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Superoxide Dismutase and Oxidative Stress in Amyotrophic Lateral Sclerosis

María Clara Franco, Cassandra N. Dennys,
Fabian H. Rossi and Alvaro G. Estévez

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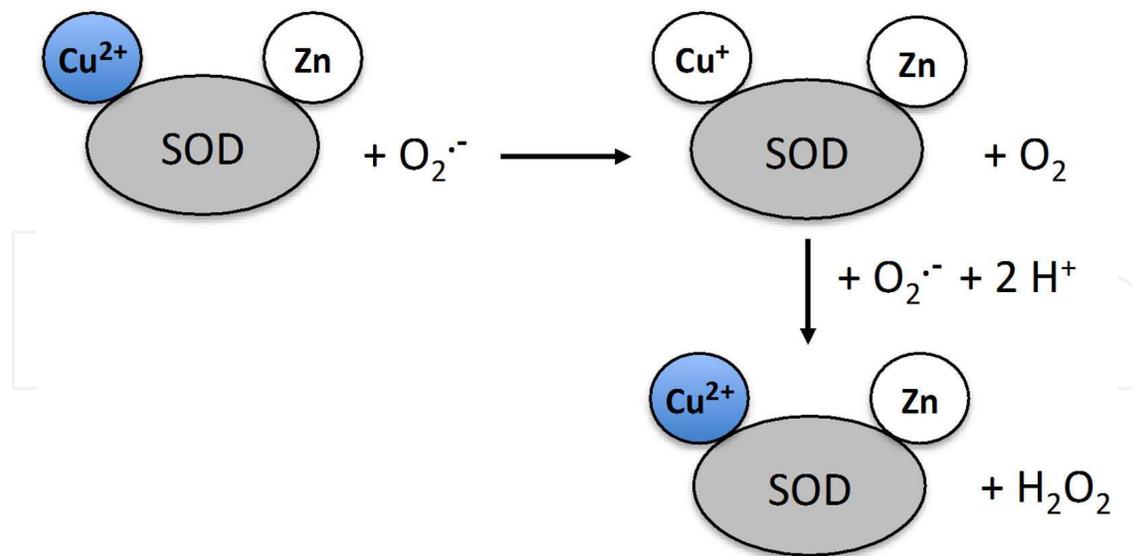
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1. Introduction

Oxidative stress is defined as the imbalance between reactive species such as free radicals and oxidants and the antioxidant defenses. Free radicals are molecules with one or more unpaired electrons, while oxidants are molecules with a high potential for taking electrons from other molecules. The more recognized reactive species are the reactive oxygen species (ROS), which include oxygen and its reduction products superoxide, hydrogen peroxide and hydroxyl radical, and the reactive nitrogen species (RNS) such as the free radical nitric oxide and its by-products, including the powerful oxidant peroxynitrite and the sub-product of peroxynitrite decomposition nitrogen dioxide.

As part of the antioxidant defense system, superoxide dismutase 1 (SOD1) is an abundant and highly conserved cytosolic enzyme responsible for the disproportionation of superoxide to molecular oxygen and hydrogen peroxide (McCord and Fridovich, 1969). SOD1 is a relatively small protein of 153 amino acids that works as a tight homodimer and requires a high stability for fast catalysis (Perry et al., 2010; Trumbull and Beckman, 2009). The stability is conferred by the quaternary structure of the protein, an eight-strand beta-barrel, as well as the binding of Cu and Zn, two metal ions with catalytic roles positioned in the active site channel (Perry et al., 2010; Trumbull and Beckman, 2009). The disproportionation of superoxide is a two-step oxidation-reduction reaction that involves the cycling of the copper atom in SOD1 from Cu^{2+} to Cu^+ and back to Cu^{2+} .

The zinc does not participate in this reaction but is essential for the structure of the active site. In addition, the formation of an intrasubunit disulfide bridge stabilizes the enzyme and plays an important role in preventing aggregation of metal-deficient SOD (Getzoff et al., 1989).



Mutations in the gene codifying for SOD1 were linked to familial ALS almost 20 years ago. Currently, over 130 point mutations on more than 70 sites on SOD1 have been described, most of these being missense single residue mutations located in critical positions that affect the stability and folding of the enzyme (Beckman et al., 2001; Perry et al., 2010; Roberts et al., 2007). The goal of this chapter is to review recent advances in our understanding of the role of oxidative stress on the gain of a toxic function associated with mutations in the gene of the copper/zinc superoxide dismutase.

