Is Amyotrophic Lateral Sclerosis a Mitochondrial Channelopathy?

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SOD1 is a cause of the fatal, paralytic disorder ALS. Although mechanisms underlying mutant SOD1 neurotoxicity remain uncertain, this protein associates with mitochondria. In this issue of *Neuron*, Israelson et al. show that mutant SOD1 binds and inhibits the mitochondrial channel VDAC1. This finding sheds light onto possible molecular links between mutant SOD1, mitochondrial dysfunction, and spinal motor neuron degeneration in inherited ALS.

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive paralytic disorder of middle and late life characterized mainly by a loss of cortical motor neurons and lower motor neurons in the brainstem and spinal cord (Pasinelli and Brown, 2006). There is no apparent genetic linkage in about 90% of ALS patients, but in the remaining cases, the disease is inherited (Pasinelli and Brown, 2006). Among the individuals with familial ALS, roughly one-fifth map to chromosome 21, where there are mutations in the gene for the antioxidant enzyme superoxide dismutase 1 (SOD1) (Pasinelli and Brown, 2006). In transgenic mice carrying the *SOD1*^{G85R} mutation, overexpression of wild-type SOD1 failed to modify the expression of the disease (Bruijn et al., 1998), supporting the notion that the mutated allele gives rise to a gain-of-function phenotype. Although in vitro studies indicate that mutant SOD1 exerts its deleterious effects via a combination of cell-autonomous and non-cell-autonomous processes, as illustrated in Nagai et al. (2007), the actual nature of the acquired adverse property remains to be established. Relevant to this outstanding issue, Israelson and collaborators have now performed a comprehensive set of investigations in the mutant SOD1 model of ALS from which has emerged, as shown in this issue of *Neuron*, an exciting and novel hypothesis: a mitochondrial channelopathy underpins neurodegeneration in this disease.

SOD1 is known to be essentially a cytosolic enzyme, but a portion of mutant SOD1, especially when overexpressed, has been identified in mitochondria (Pasinelli and Brown, 2006). This physical association has led researchers to posit that mislocalized mutant SOD1, by affecting mitochondrial functions, may contribute to the degeneration of motor neurons in this familial form of ALS. Consistent with this view, reduced respiratory chain activity, abnormally high release of apoptogenic mitochondrial molecules such as cytochrome c, and impaired mitochondrial movement have all been documented in transgenic mice expressing mutant SOD1 (Kirkinezos et al., 2005; Magrane et al., 2009; Pasinelli and Brown, 2006).

One way that mislocalized mutant SOD1 could impact on mitochondrial biology is through aberrant interactions with essential proteins associated with this organelle. For example, mutant SOD1 has already been shown to bind to Bcl-2 (Pasinelli et al., 2004) and lysyl-tRNA synthetase (Kawamata et al., 2008). Now, Israelson et al. (2010) report the remarkable finding that mutant SOD1 also interacts with the mitochondrial voltage-dependent anion channel-1 (VDAC1/porin-1). They found that VDAC1 (but not its related homolog VDAC2) coimmunoprecipitates with the catalytically active SOD1^{G93A} and inactive SOD1^{H46R} mutants but not with the wild-type SOD1 protein in solubilized mitochondrial lysates from rat spinal cords. Surprisingly, no evidence of an interaction between VDAC1 and mutant SOD1 is detected in protein extracts prepared from brain tissues despite the fact that the latter contains copious amounts of mutant SOD1 protein. The authors suggest that this regional specificity might be explained by the higher content of the known VDAC1 interactor, hexokinase-1 (Azoulay-Zohar et al., 2004), in brain compared to spinal cord, which may outcompete mutant SOD1. Yet, to support this proposal, additional experiments are still needed. For example, this proposal would be strengthened by (1) immunocytochemical studies showing that ALS-resistant ocular motor neurons express more hexokinase-1 than ALS-susceptible spinal motor neurons and (2) in vitro competition experiments showing that addition of hexokinase-1 recombinant attenuates the magnitude of SOD1/VDAC1 interaction.

