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Biochimica et Biophysica Acta 1842 (2014) 1295–1301

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Role of mitochondria in mutant SOD1 linked amyotrophic lateral sclerosis ☆

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ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 13 February 2014
Accepted 15 February 2014
Available online 22 February 2014

Keywords:
Amyotrophic lateral sclerosis
Mitochondria
Oxidative stress

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with an adult onset characterized by loss of both upper and lower motor neurons. In ~10% of cases, patients developed ALS with an apparent genetic linkage (familial ALS or fALS). Approximately 20% of fALS displays mutations in the *SOD1* gene encoding superoxide dismutase 1. There are many proposed cellular and molecular mechanisms among which, mitochondrial dysfunctions occur early, prior to symptoms occurrence. In this review, we modeled the effect of mutant SOD1 protein via the formation of a toxic complex with Bcl2 on mitochondrial bioenergetics. Furthermore, we discuss that the shutdown of ATP permeation through mitochondrial outer membrane could lead to both respiration inhibition and temporary mitochondrial hyperpolarization. Moreover, we reviewed mitochondrial calcium signaling, oxidative stress, fission and fusion, autophagy and apoptosis in mutant SOD1-linked ALS. Functional defects in mitochondria appear early before symptoms are manifested in ALS. Therefore, mitochondrial dysfunction is a promising therapeutic target in ALS. This article is part of a Special Issue entitled: Misfolded Proteins, Mitochondrial Dysfunction, and Neurodegenerative Diseases.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease [1,2], with an incidence of about 2 cases per 100,000 and a prevalence of 5 per 100,000 people per year worldwide [2]. ALS causes degeneration of upper motor neurons in the cerebral cortex and lower motor neurons in the brain stem and spinal cord, leading to muscle weakness, eventually progressing to muscle paralysis and atrophy. The most common reason of death for ALS patients is respiratory failure, usually within three to five years after the diagnosis [3,4].

In approximately 90% of cases, patients developed ALS without apparent genetic linkage (sporadic ALS or sALS), while the remaining 10% of cases are familial (fALS). The first gene discovered with ALS-causative mutations was *superoxide dismutase 1* (*SOD1*). More than 150 ALS-linked mutations have been reported in *SOD1* over the course of 20 years, which are cumulatively responsible for approximately 20% of all fALS cases [5,6]. In 2011, a genetic anomaly linked to a form of ALS associated with frontotemporal dementia (FTD) was identified as an aberrant number of expansions of a hexanucleotide repeat sequence (GGGGCC) in the non-coding region of the *C9ORF72* gene on chromosome 9 [7,8]. In addition to being involved in ~40% of fALS cases, these intronic repeat expansions have been linked to ~10% of cases previously classified as sporadic [9], making this the most abundant ALS-causative gene so far. Several other mutated genes have been identified, mainly involved in non-traditional forms of fALS or have been found in just a few families; including *VAPB* (Vesicle-associated membrane protein-associated protein B) [10], *ALS2* (alsin) [11], *VCP* (valosin-containing protein) [12], *OPTN* (optineurin) [13], *UBQLN2* (ubiquilin 2) [14], *DAO* (D-amino acid oxidase) [15], *SPG11* [16], and *hnRNPA2B1* and *hnRNPA1* [17]. Cell and animal models incorporating different mutated genes have been developed, aiming at identifying molecular mechanisms of the disease. Among them, mice harboring mutations in the human *SOD1* transgene are still the most common genetic animal models for this disease. In fact, most of our current understanding of the molecular mechanisms of ALS comes from studies done on the mutant SOD1 mouse models and will be the focus of the present review.

There is currently no cure for ALS. The only FDA approved drug, Riluzole, increases the survival in patients by a few months [18,19]. Preclinical ALS research is currently focused on the human mutant SOD1 transgenic mouse lines, which recapitulate many aspects of human ALS pathology and for which extended survival is one of the main predictors of preclinical success. Several compounds have been identified that provide some degree of improvement in survival, but none thus far has proved to be a substantial treatment option when translated in patients. There are multiple issues that could account for this discrepancy, including the study design of preclinical trials, the lack of additional animal models available for research, and insufficient

☆ This article is part of a Special Issue entitled: Misfolded Proteins, Mitochondrial Dysfunction, and Neurodegenerative Diseases.

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http://dx.doi.org/10.1016/j.bbadis.2014.02.009
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insight into pathological causes. Furthermore, studying the mutant SOD1 transgenic mouse model has identified multiple cell types and molecular mechanisms that are affected, hence single treatments that target one pathway at a time may not be enough. Recently, a number of investigators have begun to test combination therapies, which can potentially enhance the effect of single pharmacological agents [20].