2. Zn-deficient SOD1

The first proposed mechanisms linking mutations of SOD1 with ALS were based on the loss of dismutase activity (Beckman et al., 1993; Deng et al., 1993a). However, the SOD1 mutants G37R and G93A remain fully active and were linked to familial ALS (Borchelt et al., 1994; Yim et al., 1996). In addition, the mouse knockout for SOD1 developed normally and did not show signs of motor neuron deficit, although the motor neurons were more susceptible to cell death upon axonal injury (Reaume et al., 1996). This evidence indicated that a gain-of-function rather than the loss of function was responsible for motor neuron degeneration in ALS, and that the gain-of-function could be related to the redox properties of SOD1.

The discovery that mutations on the gene for an antioxidant enzyme such as SOD1 were associated with a population of familial ALS patients led to speculate on the role of oxidative stress in the pathogenesis of ALS (Beckman et al., 1993; Deng et al., 1993b; Rosen et al., 1993). From this original discovery to the present the interest on oxidative stress in ALS has been a rollercoaster. Several different groups described the presence of a variety of markers for oxidative stress in human samples and animal models of ALS, including elevated protein carbonyl and nitrotyrosine levels as well as lipid and DNA oxidation. Oxidation of proteins,

lipids, and DNA was also found in transgenic mice and cell culture models (Barber and Shaw, 2010). On the other hand, other groups failed to find markers of oxidative damage in animal models of ALS, casting doubt on the relevance of oxidative stress in the pathogenesis of the disease (Barber and Shaw, 2010). Currently, a role for oxidative stress in ALS is generally accepted but whether oxidative stress is responsible for the mutant SOD1 gain-of-function is still controversial.

2.1. Mutant SOD1 aggregation and Zn-deficiency

Mutant SOD1s have a tendency to aggregate when expressed in bacterial systems and transfected cells, and the presence of mutant and wild type SOD1-containing aggregates has been described in animal models of ALS (Bruijn and Cleveland, 1996; Watanabe et al., 2001). The formation of aggregates clogging the proteasome and containing other relevant proteins along with mutant SOD1 is one of the possible explanations for SOD1 toxic gain-of-function. However, in mice expressing the SOD1^{A4V} mutant, the most common mutation linked to familial ALS in humans, the mutant is expressed at high levels and forms protein aggregates but does not cause disease (Gurney et al., 1994). Alternatively, other groups proposed a hypothesis in which the formation of aggregates is a protective mechanism rather than cause of toxicity. *In vitro* experiments showed that both wild type SOD1 and SOD1 with mutation of the cysteine residues involved in protein aggregation were able to stabilize the mutant SOD1 enzymes, increasing their toxicity (Clement et al., 2003; Fukada et al., 2001; Sahawneh et al., 2010; Witan et al., 2009). Additionally, it was recently described that overexpression of the deubiquitinating enzyme ataxin-3 stimulates the formation of SOD1-containing aggresomes by trimming K63-linked polyubiquitin chains. The knockdown of ataxin-3 decreases the formation of aggresomes and increases cell death induced by mutant SOD1 (Wang et al., 2012). These results suggest a toxic gain-of-function for the stabilized and soluble mutant SOD1, rather than toxicity due to aggregation. Indeed, by removing the toxic soluble mutant SOD1, the formation of aggregates has been proposed to be a protective mechanism (Trumbull and Beckman, 2009). Further support is provided by recent studies of crossbreeding showing an acceleration of the disease in mutant SOD1 transgenic mice overexpressing wild type SOD1, which was linked to the formation of disulfide bridges in the enzyme by oxidation of cysteine residues, increasing the formation of aggregates (Deng et al., 2006; Furukawa et al., 2006; Wang et al., 2009). Other investigations reproduced the acceleration of the disease in animals expressing both wild type and mutant SOD1 but failed to find a correlation between expression of wild type SOD1 and protein aggregation (Prudencio et al., 2009).

The link between the gain-of-function and the redox activity of soluble mutant SOD1 as a source of oxidative stress is based on the presence of the copper atom in the active site of the enzyme as well as the loss of zinc. The requirement for copper was challenged by genetic experiments in which the chaperone that delivers the copper metal to SOD1 was deleted. The ablation of the chaperone in the G93A, G85R, or G73R-SOD1 mutant mice decreased the activity of the enzyme but had no effect on the progression of the disease (Subramaniam et al., 2002), although it may be possible for SOD1 to acquire copper from an alternative source (Beckman et al., 2002). The transgenic expression of a SOD1 with mutations that eliminate the

copper-binding site still produced disease (Prudencio et al., 2012; Wang et al., 2003). In contrast, another study showed that the mutant enzymes A4V, G85R, and G93A had a higher affinity for copper than the wild type protein, and that this aberrant copper binding was mediated by cysteine 111 (Watanabe et al., 2007), implying that the enzyme binds copper in an alternate site (Figure 1A).

