PKAN hiPS-derived astrocyte models highlight the molecular mechanism of iron accumulation.

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We utilized human-induced pluripotent stem cell (hiPSC)-derived astrocytes to model PANK2-associated neurodegeneration (PKAN). This disorder is characterized by progressive neurodegeneration and significant iron accumulation in the globus pallidus of patients. It is caused by mutations in *PANK2*, coding for the first enzyme in Coenzyme A biosynthesis. Previous co-culture experiments using hiPS-derived -neurons and -astrocytes demonstrated the neurotoxic nature of PKAN astrocytes. Further analysis of iron metabolism in PKAN astrocytes exhibited cytosolic iron accumulation, altered iron metabolism, and changes in mitochondrial morphology. They also showed a propensity to develop a stellate-like phenotype, with the degree of stellation correlating with the amount of up-taken iron loaded transferrin. This suggests potential impairments in membrane dynamics, which may underlie the observed iron overload. Analysis of constitutive exo-endocytosis, a crucial route for cellular iron intake and vesicular dynamics, using the activity-enriching biosensor SynaptoZip, revealed a general impairment in constitutive endosomal trafficking in PKAN astrocytes. Super-resolution (SRFF) experiments, combining live pulse & chase with fluorescent transferrin and retrospective immunolabeling of external mitochondrial walls, showed significantly fewer transferrin-enriched vesicles contacting mitochondria in PKAN astrocytes, indicating an impaired intracellular fate of cargo endosomes. Analysis of mitochondrial iron homeostasis and tubulin feature reveal that mitochondria display an iron deficiency status caused by an impairment of iron deliver to mitochondria due to alteration of tubulin acetylation. This mitochondrial iron deficiency caused cytosolic iron overload due to the restriction of the metal to iron-dependent mitochondrial biosynthesis that affect iron-protein regulation. The findings underscore the crucial role of mitochondria in iron homeostasis and their involvement in the pathogenesis of CoA deficiency disorders. The impairment in iron delivery to mitochondria triggers cytosolic iron overload and mitochondrial dysfunction, contributing to neurodegeneration. This study highlights potential therapeutic targets for NBIA disorders through the modulation of mitochondrial iron metabolism and tubulin acetylation.