Off the rails: axonal cargoes on the road to nowhere

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The importance of the microtubule cytoskeleton for neuronal development, organization and maintenance is reflected in the finding that mutations in various tubulin genes cause a range of nervous system abnormalities. Mutations in the β -tubulin isotype-III gene, *TUBB3*, alter the interaction between microtubules and motor proteins leading to neurological impairments. Niwa *et al* (2013) now report that *TUBB3* mutations lead to neuronal defects by impairing axonal transport.

The axon is the long, slender projection of a neuron that typically conducts electrical impulses away from the soma to the synaptic sites of muscles, glands or other neurons. The longest axons in the human body are those of motor neurons, which start in the spinal cord and extend all the way to the feet and toes, where they stimulate muscle contraction. Although axons are very narrow tubes, several building blocks, such as proteins and lipids and cellular materials, such as mitochondria and synaptic vesicles are continually

Figure 1 Model showing how tubulin mutations affect kinesin-dependent axonal transport. In the axon, kinesins normally transport mitochondria and vesicles by walking along the microtubule lattice towards the microtubule plus-ends. However, *TUBB3* mutations that disrupt the negative charge at the microtubule–motor interface impair either the attachment or the docking of kinesins on the microtubules. This leads to disruption of axonal transport and consequently to the development of neurodegenerative diseases.

shipped along the axon, to keep the nerve cell alive and functional. This process, called axonal transport, is also fundamental for the neuron's proper response to trophic and stress signals (Perlson *et al*, 2010). Impairment of axonal transport has recently emerged as a common factor underlying several neurodegenerative disorders (De Vos *et al*, 2008). It has also been suggested that disturbances in axonal transport are direct causative factors in neurodegenerative diseases, although the mechanistic link between disruptive gene mutations and neurological phenotype remain largely unknown.

The identification of disease-causing mutations in genes encoding proteins involved in neuronal transport pathways strongly support the view that defective axonal transport can directly trigger neurodegeneration. Several disruptive mutations in microtubule-based motors have been directly linked to neurological diseases in both the central and peripheral nervous system (PNS; Millecamps and Julien, 2013). For example, mutations in the kinesin motor proteins KIF5A have been linked to dominant forms of the Hereditary Spastic Paraplegia (HSP) and Charcot-Marie-Tooth disease type 2 (CMT2) in humans, whereas mutations in KIF21A contribute to congenital fibrosis of the extraocular muscles type 1 (CFEOM1). Furthermore, mutations in dynein and its accessory protein dynactin have been associated with spinal muscular atrophy, CMT disease and ALS-like motor neuron degeneration and early-onset parkinsonism called Perry syndrome (Eschbach and Dupuis, 2011). A lot of attention has focused on understanding how these mutations in motors and associated proteins contribute to axonal transport defects. Alterations in motor proteins have been classically considered as the major culprits in axonal transport defects in the context of neurodegenerative diseases. However, Niwa *et al* (2013) have found that alterations on the microtubules themselves impair axonal transport, causing severe neuropathies.

In the axon, microtubules are polarized with the minus-ends facing the cell body and the plus-ends pointing towards the axon tip (Hoogenraad and Bradke, 2009). Microtubules have a hollow tubular structure and are composed of tubulin heterodimers containing several different α - and β -tubulin isotypes, whose ratios vary from one cell to another. For example, the β -tubulin isotype-III (TUBB3) is predominantly expressed in the central nervous system during development and in the PNS into adulthood. Mutations in various α / β -tubulin isotypes cause a wide range of neurodevelopmental diseases, including lissencephaly (TUBA1A), polymicrogyria (TUBA1A, TUBB2B, TUBB3) and microcephaly (TUBB5; Tischfield *et al*, 2011). Recent studies by Tischfield *et al* (2010) and Poirier *et al* (2010) reported several heterozygous missense mutations in TUBB3. These TUBB3 mutations were associated with a spectrum of human nervous system disorders now called the TUBB3 syndromes, which included the ocular motility disorder (CFEOM3) and the late-onset axonal sensorimotor polyneuropathy. By using *in vitro* and yeast models, the authors found that some of the mutations altered microtubule dynamics, whereas others, in particular, the TUBB3 mutations E410K and D417H impaired the interaction of microtubules with kinesin motors, leading to the idea that specific defects in the microtubule surface lattice may contribute to disruption of motor protein transport.