Some SOD1 mutants bind copper and zinc and are fully active (Borchelt et al., 1994; Marklund et al., 1997) but many mutations affect the binding of zinc while copper remains tightly bound, thus favoring the formation of Zn-deficient SOD. In the SOD1^{G93A} mouse model of ALS, the dietary depletion of zinc accelerates the progression of the disease while moderate supplement of zinc provides protection (Ermilova et al., 2005). Indeed, a peak corresponding to one-metal SOD1 was detected *in vivo* in spinal cords from the SOD1^{G93A} rat model using the recently developed methodology of electrospray mass spectrometry. The one-metal peak was 2-fold larger in the disease-affected ventral spinal cord compare to that of the dorsal spinal cord (Rhoads et al., 2011), suggesting that Zn-deficient SOD1 may be present *in vivo* in the affected tissue.

ALS-linked mutant SOD1s have 5-50 fold less affinity for zinc than the wild type protein (Crow et al., 1997a; Lyons et al., 1996). The loss of zinc disorganizes the structure of the active site leaving the copper metal more expose and accessible to substrates other than superoxide, decreasing the normal activity of the enzyme. When replete with zinc, SOD1 mutants can generally fulfill the antioxidant activity of wild type SOD (Crow et al., 1997a). Early studies showed that mutant SOD1 has an aberrant chemistry and is reduced abnormally fast which allows the reaction with oxidants such as hydrogen peroxide and peroxyxynitrite (Crow et al., 1997a; Crow et al., 1997b; Lyons et al., 1996; Wiedau-Pazos et al., 1996), thus turning the antioxidant enzyme into a catalyst for oxidation. The conversion of SOD1 from antioxidant to pro-oxidant due to the loss of zinc is a simple explanation for the gain-of-function attributed to the ALS-linked SOD mutants, but is still highly controversial.

2.2. Formation of hydroxyl radical from hydrogen peroxide

In normal conditions SOD1 catalyzes the disproportionation of superoxide to hydrogen peroxide, but due to changes in mutant SOD1 conformation, the mutant enzyme can catalyze the production of hydroxyl radical from hydrogen peroxide *in vitro* (Yim et al., 1990) (Figure 1B). The G93A-SOD1 mutant has enhanced free-radical production compare to the wild type enzyme due to a more open active site, decreasing the K_m for hydrogen peroxide (Yim et al., 1996). Accordingly, an increase in the levels of hydrogen peroxide and hydroxyl radical was reported *in vivo* in the spinal cord from mice expressing the G93A mutant (Liu et al., 1999).

The aberrant chemistry of mutant SOD1 was shown to inactivate the glutamate transporter EAAT2 by oxidative reactions catalyzed by the A4V and I113T-SOD1 mutants and triggered by hydrogen peroxide (Trotti et al., 1999; Trotti et al., 1996). The function of this transporter is down regulated in human patients and animal models of ALS and its inactivation results in neuronal degeneration (Rothstein et al., 2005; Tanaka et al., 1997). Moreover, the aberrant SOD1 chemistry increases the vulnerability of a variety of cells in culture to hydrogen peroxide, with an increased susceptibility to inhibition by copper chelators. The G37R, G41D, and G85R-SOD1

mutants induce activation of caspase 1 and promoted apoptosis in N2a cells and tissue expressing mutant SOD1 when exposed to hydrogen peroxide. In NSC34 cells, a motor neuron model, mutant SOD1 induces cell death upon exposure of the cells to hydrogen peroxide (Pasinelli et al., 1998; Wiedau-Pazos et al., 1996). These findings suggest that the ALS phenotype may require both, the genetic background and an additional oxidative challenge.

