The dark side of AFG3L2 in mitochondrial degeneration

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Heterozygous mutations of a mitochondrial protease have been found in patients with SCA28. A pathological cascade starting with this mitochondrial mutation and ending in cerebellar degeneration was unravelled.

Autosomal dominant cerebellar ataxias are a group of neurodegenerative disorders whose main characteristics are imbalance, progressive unsteady gait and limb incoordination, and dysarthria (Harking, 1982). Spinocerebral ataxia 28 (SCA28) is a form of autosomal dominant spinocerebellar ataxia (SCA), whose locus is found on chromosome 18 (Cagnoli et al., 2005). SCA28 is associated with a heterozygous pathogenic mutation in AFG3L2 (Brussino, 2018). AFG3L2 (ATPase family gene 3-like-2) is a mitochondrial protease and is essential to form a complex, m-AAA (Di Bella et al., 2010). This complex has the protease activity needed for ATP-dependent degradation of products of mitochondrial translation and works as a chaperone that mediates the assembly of ATP synthase (Casari et al., 2001). No autosomal dominant spinocerebellar degeneration has been associated with mutations affecting proteins targeted to the mitochondria (Di Bella et al., 2010).

Mitochondria are the primary energy-generating systems that regulate calcium homeostasis and aid in signaling pathways, including cell death cascades. (Maltecca et al., 2009; McBride et al., 2006). Hence, mitochondria are essential for neuronal health, and alteration of mitochondrial physiology is linked with some neurodegenerative disorders and aging (Chang et al., 2006; Kwong et al., 2006; Maltecca et al., 2009). Several of these diseases are caused by mutations in nuclear genes, which code for mitochondrial proteins (Maltecca et al., 2009; Schapira, 2006). Some of these are loss-of-function mutations of the paraplegin-coding gene, which lead to recessive hereditary spastic paraplegia (HSP) due to degeneration of the longest motor and sensory axons of the central nervous system (CNS) (Casari et al., 2001; Maltecca et al., 2009). Paraplegin is part of the AAA-protease superfamily, and it bonds with its homologous protein, AFG3L2, to form the m-AAA complex in the inner membrane. This complex aids in the mediation of the complete degradation of organellar proteins. The m-AAA complex is also necessary for the assembly of the respiratory chain complexes (Maltecca et al., 2008; Maltecca et al., 2009). Despite paraplegin and AFG3L2 being in the same family of mixed-composition oligomers, only AFG3L2 can form high-molecular-weight significant proteins (Maltecca et al., 2009; Koppen and Langer, 2007; Koppen et al., 2007). Losses of function of AFG3L2 and paraplegin have different physiological outcomes. A paraplegin mutation results in late-onset axonal degeneration, whereas AFG3L2's mutation results in impaired axonal development, delayed myelination, and neuropathological alterations in the CNS and peripheral nervous system (PNS) (Maltecca et al., 2008; Maltecca et al., 2009). Furthermore, loss of AFG3L2 strongly affects the cerebellum, unlike the paraplegin mutation (Maltecca et al., 2008; Maltecca et al., 2009). Mice with a heterozygous AFG3L2 mutation show progressive loss of motor coordination and balance, related to mitochondrial dysfunction and Purkinje Cell (PC) dark degeneration (Maltecca et al., 2009). Simultaneously, AFG3L2 mutations have been found in patients with SCA28 (Cagnoli et al., 2005; Di Bella et al., 2010).

The researchers hypothesized that the pathological cascade leading to this form of SCA was related to a heterozygous mutation in AFG3L2. This mutation resulted in mitochondrial dysfunction, more specifically abnormal respiratory chain reactive oxygen species, which consequently led to Purkinje cell dark degeneration (Maltecca et al., 2009).

