Poster for the meeting of the American Society for Cell Biology (December 2017)

A new genetic mouse model for SPAST-based Hereditary Spastic Paraplegia reveals the importance of toxic gain-of-function mechanisms

Authors: Liang Qiang, Emanuela Piermarini, Lanfranco Leo, Hemalatha Muralidharan, Guillermo Alexander, Laura Hennessy, Silvia Fernandes, Wenqian Yu, Terry Heiman-Patterson and Peter. W. Baas

Mutations of the *SPAST* gene, which encodes for the microtubule-severing protein spastin, are the most common cause of Hereditary Spastic Paraplegia (HSP), a debilitating neurological disease, typically adult-onset, in which corticospinal degeneration leads to spasticity and gait deficiencies. Haploinsufficiency is the most prevalent opinion as to the mechanism of the disease, but several lines of evidence suggest a different mechanism, namely gain-of-function toxicity of the mutant spastin proteins. *SPAST* has two start codons, producing a full-length isoform called M1 and a slightly shorter isoform called M87. The region specific to M1 is hydrophobic and may cause its mutant or truncated forms to misfold and produce cytotoxic effects. Here we report a new genetic mouse that expresses human M1 and M87 spastins harboring a pathogenic HSP mutation (C448Y), while still also expressing endogenous rodent spastin. The particular mutation was chosen in part because previous studies indicated that the mutant spastin does not act in dominant-negative fashion to reduce endogenous spastin activity, and this conclusion was supported by observations on the mouse. For example, staining for acetylated tubulin suggest a decrease in microtubule stability, which is the opposite of the expectation of a loss-of-function mechanism. Expression of the mutant proteins was detected by Western blotting from fetus to adult, but spasticity-like tremor and gain defects were only identified in adults. No symptoms inconsistent with HSP were observed. Results of histological and tracer studies were consistent with dying back of corticospinal axons, which is characteristic of HSP. Cultured newborn cortical neurons from the mouse showed no cellular morphological defects compared to wild-type counterparts, but displayed defects in lysosome transport that were worse in homozygotes than heterozygotes and worse yet when endogenous mouse spastins was mostly depleted by siRNA. These results indicate that the toxic gain-of-function effects of the mutant spastins are present in cells long before behavioral symptoms are apparent in the animal. Interestingly, the defects in lysosome transport were not correctable when the human mutant spastins were mostly depleted by siRNA, which is consistent with previous indications that only vanishingly small amounts of the mutant protein, presumably M1, are needed to elicit the cellular pathology. We posit work that the HSP phenotype is produced by toxic gain-of-function mechanisms of mutant spastins, but that haploinsufficiency can exacerbate the symptoms. The mouse will be useful for testing therapies.