2.3. Production of peroxynitrite

Nitric oxide alone is not toxic to normal motor neurons (Estévez et al., 1999), but when superoxide is also produced it can react with nitric oxide to form the powerful oxidant peroxynitrite, responsible for the induction of cell death. Overexpression of mutant SOD1 makes motor neurons vulnerable to exogenous and endogenous production of nitric oxide. The increased vulnerability is linked to the activation of the Fas death pathways (Raoul et al., 2002). More recently it was shown that motor neurons from mutant SOD1 transgenic animals have lower levels of a calcium-binding ER chaperone calreticulin. A decrease in the expression of this protein is necessary and sufficient to activate the Fas/NO pathways in motor neurons. Further evidence *in vivo* shows that this protein is decreased in the spinal motor neurons of SOD1^{G93A} transgenic animals prior to muscle denervation (Bernard-Marissal et al., 2012). Therefore, motor neurons expressing mutant SOD1 may produce superoxide making them susceptible to the formation of peroxynitrite in the presence of nitric oxide. In the presence of reductants, Zn-deficient SOD1 is able to produce superoxide. For instance, ascorbate reduces the copper on Zn-deficient SOD1 from Cu²⁺ to Cu⁺. In turn, Zn-deficient SOD1 can transfer the electrons from ascorbate to oxygen to produce superoxide slowly but significantly over a period of minutes. Indeed, Zn-deficient SOD1 is able to oxidize ascorbate 3000-fold faster than mutant or wild type Cu,Zn-SOD1 *in vitro* (Estévez et al., 1999). In the cells, ascorbate and other cellular antioxidants such as glutathione, urate, and cysteine could have a similar effect. Normally, superoxide would be removed by the dismutase activity of the remaining and fully active Cu,Zn-SOD1. However, if nitric oxide is also produced it can effectively compete with Cu,Zn-SOD1 for superoxide to produce peroxynitrite. Because nitric oxide is a small molecule able to diffuse 10-fold faster than a small size protein, the reaction of nitric oxide with superoxide occurs 10 times faster than that with SOD1 (Beckman et al., 2001; Estévez et al., 1999; Franco and Estévez, 2011; Nauser and Koppenol, 2002) (Figure 1B). Wild type Cu,Zn-SOD1 can also produce peroxynitrite by a similar mechanism but requires superoxide in the initial step to be efficiently reduced (Beckman et al., 2001).

2.4. Catalysis of tyrosine nitration

Cu,Zn-SOD1 is not only responsible for the production of peroxynitrite but it can also catalyze tyrosine nitration *in vitro* (Beckman et al., 1993; Crow et al., 1997b; Ischiropoulos et al., 1992). The mechanism for tyrosine nitration depends on the copper atom in SOD1 that reacts with peroxynitrite. The loss of zinc from Cu,Zn-SOD1 increases by 2-fold the efficiency of the enzyme to catalyze tyrosine nitration (Crow et al., 1997a) (Figure 1B). Moreover, SOD1 is not inactivated by peroxynitrite and can catalyze tyrosine nitration indefinitely. Indeed, reactivity for nitrotyrosine was found *in vivo* in the SOD1^{G93A} mouse model and in patients with ALS

(Beal et al., 1997; Ferrante et al., 1997). In spite of the indirect evidence of mass spectrometry showing a peak corresponding to a one-metal SOD1 in a rat model of ALS (Rhoads et al., 2011), whether Zn-deficient SOD1 is present *in vivo* and catalyzes tyrosine nitration is still source of debate and remains to be determined.

3. Regulation of NADPH oxidase activity by mutant SOD1

Several lines of evidence support the role of oxidative stress in mutant SOD1 toxicity, but some evidence suggest that interactions other than the redox properties of the enzyme stimulate oxidative stress by different mechanisms. Mutant SOD1 can induce oxidative stress by disruption of the redox-sensitive regulation of NADPH oxidase (Nox) in microglial cells. Noxs are transmembrane proteins that catalyze the reduction of oxygen to superoxide using NADPH as an electron donor (Brown and Griendling, 2009). Superoxide is then converted to hydrogen peroxide by SOD1. Under reducing conditions, SOD1 regulates Nox2 activation by binding and stabilizing Rac1. The oxidation of Rac1 by hydrogen peroxide disrupts the complex with SOD1 and inactivates Nox2. Upon expression of certain ALS SOD1 mutants, the dissociation of Rac1 from SOD1 is impaired and Nox2 remains active (Figure 1C). In addition, the expression of Nox2 is upregulated in the SOD1^{G93A} mouse model and in ALS patients. In fact, gene deletion of Nox1 or Nox2 provides the larger protection to date in animal models of ALS (Harraz et al., 2008; Marden et al., 2007).