Niwa *et al* (2013) expanded on this new concept and directly tested whether TUBB3 mutations impaired axonal transport and influenced neuronal development *in vivo*.

To verify whether tubulin defects can influence axonal transport, Niwa *et al* (2013) co-transfected cultured hippocampal neurons of the central nervous system with several different β -tubulin mutations and the well-established synaptic vesicle transport markers VAMP2 and Rab3A. The authors found that of the 14 β -tubulin mutants tested, only the TUBB3 mutations E410K and D417H impaired the axonal transport of synaptic vesicles. TUBB3 mutations have also been implicated in peripheral neuropathies, and several studies have shown that major causes of these neuropathies are abnormalities in mitochondrial transport and function. Thus, the authors assessed the effect of TUBB3 mutations E410K and D417H on the transport of mitochondria in sensory neurons of the PNS and found that these two mutations also reduced the number of moving mitochondria.

Next, Niwa *et al* (2013) investigated in more detail the mechanism that led to the defects in cargo transport. Since the synaptic vesicles and mitochondria are transported by kinesin-1 and kinesin-3, a key experiment was to test whether these motors were still able to bind to microtubules containing mutant TUBB3. The authors found that in the presence of TUBB3(E410K) and TUBB3(D417H), kinesin-1/3 motors were unable to bind to microtubules *in vitro*, even when tail-less constitutively active forms were used. To further test the link between TUBB3 mutations and impaired motor protein transport, Niwa *et al* (2013) sought to quantify the translocation of kinesins into the axon by measuring the accumulation of kinesin at the axonal tips. Their data confirmed the biochemical analysis and demonstrated that TUBB3 mutations E410K and D417H can change the kinesin localization in neurons. This is also consistent with previous *in vitro* studies, where the residues E410 and D417 were found to be crucial for the kinesin-microtubule interaction and the size of kinesin stall force (Uchimura *et al*, 2006). One of the questions that remain unanswered is whether the TUBB3 mutants impair the movement of cargoes by inducing the loss of kinesin attachment during transport or by reducing the initial kinesin docking events on the altered microtubules. Setting up *in vitro* motility assays and purifying mutant tubulin isotypes would allow measuring these motor parameters along TUBB3-altered microtubules.

So how do TUBB3(E410K) and TUBB3(D417H) mutations inhibit axonal transport? The E410 and D417 residues are negatively charged and located on the H12 helix of TUBB3. Interestingly, no previous work had analysed the importance of the charge on H12 helix in relation to axonal transport. Niwa *et al* (2013) tackled this question by changing the negative charge of other TUBB3 residues at the microtubule surface and analysing the localization of kinesins and axonal cargoes. Their data demonstrated that β -tubulin mutants that disrupt the negative charge on tubulin severely impairs axonal transport. The authors took their experimental approach further and asked what would happen if these mutations were introduced into other β -tubulin isotypes. In other words, is the effect of these mutations specific for TUBB3? The authors convincingly showed that the mutations in other β -tubulin isotypes also inhibited axonal transport and mislocalized kinesins away from the axon tips.

Furthermore, to present more direct evidence that axonal transport was impaired because of the presence of mutant β -tubulin in the microtubule lattice, the authors added a new mutation (S172P) to TUBB3(E410K), which is known to impede β -tubulin incorporation into microtubules. In this case, axonal transport was normal and kinesin did accumulate at the axon tip to the same extent as controls, demonstrating that incorporation of the TUBB3(E410K) to microtubules inhibits axonal transport.

So far, Niwa *et al* (2013) provide compelling evidence that negative charges on β -tubulin residues at the microtubule—motor interface are important for axonal transport (see Figure 1). This raises many interesting questions as their data indicate that other disease-causing mutations in tubulin that perturbate the microtubule—kinesin interface could affect the axonal transport pathways. This model asks for a clinical re-classification of tubulin-related disorders (Breuss *et al*, 2012), where the neurological phenotype must be defined not only genetically by the mutations in α/β -tubulin gene but also by the functional effects of the specific

alterations. Disease-causing alterations in tubulin could disturb many different functions, including the stability of α/β -tubulin heterodimers, their incorporation into microtubules, microtubule dynamics and function, as well as their motor protein and/or MAP interactions (Tischfield *et al*, 2011). In the future, it will be intriguing to find out the various other mechanisms by which disease-related tubulin mutations disrupt microtubule function in the developing nervous system.

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Conflict of interest

The authors declare that they have no conflict of interest.

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