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The secret life of degradative lysosomes in axons: delivery from the soma, enzymatic activity, and local autophagic clearance

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ABSTRACT

Lysosomal degradation of protein aggregates and damaged organelles is essential for maintaining cellular homeostasis. This process in neurons is challenging due to their highly polarized architecture. While enzymatically active degradative lysosomes are enriched in the cell body, their trafficking and degradation capacity in axons remain elusive. We recently characterized the axonal delivery of degradative lysosomes by applying a set of fluorescent probes that selectively label active forms of lysosomal hydrolases on cortical neurons in microfluidic devices. We revealed that soma-derived degradative lysosomes rapidly influx into distal axons and target to autophagosomes and Parkinson disease-related SNCA/α-synuclein cargos for local degradation. Disrupting axon-targeted delivery of degradative lysosomes induces axonal autophagic stress. We demonstrate that the axon is an active compartment for local degradation, establishing a foundation for future investigations into axonal lysosome trafficking and functionality in neurodegenerative diseases and lysosomal storage disorders associated with axonal pathology and macroautophagy/autophagy stress.

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A long-standing question in neurobiology is whether axons are active degradation compartments with the capacitiy to locally eliminate protein aggregates and autophagosomes. Neurons are polarized cells consisting of a highly extended long axon. Whereas degradative lysosomes are enriched in the soma, endolysosomal organelles, often labeled by LAMP1 (lysosomal-associated membrane protein 1), have also been described in axons. Because LAMP1 is widely distributed among degradative and non-degradative endolysosomal organelles, the degradative capacity of these axonal organelles remains elusive. The current accepted model is that mature lysosomes are mainly distributed in the soma, so that cargos destined for degradation, such as those within autophagosomes, need to be retrogradely transported into the soma for degradation. Interestingly, genetic or pharmacological disruption of lysosomal function leads to axonal dystrophy, characterized by axonal swellings containing accumulated cargo for degradation, highlighting the importance of lysosomes in maintaining axonal homeostasis. Lysosomal dysfunction, autophagy stress, and axonal dystrophy contribute to the pathogenesis of neurodegenerative diseases including Alzheimer and Parkinson diseases, and amyotrophic lateral sclerosis. These clinical implications prompted us to address 3 fundamental questions as to whether (1) neurons recruit enzymatically active degradative lysosomes from the soma into distal axons to maintain local degradation capacity; (2) those degradative lysosomes have unique motility and distribution patterns in distal axons; and (3) these axon-targeted lysosomes are locally degrading autophagosomes and aggregate-prone mutant proteins.

We first tested whether degradative lysosomes are found in distal axons using a set of activity-based fluorescent probes that label active forms of the lysosomal hydrolases CTSD (cathepsin D), CTSB, CTSL, and GBA/β-glucosidase/glucocerebrosidase/GCase (glucosidase, beta, acid) [1]. We used microfluidic devices to physically separate axons from cell bodies and dendrites of cortical neurons by an array of 450-μm-long microgrooves. This separation allows clear visualization of degradative lysosomes distributed in distal axons. We found that cathepsin-positive lysosomes are distributed along axon bundles and enriched in distal axon tips. We also examined the axonal distribution of active GBA, which can be detected by the activity-based fluorescent probes MDW933 (green) and MDW941 (red). Similarly, active GBA puncta are detected along axon bundles and accumulate in distal tips. Thus, while enzymatically active degradative lysosomes are enriched in the soma, they are also positioned along axons and abundant in distal tips.

The axonal positioning of degradative lysosomes suggests their delivery from the soma where degradative lysosomes are highly enriched. To test whether lysosomes from the soma transport into distal axons, we spatially loaded MDW941 for 1 h in the soma-dendritic chamber followed by time-lapse imaging of axon bundles in the microgrooves. We detected robust anterograde flux of degradative lysosomes toward distal axons with a mean velocity of 1.91 ± 0.04 μm/sec. Disrupting microtubules using nocodazole abolishes the delivery of MDW933-labeled degradative lysosomes to distal axonal tips, confirming microtubule-based transport. Degradative

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lysosomes are also delivered from the soma into the axon in synaptically connected mature cortical neurons in regular culture dishes.

We next examined whether degradative lysosomes locally cleave cargos in distal axons. We spatially loaded Magic Red CTSB or CTSL fluorogenic substrates in the axon chamber. These substrates become fluorescent upon proteolytic cleavage by CTSB or CTSL, respectively, within acidic lysosomes. Live imaging reveals the presence of vesicular structures containing cleaved CTSB or CTSL substrates along distal axons, which are largely suppressed when lysosome acidification is blocked using bafilomycin A_1. Furthermore, we observed that soma-derived degradative lysosomes are recruited to the distal tip where they colocalize with LC3-labeled autophagic vacuoles (AV) to form autolysosomes. The majority of AVs in distal axons colocalize with active GBA and contain locally degraded CTSB and CTSL substrates, reflecting their degradative nature and suggesting that autophagic cargos can be locally degraded within distal axonal autolysosomes. We also observed that Parkinson disease-related SNCA/α-synuclein colocalizes with degradative lysosomes in distal axons.

These findings raise a question as to whether reduced axonal delivery of degradative lysosomes affects axonal clearance of autophagosomes and pathogenic proteins. To address this, we used cultured DRG (dorsal root ganglion) neurons from mice at postnatal day 30–40 to examine outcomes of impaired lysosome delivery by knocking down Arl8, a small GTPase that mediates anterograde transport of endolysosomes. Arl8 depletion significantly reduces the density of degradative lysosomes in axons and increases AV axonal density, reflecting axonal autophagic stress, which is rescued by expressing an siRNA-resistant *Arl8b* mutant. Furthermore, impaired axonal delivery of degradative lysosomes compromises the clearance of A53T mutant SNCA/α-synuclein in distal axons. These results demonstrate that axonal delivery of degradative lysosomes is required for the maintenance of axon cellular homeostasis under physiological and pathological conditions.

Our study provides evidence of the axonal delivery of degradative lysosomes using a unique combination of 2 approaches: (1) by implementing microfluidic devices to physically isolate axons from the soma, which allows selective labeling of degradative lysosomes in the soma chamber for tracing their influx into distal axons, and (2) by applying 4 different activity-dependent probes for labeling active forms of cathepsins as well as GBA, to characterize axonal trafficking and positioning of degradative lysosomes. Our study demonstrates that degradative lysosomes are dynamically delivered to distal axons in developing and mature neurons from both central and peripheral nervous systems; disrupting the axonal delivery of degradative lysosomes induces axonal autophagic stress with buildup of autophagosomes and mutant SNCA/α-synuclein cargos. Thus, axonal degradation capacity is maintained by delivery of "fresh" degradative lysosomes from the lysosomal reservoir in the soma. Our study provides experimental guidelines for investigations into the regulation of transport, distribution, and degradation capacity of axonal lysosomes, thus advancing our understanding of neurodegenerative diseases and lysosomal storage disorders characterized by axonal dystrophy and autophagic stress.

Disclosure statement

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