4. Mutant SOD1 translocation to mitochondria

Mitochondria are one of the major sources of cellular ROS formed as by-products of oxidative phosphorylation. Abnormalities in the mitochondrial structure, localization and number as well as altered activity of the electron transport chain have been described in both, sporadic and familial ALS (Manfredi and Xu, 2005). The mitochondrial electron transport chain and ATP synthesis are severely impaired at disease onset in spinal cord and brain of SOD1^{G93A} transgenic mice (Lin and Beal, 2006). Both, wild type and mutant SOD localize in mitochondria in the central nervous system (Higgins et al., 2002). Mutant human SOD1 was found in the mitochondrial outer membrane, intermembrane space and matrix in transgenic mice, while inactive mutant SOD1 accumulates and forms aggregates in the mitochondrial matrix in the brain (Vijayvergiya et al., 2005). Aggregates of the mutant enzyme are also selectively found in the mitochondrial outer membrane in spinal cord from mouse models of ALS (Liu et al., 2004). Interestingly, the anti-apoptotic protein Bcl-2 binds to mutant SOD1 and aggregates in spinal cord mitochondria from patients and a mouse model of ALS, suggesting that mutant SOD1 may be toxic by depleting motor neurons of this anti-apoptotic protein (Pasinelli et al., 2004). Mutant SOD1 targeted to the mitochondrial intermembrane space in NSC34 cells induces cell death upon exposure of the cells to hydrogen peroxide (Magrane et al., 2009). In addition, the increase in carbonylated proteins and lipid hydroperoxides in mitochondria, as well as the abnormally high rates of production of hydrogen peroxide in SOD1^{G93A} transgenic

mice (Mattiuzzi et al., 2002; Panov et al., 2011) support the mutant SOD1 aberrant catalytic gain-of-function. Indeed, it was shown that metal-deficient SOD1s are prone to mitochondrial translocation and are found in the mitochondrial intermembrane space (Okado-Matsumoto and Fridovich, 2002). The mitochondria contain the majority of the cellular copper because is required by the oxygen-consuming proteins. The insertion of copper into the translocated metal-deficient SOD would result in the formation of Zn-deficient SOD inside the mitochondria (Figure 1A). This could explain why the mitochondria are affected early in the onset of the disease (Beckman et al., 2002). The ROS-linked toxic gain-of-function of mutant SOD1 would produce hydroxyl radical from H₂O₂ as well as peroxynitrite in the mitochondria. The mutant enzyme could then catalyze the nitration of mitochondrial proteins such as cyclophilin D and the adenine nucleotide translocator (Martin, 2010). Due to these toxic effects of mutant SOD1 on mitochondria, it has been proposed that the abnormal activity of the mitochondria in ALS may account for the initiation and progression of the disease. However, whether the mitochondrial localization of mutant SOD1 is cause or a consequence of pathology needs to be established.

5. Expression of mutant SOD1 in motor neurons and neighboring cells

A new mechanism integrating the autonomous and non-autonomous induction of motor neuron death in ALS is emerging. In this scenario, the role of motor neurons and surrounding cells in the onset and progression of ALS is temporally determined. Several studies were conducted where mutant SOD1 was selectively expressed *in vivo* either in motor neurons or microglia of chimeric mice, or in culture in embryonic primary or stem cell-based models, allowing the study of the role of individual population of cells in the onset and progression of ALS. The cell-autonomous degeneration of motor neurons expressing mutant SOD1 seems to be more relevant for the onset and early progression of the disease, while microglia, peripheral macrophages, and astrocytes would play a role in the late disease progression.

5.1. Expression of mutant SOD1 in motor neurons

ALS is a motor neuron disease characterized by the gradual and selective loss of both, upper and lower motor neurons. Expression of mutant SOD1 in spinal motor neurons and interneurons of chimeric mice is enough to induce neuronal degeneration (Boillee et al., 2006; Wang et al., 2008). The mice do not develop clinical ALS but the motor neurons expressing mutant SOD1 exhibit pathological and immunohistochemical abnormalities, while motor neurons negative for mutant SOD1 expression do not. These observations indicate that in the chimeric mice the degeneration of motor neurons can be cell-autonomous. The fact that only some of the motor neurons express mutant SOD1 in this model may explain why the animals do not develop the disease (Wang et al., 2008). Indeed, normal motor neurons can prevent or delay the degeneration of mutant SOD1-expressing motor neurons (Clement et al., 2003). In addition, decreased expression of mutant SOD1 in motor neurons has a modest effect on the duration of the disease but significantly delay the onset and early phase of the disease progression (Wang et al., 2008). Similar results were observed in culture, where primary spinal motor

