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Insights into the neurodegenerative mechanisms associated with KIF5A mutations

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Introduction and objectives: KIF5A is a neuron-specific kinesin driving anterograde axonal transport. It comprises an N-terminal motor domain for ATP-dependent microtubule binding, a coiled-coil stalk for conformational changes and dimerization, and a C-terminal tail domain for cargo binding and autoinhibition (Hirokawa et al., 2009). Mutations targeting the three KIF5A domains give rise to distinct neurodegenerative diseases (Sleigh et al., 2019), but the processes underlying such phenotypic heterogeneity are not yet known. Our aim is to investigate the pathogenetic mechanisms behind KIF5A-related neurodegeneration by functionally characterizing four disease-associated KIF5A mutations (R17Q, R280C, R864X, N999Vfs*39), which target the different domains of the protein.

Results: Altered protein turnover was evidenced for R17Q and N999Vfs*39 KIF5A upon overexpression, with the two mutants displaying shorter half-life compared to the wild-type (WT) protein. At the same time, R864X and N999Vfs*39 KIF5A showed abnormal intracellular distribution by preferentially localizing within neurites instead of being diffused in the whole cytoplasm like WT KIF5A. Such aberrant distribution pattern is consistent with impaired R864X and N999Vfs*39 KIF5A autoinhibition, respectively depending on loss or alteration of KIF5A tail domain. More in detail, while the R864X mutant was diffused within neurites, N999V*fs39 KIF5A formed puncta colocalizing with the ubiquitin-binding protein p62. Proteasomal blockage induced significant R17Q and N999Vfs*39 KIF5A accumulation within detergent-insoluble inclusions, indicating that the two mutants are mainly degraded by the ubiquitin-proteasome system and that they may form harmful aggregates when proteostasis is impaired. On the other hand, no involvement of the autophagic pathway was found in mutant KIF5A degradation. Finally, the abnormal distribution pattern characterizing R864X and N999Vfs*39 KIF5A was paralleled by limited colocalization with mitochondria, whose axonal transport largely relies on KIF5A, and by WT KIF5A sequestration within neurites.

Conclusions: Together, our preliminary results suggest that both unique and shared pathogenetic mechanisms underpin mutant KIF5A-dependent neurodegeneration.

Bibliography:

Hirokawa N, et al. Kinesin superfamily motor proteins and intracellular transport. *Nat. Rev. Mol. Cell Biol.* (2009) 10: 682–696.

Sleigh JN et al. Axonal transport and neurological disease. *Nat. Rev. Neurol.* (2019) 15: 691–703.