To assess this hypothesis, the researchers used mouse models. In the first set of experiments, it was verified that heterozygous mice had half the amount of protein compared to wild-type mice. AFG3L2 heterozygous mice display defects in motor coordination, and then wild-type and mutant mice's gait, pelvic elevation, and negative-geotaxis reflex were compared. It was also found that defects caused by the AFG3L2 mutation worsen with age. After 12 months, they presented uncoordinated hind limb movement. The mice's balance was also analyzed, and it was found that AFG3L2 +/Emv66 mice from 4 months onward could not maintain balance on the rotarod; additionally, it progressively worsened. It was also found that wild-type mice could walk along the beam; mutant mice could barely transverse it, causing their feet to slip more often. This evidence prompted the researchers to investigate cerebellar degeneration in mice. The cerebellar studies of AFG3L2 +/Emv66 mice show an alteration of the Purkinje cell dendritic tree. It was found that in mutant mice, several Purkinje cells disappear, resulting in empty spaces in the Purkinje cells' monolayer. The correlation between decreased motor coordination and cerebellum degeneration was determined through the analysis of semithin sections of the cerebellum from AFG3L2 +/Emv66 mice, in which progressive degeneration could be identified. It revealed a significant loss of Purkinje cells, which was already clearly noticeable in 6- and 12-month-old mutant mice. In 6-month-old mice, their mutant Purkinje cells display evident shrinkage and darkening of the cytoplasm, as well as a condensed nucleus. In 12-month-old mice, most of the remaining cells are degenerating Purkinje cells. At 12 months, changes in granule cells were also noticeable. These cells had an empty, swollen cytoplasm and a shrunken nucleus. To understand the degeneration of Purkinje and granule cells, the researchers investigated the mitochondria and possible morphological abnormalities. It was found that as early as 4-month-old mice already presented great defects in mitochondrial morphology and distribution. These findings are contemporaneous with early-onset incoordination. Mutant mitochondria were enlarged and lost their elongated shape. These mitochondria were also detected in Purkinje cells' dendrites. At 12 months, several organelles with odd shapes and unusual spatial organization were identified.

From 6 months onwards, many granule cells displayed empty cytoplasm and fully condensed chromatin. These features corresponded to late-stage apoptosis (Ihara et al., 1998), meaning that granule cells were dying of apoptosis. However, it was confirmed that Purkinje cells were not dying of apoptosis. Instead, the cerebella exhibited highly activated astrocytes. Despite the expectation that AFG3L2 +/Emv66 mice's spinal cords would be equally affected as the mice's cerebellums, semithin sections of the spinal cord revealed no degeneration in lumbar motoneurons. This data verifies the hypothesis that different neuronal populations have different sensitivities to m-AAA defects.

The researchers were able to prove that the defect has a mitochondrial origin, since morphological mitochondrial alterations precede all the other signs of dark cell degeneration. These abnormal mitochondria were found in Purkinje cell dendrites, where they would have been a crucial feature for ion homeostasis in synaptic sites, especially in the dendritic spines of glutamatergic synapses. Due to mitochondrial dysfunction, Purkinje cells suffer an ongoing process of dark degeneration as a result of excessive glutamate stimulation. Furthermore, the number of Purkinje cells under oxidative stress is correlated with the percentage of Purkinje cells undergoing dark degeneration.

A consequence of cells undergoing oxidative stress is the alteration of a gradient essential for mitochondrial uptake of calcium ions (Maltecca et al., 2009; Brookes et al., 2004). This results in an excessive, pathological accumulation of calcium ions in the cell. This leads to excitotoxic-mediated apoptosis, meaning toxic actions by excitatory neurotransmitters. Glutamate is the main culprit; these actions lead to dark degeneration, meaning the failure of cell physiology as well as nuclear and cytoplasmatic condensation of Purkinje cells (Maltecca et al., 2009; Barenberg et al., 2001).

In Purkinje cells with an AFG3L2 heterozygous mutation, the synthesis of ATP is altered, especially in dendritic mitochondria. Due to oxidative stress, these abnormal mitochondria cannot uptake calcium ions, meaning that there is an unrestricted accumulation of calcium ions in the cell, which leads to excessive glutamate stimulation. Excitotoxicity causes Purkinje cell dark degeneration, characterized by nuclear and cytoplasmatic condensation, overall shrinkage of the neuron, and loss of neuronal function. On a large scale, loss of Purkinje cell function re-

sults in degeneration of the cerebellum and, consequently, SCA28.

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