

# Bringing SOD1 into the fold

Sami Barmada & Steven Finkbeiner

**Could similar changes in superoxide dismutase 1 (SOD1) underlie both familial and sporadic amyotrophic lateral sclerosis (ALS)? A new study finds that wild-type SOD1 from sporadic ALS tissues shows conformational changes similar to those seen in familial ALS and may be pathogenic as a result of the same mechanism.**

ALS is an invariably fatal disorder, characterized by the relentless progression of muscle weakness in the absence of sensory symptoms<sup>1</sup>. The majority of ALS is acquired spontaneously (sALS), with inherited disease accounting for only 10–15% of all cases. Although several different mutations have been implicated in familial ALS (fALS), mutations in the gene encoding SOD1 are the most common cause of inherited motor neuron disease<sup>2</sup>. In this issue of *Nature Neuroscience*, Bosco *et al.*<sup>3</sup> provide compelling evidence that the misfolding of SOD1 into a disease-specific and toxic conformer underlies both sporadic and inherited ALS. As such, their results could change how we view motor neuron disease classification, pathogenesis, and the design and evaluation of therapies in clinical trials.

In healthy cells, SOD1 neutralizes superoxide, a reactive and potentially dangerous byproduct of energy-generating reactions in the mitochondria. Pathogenic mutations in *SOD1* have little effect on the enzymatic activity of the protein; instead, mutant SOD1 (mSOD1) exhibits toxic traits that may be important for the initiation and progression of disease<sup>4</sup>. In addition, post-translational modifications of wild-type SOD1, such as oxidation, can induce toxic gain-of-function properties in the protein that closely resemble those of mSOD1 (ref. 5). Reactive oxygen species (ROS) accumulate in the motor neurons of individuals with ALS and increased levels of oxidized protein can be detected in the same cells<sup>6</sup>.

In an elegant series of experiments, Bosco *et al.*<sup>3</sup> found that oxidized SOD1 shares important structural and toxic features with mSOD1.

Oxidation of SOD1 occurred primarily at a single amino acid residue (cysteine at position 111) that lies in exon 4, the same region of the protein that is often mutated in fALS. Moreover, oxidation changed the structure of SOD1 so that it became detectable with a conformation-specific monoclonal antibody, C4F6. This antibody was raised against nascent mSOD1 and exclusively recognizes a misfolded conformation in exon 4. These results suggest that oxidation of SOD1 changes the conformation of the protein such that it exhibits the same three-dimensional epitope as mSOD1. Notably, the authors found that C4F6-reactive SOD1 inhibited axonal transport, a fundamental cellular process that is essential for maintaining motor neuron survival.

The *in situ* oxidation of SOD1 in motor neurons of humans would be expected to generate similar C4F6-reactive and toxic conformers of SOD1. Might misfolded SOD1 be important for the pathogenesis of sALS? To address this question, Bosco *et al.*<sup>3</sup> used the C4F6 antibody to probe tissue samples from individuals with sALS and controls. Surprisingly, they detected C4F6-reactive SOD1 in over 50% of sALS samples. Furthermore, SOD1 purified from tissue samples of individuals with sALS, but not SOD1 isolated from control samples, inhibited axonal transport. The presence of C4F6-reactive SOD1 in tissue samples of individuals with ALS and the ability of this conformer to suppress axonal transport suggest that abnormal conformations of SOD1 could indeed mediate motor neuron toxicity in sALS.

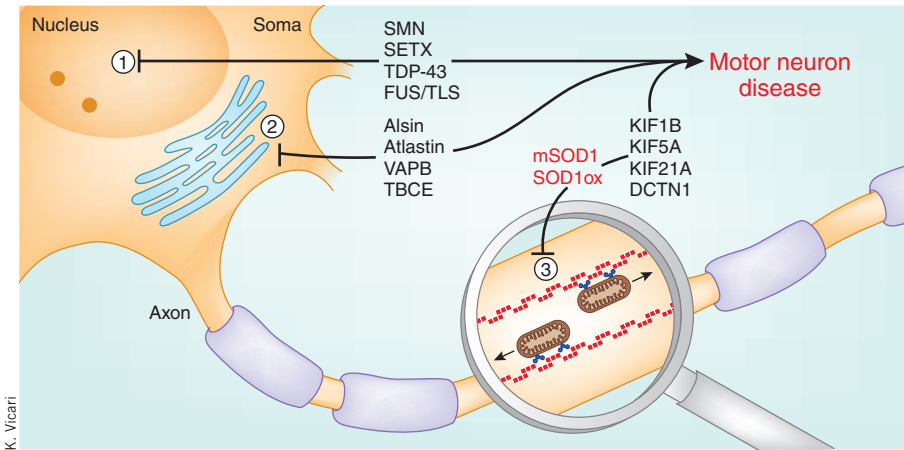
Accumulating evidence points toward abnormalities in axonal transport as a final common pathway in motor neuron disease. Deficits in axonal transport might occur through three independent mechanisms (Fig. 1). Initially, axonal transport could be prevented by improper transcription of essential genes, abnormal processing of the gene