By using an antibody that binds to a disease-specific epitope inaccessible on correctly folded SOD1, the authors were able to demonstrate that the VDAC1/SOD1 interaction involves, at least in part, misfolded SOD1 protein, which may represent the actual toxic species. Misfolded SOD1 protein—and consequently its interaction with VDAC1—is detected only in spinal cord mitochondrial fractions of transgenic mutant SOD1 rats and only in symptomatic animals. These results suggest that the binding of the noxious misfolded SOD1 conformer to VDAC1 parallels the time course of the disease and is restricted to areas most affected in this ALS animal model.

Next, to examine the potential functional significance of the VDAC1/SOD1 interaction, Israelson et al. (2010) measured ion channel conductance of purified VDAC1 reconstituted into a planar lipid bilayer by voltage clamp, as before

(Azoulay-Zohar et al., 2004). They found that mutant SOD1 proteins ($SOD1^{G93A}$ and $SOD1^{G85R}$), but not the wild-type SOD1 protein, reduce the ion conductivity of VDAC1. To assess another important function of VDAC1, but this time in a more genuine setting, the authors assessed the uptake of ADP by mitochondria isolated from spinal cords of transgenic $SOD1^{G93A}$ rats. This experiment showed a reduced ADP accumulation in mitochondria from presymptomatic animals and, even more so, from symptomatic animals. Collectively, these functional data suggest that, in the presence of mutant SOD1, VDAC1 is partially closed, a conformational state thought, at least by some, to adversely impact on normal mitochondrial functions (Mannella and Kinnally, 2008). Of note, high concentrations of the hexokinase reaction product glucose 6-phosphate can reopen VDAC1 (Azoulay-Zohar et al., 2004). Thus, should mutant SOD1-mediated VDAC1 closure be deleterious and should glucose 6-phosphate be able to alleviate the effect of mutant SOD1 on VDAC1 conductance, this small molecule may have to be considered as a potential neuroprotective compound for the treatment of ALS.

In the last part of their study, Israelson et al. (2010) wanted to determine the in vivo significance of a loss of VDAC1 conductance on the ALS phenotype by crossing transgenic $SOD1^{G37R}$ mice with mutant mice carrying one or two VDAC1 null alleles. The outcome of this experiment is striking, in that $SOD1^{G37R}$/$VDAC1^{-/-}$ mice reached end-stage paralysis ~16% sooner than their $SOD1^{G37R}$/$VDAC1^{+/+}$ counterparts. However, if the mutant SOD1/VDAC1 interaction were pathogenically important in SOD1-linked ALS, one would predict that abrogation of VDAC1 should have instead extended lifespan of animals. However, in the absence of histological data, whether the shortened lifespan in transgenic $SOD1^{G37R}$ mice deficient in VDAC1 reflects a true exacerbation of the neurodegenerative process or is just a confound due to functional abnormalities in cardiac and skeletal muscles (Anflous et al., 2001) cannot be sorted out.

Although the work of Israelson et al. (2010) opens a compelling new way of thinking about the neurobiology of ALS, at this point, it seems that the jury is still out as to whether the loss of channel conductance plays a pathogenic role in this inherited form of ALS. VDAC1 plays a key role in mitochondrial-dependent apoptosis (Mannella and Kinnally, 2008), a form of cell death that is thought to drive the ultimate demise of motor neurons in ALS (Guégan and Przedborski, 2003) and in mitochondrial turnover by autophagy (Geisler et al., 2010). Furthermore, VDAC1 is a major component of mitochondria-associated endoplasmic reticulum (ER) membranes (MAM); these are zones of physical interaction between mitochondria and the ER. Interestingly, the MAM has pivotal roles in a host of cellular functions, including Ca^{2+} signaling, lipid transport, energy metabolism, and cellular survival (Hayashi et al., 2009), and, in addition to mitochondria, mutant SOD1 also localizes to the ER (Kikuchi et al., 2006). Therefore, whether mutant SOD1-associated VDAC1 alterations promote neurodegeneration here through inhibition of channel conductance or one of these alterative VDAC1 functions, or a combination of both, may have to be considered in future studies designed to bring closure to this channelopathy story.

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