Cytoskeletal Transport in Neurological Disease: 2022 Highlights

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To ensure neuronal homeostasis and function, a variety of cargoes are bi-directionally shuttled between the neuronal cell body and distal axon terminals via the process of axonal transport. Driven by motor proteins running along microtubule networks, axonal transport is essential for neuronal survival, as evidenced by perturbations observed in disease models, as well as mutations in transport machinery causing neurodegeneration.[1] Axonal transport is finely controlled[2], is closely linked with organelle maturation[3], and has been shown to be coupled with mRNA tethering to facilitate local protein translation.[4] This summary highlights recent advances supporting the emergence of alterations in the axonal cytoskeleton and trafficking as drivers of neurological diseases.

Axonal transport is regulated by microtubule post-translational modifications (PTMs), including polyglutamylation, in which glutamate chains are added onto microtubules by tubulin tyrosine ligase-like (TTLL) proteins and removed by cytosolic carboxypeptidases (CCPs). Impaired function of deglutamylase CCP1 causes increased tubulin polyglutamylation driving Purkinje neurons and peripheral nerve degeneration in both mice and humans. The two main neuronal polyglutamylases were recently shown to possess distinct enzymatic specificities: TTLL1 modifies α-tubulin, whereas TTLL7 modifies β-tubulin.[5] TTLL1, but not TTLL7, knockout rescued cerebellar and motor neuron defects in CCP1 loss-of-function mice, indicating that polyglutamylation of α-tubulin is sufficient to cause neurodegeneration. Mitochondrial mobility was perturbed in hippocampal axons lacking TTLL1, but not TTLL7, suggesting that different polyglutamylation patterns, and by extension, alternative microtubule PTMs, such as acetylation and detyrosination, selectively affect axonal transport to trigger neurodegeneration.

Mutations in *KIF5A*, encoding the main subunit of the anterograde kinesin motor, cause spastic paraplegia and amyotrophic lateral sclerosis (ALS). In an axonal proteome screen of rat retinal ganglion cells (RGCs), optic nerve injury was shown to impair anterograde transport of several proteins, of which Kif5A was the most affected.[6] In a glaucoma model, synthesis of Kif5A, but not Kif5B, was also reduced, consistent with the selective enrichment of Kif5A in RGCs and supporting the importance of Kif5A-mediated transport in optic neuropathy models. Crush-affected proteins were cross-referenced with a dataset generated from mice with RGC-specific *Kif5a* knockout, revealing common perturbations in mitochondria-associated genes. Accordingly, *Kif5a* knockdown and overexpression in cultured RGCs impaired and enhanced anterograde axonal transport of mitochondria, respectively. Crucially, Kif5a reduction caused progressive RGC degeneration, strengthening the link between axonal transport perturbations and neurodegeneration.

Dysfunction of KIF2A, an atypical kinesin that catalyses microtubule depolymerisation and is critical to cell division, causes progressive malformations of cortical development through impairments in neurogenesis and neuronal migration. However, through selective ablation in progenitor, nascent or mature cortical neurons of mice, new evidence indicates that KIF2A is dispensable for neurogenesis, but critical for neuronal connectivity, maturation and maintenance.[7] Loss of Kif2A perturbed microtubule-dependent processes, including axonal transport of lysosomes. These experiments identify new KIF2A functions and indicate that aetiology of the KIF2A-associated brain diseases should be revisited.

The assessment of axonal transport in neurons *in situ* prevents the disruptions caused by *ex vivo* preparations or *in vitro* culturing. Through injection of a radiolabelled, non-toxic fragment of tetanus neurotoxin into the mouse gastrocnemius muscle, it was recently shown that single

photon emission computed tomography applied to the lumbar spinal cord can be used to quantify net retrograde axonal transport of endosomes *in vivo*.[8] Confirming evidence obtained using intravital imaging approaches,[1,9] this technique identified trafficking decline with aging, as well as disruptions in mice bearing mutations in the ALS/frontotemporal dementia (FTD) genes *SOD1*, *C9ORF72* and *PFN1*. Notably, transport disruption was rescued in gene-edited mutant *SOD1* mice, with the benefit being detectable well before behavioural improvements. Since immunisation against tetanus does not prevent transport analysis, this method has clinical potential as a physiological biomarker in motor neuron diseases and possibly other neurological conditions, for early identification of therapeutic efficacy.

Mutations in *C9ORF72* and *TBK1* are linked to ALS/FTD, two neurodegenerative diseases sharing clinicopathological features, including the formation of protein aggregates containing TDP-43. Combined TBK1 loss-of-function with *C9ORF72* hexanucleotide repeat expansion, which results in the generation of toxic poly(GA) dipeptide repeats, causes a more aggressive FTD-ALS. Expression of poly(GA) in mouse brain causes TBK1 sequestration into cytoplasmic aggregates, with consequent reduced kinase activity.[10] Poly(GA) expression caused neuroinflammation, cortical neurodegeneration, and motor defects, all of which were exacerbated in mice harbouring a *TBK1* loss-of-function mutation. Notably, disrupted endosome maturation leading to formation of poly(GA)-positive TDP-43 inclusions, was also more pronounced in *TBK1* mutants, suggesting that *C9ORF72* and *TBK1* mutations converge on the endolysosomal pathway to drive TDP-43 pathology and neurodegeneration. Endosome maturation within axons is closely linked to retrograde transport[3], thus it will be of interest to determine whether this perturbed network directly contributes to axonal endosome dysfunction.

Late endosomes were recently identified as platforms for local translation to support mitochondrial function within axons, prompting a new area of research focused on mRNA hitchhiking on organelles for axonal delivery and local protein expression.[4] A recent study showed that *Pink1* mRNA is tethered to mitochondria for co-trafficking along axons and dendrites to enable on-demand Pink1 protein production.[11] This finding solves the conundrum of the prohibitively short half-life of Pink1 that prevents its timely delivery from cell body to distal compartments. This neuron-specific tethering mechanism, which is shared by several other transcripts, ensures the sufficient supply of Pink1 throughout neuronal processes that is required for removal of damaged mitochondria by mitophagy, an essential pathway that becomes dysfunctional in Parkinson's disease.

Overall, these discoveries highlight the importance of a functional cytoskeletal transport network in axons and dendrites to ensure neuronal integrity and survival. Novel, exciting targets have been identified, including microtubule PTMs, motor complexes and the endolysosomal pathway, which may be now exploited in innovative therapeutic strategies.

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