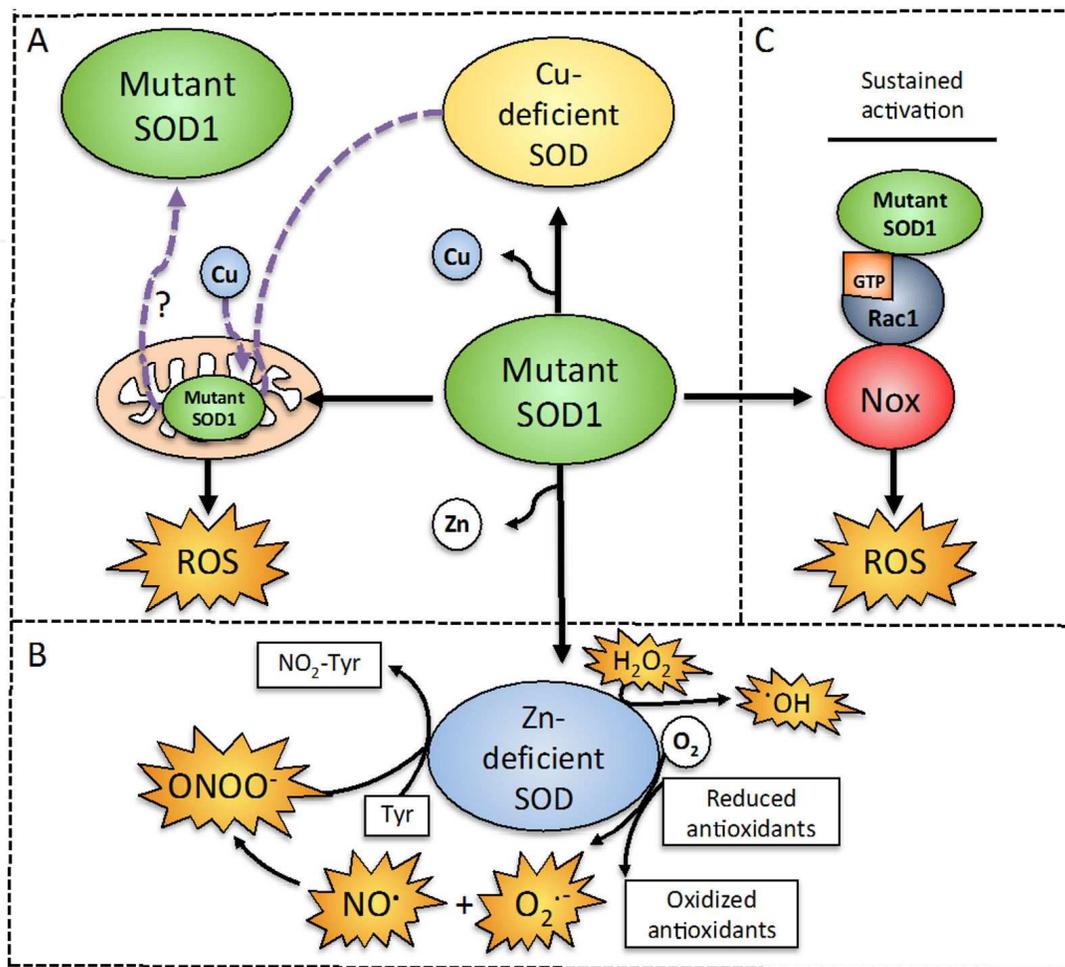


Figure 1. Role of oxidative stress in mutant SOD1 toxic gain-of-function. A. The toxic gain-of-function depends on the redox properties of the enzyme and relies on the copper atom. The mutant SOD1 translocates to mitochondria, while the metal-deficient enzyme may translocate and bind copper in the organelle. B. Zn-deficient SOD1 as catalyst of ROS production and tyrosine nitration. C. Mutant SOD1 regulation of ROS production by Noxs. NO₂-Tyr: nitrotyrosine, HO: hydroxyl radical, O₂: molecular oxygen, ONOO: peroxynitrite, NO: nitric oxide, O₂^{•-}: superoxide.

neurons as well as embryonic stem cell-derived motor neurons expressing mutant SOD1 showed changes characteristic of neurodegeneration (Di Giorgio et al., 2007; Raoul et al., 2002). Primary embryonic motor neurons from SOD1^{G93A} and SOD1^{G85R} transgenic animals exposed to endogenously produced or exogenously added nitric oxide show an increased susceptibility to cell death in culture (Raoul et al., 2002). Thus, motor neurons expressing mutant SOD1 are susceptible to cell death stimulated by oxidative stress.

