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Primary lysosomal dysfunction causes cargo-specific deficits of axonal transport leading to Alzheimer-like neuritic dystrophy

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Abnormally swollen regions of axons and dendrites (neurites) filled mainly with autophagy-related organelles represent the highly characteristic and widespread form of "neuritic dystrophy" in Alzheimer disease (AD), which implies dysfunction of autophagy and axonal transport. In this punctum, we discuss our recent findings that autophagic/lysosomal degradation is critical to proper axonal transport of autophagic vacuoles (AVs) and lysosomes. We showed that lysosomal protease inhibition induces defective axonal transport of specific cargoes, causing these cargoes to accumulate in axonal swellings that biochemically and morphologically resemble the dystrophic neurites in AD. Our findings suggest that a cargo-specific failure of axonal transport promotes neuritic dystrophy in AD, which involves a mechanism distinct from the global axonal transport deficits seen in some other neurodegenerative diseases.

Within dystrophic neurites in the AD brain, the accumulation of organelles in axon swellings implies a failure of their axonal transport; however, the mechanism(s) underlying this transport disruption have been unclear. Furthermore, whether neurite dystrophy in AD reflects a transient and possible compensatory response to a specific injury or is a sign of impending irreversible degeneration, is not known. Neuritic dystrophies are often interpreted as axonal "traffic jams" that are the consequence of a global failure of axonal transport and, indeed, focal disruption of the transport mechanisms in axons usually leads to local accumulations of diverse organelles. However, in some diseases, including AD, more selective classes of organelles accumulate. The organelle composition may vary suggesting different underlying mechanisms in these diseases. We set out in our studies to investigate mechanisms that could explain the particular pattern of dystrophy seen in AD and be generally relevant to sporadic as well as familial forms of AD.

Mounting evidence has emerged that lysosomal dysfunction is a pathogenic factor in AD. Our laboratory has demonstrated that Presenilin-1 (PS1) is essential for the acidification of lysosomes required to degrade autophagy substrates. The mechanism involves deficient glycosylation and lysosomal delivery of the V01a subunit of vacuolar-ATPase (v-ATPase), the proton pump that maintains the low pH needed to activate lysosomal acid hydrolases. Ablation of PS1 or mutations of PS1 that cause familial early-onset AD result in an unstable form of the V01a subunit, reduced assembly of v-ATPase on lysosomes, and failure of lysosomal acidification and proteolysis. To support the hypothesis that lysosomal proteolytic impairment directly contributes to AD-specific neuropathology, we recently demonstrated that enhancing activities of lysosomal proteases by deleting an endogenous cysteine protease inhibitor, cystatin B, substantially prevents intracellular lysosomal clearance deficits, β-amyloid and Aβ accumulation, and the development of cognitive dysfunction in AD model mice overexpressing mutant APP. These

observations strongly suggest that the relatively selective accumulation of incompletely digested substrates in autophagic vacuoles (AVs) and lysosomes within dystrophic neurites of AD brains is principally due to impaired lysosomal degradation.

Based on these findings, we investigated how impaired lysosomal degradation affects axonal transport of AVs and other organelles. We used two independent approaches to inhibit lysosomal proteolysis and compared the effects with those seen after strongly inducing autophagy, another possible basis for selective AV accumulation. As others have reported in non-neuronal cells, autophagy induction by rapamycin does not alter axonal transport of autophagosomes or lysosomes although these organelles increase in number. However, either inhibiting cysteine protease activities with leupeptin or neutralizing lysosomal pH with bafilomycin significantly delays or halts the dynamic movement of late endosomes, lysosomes, and autophagosomes. Importantly, the transport deficit is restricted to degradative organelles and does not affect mitochondria or early endosomes, which contain little or no cathepsin. Furthermore, these effects are reversible: upon removing the protease block, normal transport is restored. The delayed axonal transport of degradative organelles causes these vesicles to accumulate selectively in axonal swellings, as viewed ultrastructurally. Also enriched in these swellings are other markers of AD dystrophic neurites in vivo, including APP and ubiquitinated proteins, which are common constituents of AVs, and hyperphosphorylated neurofilaments that likely reflects the local activation of protein kinases within the dystrophic region of the neurite. The morphological and biochemical similarities between axonal swellings after lysosomal protease inhibition and in dystrophic neurites observed in AD brain provides an explanation for why autophagic and lysosomal vesicles are the overwhelmingly predominant organelles in AD dystrophic neurites. Moreover, the formation of AD-like neuritic pathologies by lysosomal protease impairment in wild-type primary neurons without mutations or overexpression of familial-AD proteins further highlights the potential significance of lysosomal dysfunction as a risk factor in all types of AD.

Since late endosomes and AVs mostly undergo retrograde transport, we speculate that the specific impairment of AV and endo-lysosome transport by proteolysis inhibition may involve a dynein-interacting protein residing specifically on these cargoes, as dysfunction in the dynein complex itself would affect all or most dynein substrates, including mitochondria and early endosomes. Mutations in dynein-interacting proteins, however, can produce a range of intracellular pathologies. For example, overexpression of the dynactin subunit dynamitin impairs dynein-dynactin interactions and leads to axonal dystrophies with prominent neurofilament accumulation. Mutations in dynactin cause the protein to aggregate in the cell body near the endoplasmic reticulum and Golgi vesicles; whereas mutation of the dynein heavy chain leads to inclusion body formation, neither of which pathologies are prevalent in AD brains. Thus, the retrograde transport deficit selectively affecting proteolytically impaired autophagic-lysosomal cargoes is most likely to involve a dynein-interacting protein that specifically recognizes these cargoes. In future studies, it will be interesting to examine how changes in the degradative capacity of AVs, lysosomes, and endosomes alter the composition of membrane proteins that may interact with molecular motor proteins.

Thus, although dystrophies are observed in many neurodegenerative diseases, we propose that transport failure affecting specific cargoes characterizes the neuritic dystrophy seen in AD, and that transport deficits involving different specific organelle populations may characterize certain other neurological disorders. For example, in disease models of hereditary spastic paraplegia type 2, SPG2 mutations produce axonal swellings selectively enriched in membrane-bound organelles resembling those seen in paraplegin and spastin mutants but distinguishable from AD dystrophic neurites, which are predominantly composed of autophagosomes and lysosome-related compartments. As in AD, the subtypes of cargoes that accumulate are likely to reflect the underlying subcellular pathobiology in each disorder.

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