, is tau secretion reported by Lee et al. clinically relevant? The release of tau from HEK cells was observed when both tau and the syntaxins were overexpressed, yet the endogenous level of STX6 did not appear to have any detectable effect on tau release. This begs an important question, is there evidence of STX6 or STX8 overexpression in tau pathology? While a quick pubmed search did not uncover any direct proof, there is data showing oxidative stress, a major risk factor for Alzheimer's disease, could induce the overexpression of syntaxin5 (Golgi paralog of STX6 and STX8) in hippocampal cells of the

rat brain [27]. As properly concluded by the authors, whether the tau secretory activity of STX6 (or STX8) contributes to disease onset and progression remains to be shown.

Finally, the tau protection assay described in the study is not the ultimate test of protein translocation across the membrane. There is a possibility that by simply binding to membrane-anchored TM domains, the protease cleavage sites on tau could be shielded, which would slow down the rate of tau degradation. How does one explain STX6/STX8-mediated tau secretion from transfected HEK 293T cells then? In one plausible scenario, tau could get loaded into exosomes after they are recruited to MVBs by STX6 or STX8 (Figure 1). Since both STX6 and STX8 have been shown to interact with E3 ubiquitin ligases [28,29], they might be sort into exosomes via the ESCRT pathway upon monoubiquitination [11]. Of course, the TM domains alone lack lysine residues required for ubiquitination, but tau itself can be monoubiquitinated [30], and may not need any help from syntaxins to enter exosomes after it is recruited to the MVBs. It would be logical, as part of the future study, to demonstrate if TM domains could indeed cause tau translocation, perhaps by reconstituting TM domains in giant unilamellar vesicles (GUVs; $d > 1\ \mu m$) [31,32], which would allow any luminal distribution of tau to be discerned. In addition, TM domains from different syntaxins and other late endosome markers (e.g. VAMP8 and CD63) should be tested for their effect on tau release.

Like any interesting and impactful study, Lee et al. generated more questions than they answered. It opened a door for future investigations to unearth the molecular details that underscore tau secretion in the progression of tau pathology. These studies will also shed light on the propagation of other neurodegenerative diseases that are caused by the misfolding of neuronal proteins including αsynuclein, TDP-43 and SOD1 [33], all of which can be actively secreted from cells in ways not fully understood.

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Abbreviations

CBD	corticobasal degeneration
CTTR	C-terminal tail region
EGFP	enhanced green fluorescent protein
ER	endoplasmic reticulum
ERAD	ER-associated degradation
FTDP-17	frontotemporal dementia with parkinsonism linked to chromosome 17
GUV	giant unilamellar vesicle

HIV	human immunodeficiency virus
MVB	multivesicular body
PSP	progressive supranuclear palsy
RAMP1	receptor activity modifying protein 1
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SOD1	superoxide dismutase 1
STX6	syntaxin6
STX8	syntaxin8
TDP-43	TAR DNA-binding protein 43
TGN	trans-Golgi network
TM	transmembrane
t-SNARE	target-SNARE
VAMP	vesicle associated membrane protein
v-SNARE	vesicle-SNARE

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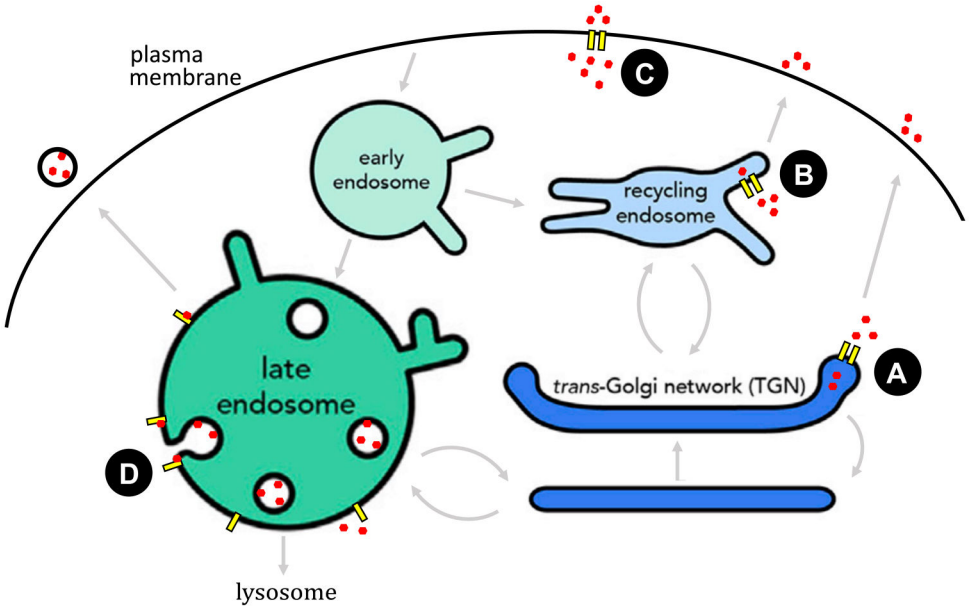

Figure 1. Models of syntaxin-mediated tau secretion.

Based on evidence provided by Lee et al. it is conceivable that selected syntaxins and their TM domains could facilitate tau entry into the secretory pathway by forming a proteinaceous pore in the classical secretory compartments, including (**A**) TGN, (**B**) recycling endosomes (altered cholesterol levels have been shown to trigger STX6 accumulation in recycling endosomes [34]), and Golgi-derived vesicles (not depicted in the figure). A proteinaceous pore could also be formed by syntaxins localized to the cell surface (e.g. syntaxin1) (**C**), to directly mediate tau transport across the plasma membrane [14]. On the other hand, tau may exploit interactions with MVB-specific SNAREs (e.g. STX8 and VAMP8) to get loaded into exosomes (**D**), accounting for the extracellular accumulation of exosomal tau in Alzheimer's patients and transgenic mice that model tau pathology [14]. Yellow objects represent syntaxins; red objects represent tau. This figure is adapted from Day et al. [35].