5.2. Expression of mutant SOD1 in glial cells

Neighboring cells also seem to play a role in mutant SOD1 toxicity. Normal motor neurons in the context of a mutant SOD1-expressing chimera show signs of neurodegeneration, while non-neuronal cells negative for mutant SOD1 expression delay neuronal degeneration and significantly extend survival of mutant-expressing motor neurons (Clement et al., 2003). In the

last few years, a role for microglia and astrocytes in the induction of motor neuron death has become evident.

5.2.1. Role of microglia in the induction of motor neuron death

Activated microglia is found in the spinal cord of SOD1^{G93A} transgenic mice, suggesting that it may play a role in the neurodegeneration of neighboring motor neurons (Beers et al., 2006). Reducing the expression of mutant SOD1 in microglia and peripheral macrophages in chimeric mice leads to a delay in the late progression of ALS but has little effect on the onset and early disease progression (Boillee et al., 2006). Likewise, in the PU.1^(-/-)/SOD1^{G93A} mice unable to synthesize myeloid cells, the replacement of microglia, monocyte, and macrophage lineages with genotypically identical wild type cells slows disease progression and extends overall survival (Beers et al., 2006), suggesting that non cell-autonomous effects contribute to ALS progression independently of disease onset. Comparable findings were observed in co-culture studies where glial cells expressing mutant-SOD1 had a direct adverse effect on motor neuron survival (Di Giorgio et al., 2007). Microglia expressing G93A-SOD1 is toxic to primary motor neurons *in vitro*. In addition, SOD^{G93A} microglia show an increase in superoxide and nitric oxide production and release respect to wild type microglia. Treatment with lipopolysaccharide further increases SOD^{G93A} microglia activation and induction of motor neuron death (Beers et al., 2006). Hence, mutant SOD1-expressing microglia is activated, is more susceptible to activation, and it is capable of inducing motor neuron death *in vitro* (Beers et al., 2006). Interestingly, PU.1^(-/-) mice transplanted with bone marrow from a SOD1^{G93A} donor do not develop clinical or pathological evidence of ALS, suggesting that expression of mutant SOD1 in microglia is not enough to induce motor neuron disease *in vivo* (Beers et al., 2006). The fact that expression of mutant SOD1 in microglia alone does not induce motor neuron degeneration suggests that motor neurons and other glial cells play a role in the pathological process. Indeed, motor neurons expressing mutant SOD1 are more susceptible to cell death induced by exposure to nitric oxide or Fas activation (Raoul et al., 2002).

5.2.2. Role of astrocytes in the induction of motor neuron death

Astrocytes are the most abundant non-neuronal cells in the nervous system. The co-culture of normal primary embryonic or stem cell-derived motor neurons with astrocytes expressing mutant SOD1 result in motor neuron death. The death pathway is triggered by a toxic factor released by the astrocytes (Aebischer et al., 2011; Nagai et al., 2007). A population of phenotypically aberrant astrocytes was recently described in the SOD1^{G93A} mouse model of ALS (Diaz-Amarilla et al., 2011). These astrocytes, referred to as "AbA cells", have an increased proliferative capacity and secrete soluble factors that are 10 times more potent than neonatal SOD1^{G93A} astrocytes for the induction of motor neuron death. AbA cells are present in degenerating spinal cord of SOD^{G93A} rats surrounding affected motor neurons, and their number increases dramatically after disease onset, highlighting the importance of this finding. Interestingly, the levels of interferon- γ (IFN γ) are significantly increased in mutant SOD1-expressing astrocytes, and IFN γ induces motor neuron death (Aebischer et al., 2011), suggesting that this cytokine may be one of the toxic factors mediating induction of cell death (Figure

2). The role of astrocytes in the induction of motor neuron death was recently confirmed in astrocytes generated from post-mortem tissue of familial and sporadic ALS patients, additionally providing an *in vitro* model system for the study of these mechanisms (Haidet-Phillips et al., 2011).