transcripts or translational inhibition. This mechanism likely underlies motor neuron disease as a result of mutations in nucleic acid-binding proteins, such as TDP-43, FUS/TLS, SETX and SMN<sup>7</sup>. The intracellular trafficking of microtubules and endosomes is also essential for proper axonal transport and mutations in the genes encoding proteins important for this process (Alsin, Atlastin, VAPB and TBCE) likewise cause motor neuron degeneration<sup>8,9</sup>. Mutations in the genes encoding kinesins (KIF1B, KIF5A and KIF21A) and the dynactin complex (DCTN1) directly suppress axonal transport by disrupting motor protein function<sup>10</sup>. Bosco *et al.*<sup>3</sup> found that mSOD1, found in fALS due to SOD1 mutations, and misfolded SOD1, created by oxidation of SOD1, specifically inhibit anterograde axonal transport through disproportionate activation of p38 kinase. Suppression of axonal transport is lethal in motor neurons and p38 kinase activity is strongly associated with motor neuron degeneration in animal models of ALS<sup>11</sup>, confirming the potential of misfolded SOD1 to cause motor neuron disease through the same pathway. Indeed, small molecule inhibitors of p38 kinase show promise in animal models of ALS<sup>12</sup>, highlighting this mechanism as a major target for drug development in humans.

The study of neurodegenerative diseases, including ALS, has benefited greatly from genetic models that are based on inherited mutations in disease-associated genes. However, for several disorders, including Alzheimer's disease, Parkinson's disease and ALS, familial syndromes account for only a minority of the total disease burden<sup>13</sup>. Are the genetic models that we commonly employ accurate representations of the pathogenesis that occurs in sporadic disease and can we use these models to identify new therapeutics? The clinical and genetic heterogeneity of motor neuron disease implies that ALS may

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**Figure 1** Mechanisms of motor neuron degeneration. A schematic representation of a motor neuron and an enlarged portion of the axon are pictured to highlight three separate mechanisms that lead to motor neuron disease. (1) Mutations affecting proteins involved in essential nuclear processes, including DNA translation, RNA processing, microRNA biogenesis, and mRNA splicing and transport, often result in the selective degeneration of motor neurons. (2) Endosomal trafficking and the stabilization of microtubule networks are critical for the maintenance of motor neuron health. Interference with any of these activities is sufficient to induce motor neuron toxicity *in vivo* and *in vitro*. (3) Motor neurons are exquisitely dependent on functional axonal transport for growth signals, synaptic transmission and membrane turnover. Mutations affecting anterograde and retrograde motor proteins alike cause motor neuron dysfunction and eventually death. Both mSOD1 and oxidized SOD1 (SOD1ox) cause motor neuron toxicity by inhibition of anterograde axonal transport. DCTN1, dynactin 1; FUS/TLS, fused in sarcoma/translated in liposarcoma; KIF1B, kinesin family member 1B; KIF5A, kinesin family member 5A; KIF21A, kinesin family member 21A; SMN, survival motor neuron; SETX, senataxin; TBCE, tubulin-specific chaperone E; TDP-43, TAR (transactive response element) DNA binding protein of 43 kDa; VAPB, vesicle-associated protein B.

not be a single disorder<sup>2</sup>. Instead, what we call ALS may represent a collection of separate diseases that are characterized pathologically by motor neuron loss and clinically by weakness. Nevertheless, the results of Bosco *et al.*<sup>3</sup> suggest that the pathogenesis of fALS linked to *SOD1* mutations is, in fact, closely linked to that of sALS. This is reassuring evidence in support of the relevance of genetic ALS models to sporadic disease. Inhibition of axon transport may be the final common pathway in motor neuron disease, one that is susceptible to genetic and environmental insults (such as oxidation), perhaps representing a pathogenic mechanism that is common to both the familial and sporadic forms of ALS (Fig. 1).

Bosco *et al.*<sup>3</sup> also raise intriguing questions about the importance of protein misfolding in neurodegenerative disorders. Misfolded proteins with toxic properties have been implicated in the development of ALS and other neurodegenerative conditions<sup>14,15</sup>, but their contribution to disease pathogenesis is still unclear. In part, this is because we lack the appropriate tools with which to examine the role of protein misfolding in neurodegeneration. Although misfolded proteins can be recreated and studied *in vitro*, their prevalence and potential effects *in situ* are largely unknown. Conformation-specific antibodies such as C4F6 represent extremely powerful reagents that may help to illuminate the effects of protein misfolding on neuronal health.

Does protein misfolding occur to appreciable degrees *in situ*? The detection of C4F6-reactive species in the motor neurons of individuals with sALS shows that, at least to some extent, it does. How is the toxicity of misfolded SOD1 related to the C4F6 epitope? The misfolded conformer detectable by C4F6 also inhibited axonal transport, suggesting that the C4F6 epitope may be important for the acquisition of toxic gain-of-function properties. Is the C4F6 epitope directly responsible for the toxicity of misfolded SOD1? If so, then the C4F6 antibody, or other molecules that mask or alter the C4F6 epitope, may prove to be useful as therapeutics. The utility of such treatments could be maximized by first identifying individuals with detectable accumulations of misfolded proteins using conformation-specific antibodies, such as C4F6. Thus, in the future, these antibodies may be used to prevent or treat neurodegenerative disease and to identify the subset of individuals who stand most to benefit from the treatment itself.

#### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. [AU: Please clarify “mSOD1, found in fALS due to SOD1 mutations”. Mutant SOD1 is a result of SOD1 mutations?]

## **Production Query\_NN2675**

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