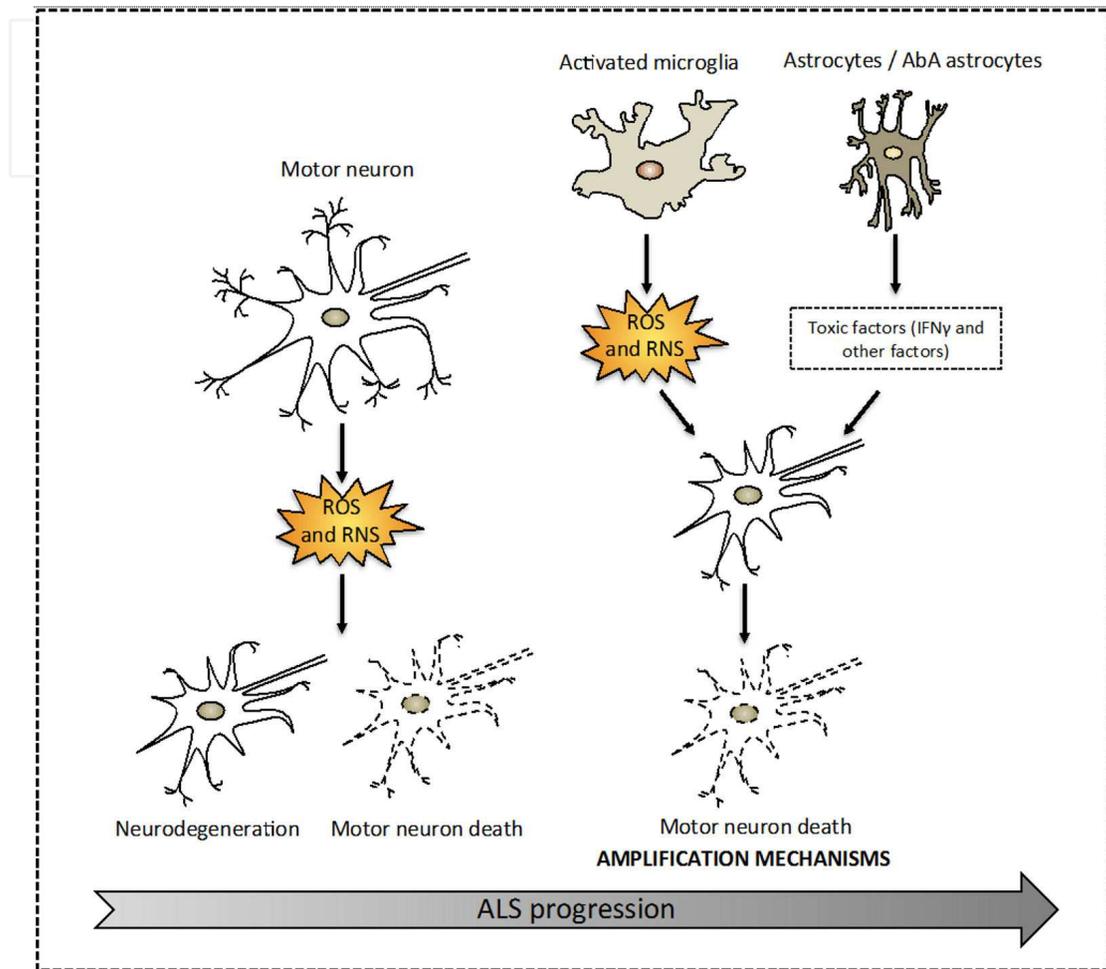


Figure 2. Model for the role of motor neurons and glia in the ROS-mediated ALS progression. Different cell types are affected and play a role at different stages of the disease. During the onset and early disease progression, motor neurons undergo degeneration and cell death by cell-autonomous mechanisms. Later in the disease progression, activated microglia and astrocytes release ROS, RNS, and toxic factors that magnify the injury (cell death amplification mechanisms).

6. Conclusion

In summary, the mechanism of mutant SOD1 toxicity is unknown and highly controversial but there is strong evidence suggesting that the mutant SOD1 toxic gain-of-function is related to an alteration of its redox properties and the induction of oxidative stress. In this scenario, the aberrant chemistry of mutant SOD1 turns the enzyme from antioxidant to pro-oxidant. Zn-

deficient SOD1 reacts with hydrogen peroxide, produces superoxide and peroxynitrite, and is able to catalyze tyrosine nitration, altering the cellular redox balance. In addition, although not related to the redox properties of the enzyme, the interaction of mutant SOD1 with mitochondria and Nox, the two major sources of cellular ROS, further support the involvement of oxidative stress in the toxic gain-of-function. The cell type affected by mutant SOD1 is also controversial. A picture in which several cell types are affected and play a role at different stages of the disease seems to be emerging. In this context, during onset and early stages of the disease SOD1-expressing motor neurons undergo neurodegeneration and cell death by cell-autonomous processes. The activation of microglia and astrocytes may work as an amplification mechanism in the induction of motor neuron death in the late progression of the disease (Figure 2).

Author details

María Clara Franco^{1,2}, Cassandra N. Dennys^{1,2}, Fabian H. Rossi^{1,2} and Alvaro G. Estévez^{1,2}

1 Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, USA

2 Orlando VA Healthcare System, Orlando, USA

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