Many cellular and molecular mechanisms have been proposed to explain the loss of motor neurons seen in ALS, including glutamate-induced excitotoxicity, endoplasmic reticulum stress, proteasome inhibition, mitochondria-mediated damage, secretion of toxic factors by non-neuronal cells, oxidative stress, axonal disorganization, neuromuscular junction abnormalities, aberrant RNA processing [21]. In this article, we will review the role of mitochondria and mitochondria-mediated mechanisms of cell damage in ALS, focusing primarily on the function played by mitochondria in the pathogenesis of mutant SOD1-ALS, since most of the mechanistic studies on mitochondrial dysfunction have been done using models of mutant SOD1-mediated ALS.

2. Mitochondria and ALS pathogenesis

That mitochondria are compromised in ALS is apparent from multiple studies performed using cellular or animal models of disease and in patients. Early studies on post-mortem tissues of ALS patients identified at the electron microscopic level structural and morphological abnormalities in mitochondria of skeletal muscle, liver, spinal cord neurons and motor cortex [22–24]. Defects in mitochondria Ca^{2+} buffering capacity [25–30] and in the activity of the electron transport protein complex occurring during the pre-symptomatic phase of disease were also reported in the spinal cord of mutant SOD1 mice [25–30]. Aberrant mitochondria have also been identified in more recent models of disease, as mutant FUS and TDP-43 expressing cells as well as flies, which exhibit aberrant, and perhaps, non-functional mitochondria [31]. In the SOD1-G93A mouse model the appearance of mitochondria with dilated and disorganized cristae has been reported, both in the axons and dendrites of motor neurons at onset of disease [28]. In these mice, the onset of the disease is immediately preceded by a rapid increase in degenerating mitochondria with almost absent motor neuron death, which progressed during the symptomatic stage to a vacuolar pattern likely originating from expansion of the mitochondrial intermembrane space and extension of the outer mitochondrial membrane [32]. Mitochondria displaying swollen morphology and increased cristae were reported in the soma and proximal axons of motor neurons in the anterior horns of patients with sporadic ALS (sALS) [33]. Since these morphological abnormalities appeared in mice before the onset of symptoms and motor neuron degeneration, it has been concluded that mitochondrial impairment plays a key role in initiating motor neuron degeneration in ALS.

2.1. Mitochondria and protein misfolding in ALS

One of the pathological hallmarks of ALS is aggregation of ubiquitinated proteins in motor neurons [34]. Most of the proteins encoded by the known ALS causative gene mutations have been identified as part of these ALS-linked cellular aggregates, which include SOD1, FUS, TDP-43, OPTN, and UBQLN2. Mutations in proteins alter their normal conformation making them unstable [35]; segregation of this phenomenon with disease in familial and even sporadic cases (i.e. TDP43) [36] suggests a causal relationship between protein instability, aggregates and disease. However, whether aggregates represent a pathogenic or simply a pathological feature of the mutated protein is still debated. Whether there is a causal relationship between misfolded proteins and mitochondrial dysfunction is still largely unknown for the newly discovered ALS-linked mutations such as FUS, TDP43 and C9ORF72, but it has been quite extensively studied in the case of mutant SOD1. Mutant SOD1 forms insoluble aggregates in mitochondria at the surface of outer membrane [37–39], raising the prospect of a direct cause–effect mechanism by which mutated SOD1 directly impact mitochondrial function, ultimately leading to cell death. Indeed, isolated mitochondria exposed *in vitro* to purified mutant SOD1 showed increased susceptibility to oxidative stress and structural damage, which ultimately caused cytochrome c release [39,40].

While misfolding and oligomerization is pronounced in the perturbed mutant SOD1 protein, it was reported that also wild type SOD1 has the tendency to self-oligomerize, particularly in its apo state [41,42]. Interestingly, the aggregation propensity of wild type SOD1 seemed to be balanced by the rate of self-dissociation [43]. This equilibrium may be disturbed by structural alterations introduced, for instance, by disease-causative mutations, or by age- and/or stress-induced post-translational modifications, such as oxidation. Intriguingly, it has been reported that posttranslationally modified wild type SOD1 is a potential risk factor in ALS [44,45]. Oxidized SOD1 (OxSOD1) is conformationally altered and is more prone to aggregation than its wild type counterpart [46], similarly to what has been described for mutant SOD1-G93A [47]. Oxidized forms of wild type SOD1 have also been identified in sALS and conformational alterations in wild type SOD1 have been proposed as a possible pathogenic link between sALS, or at least a subset of sALS cases, and mutant SOD1-linked fALS [47,48]. OxSOD1 and mutant SOD1 may also hetero-oligomerize with wild type SOD1 to form aggregates that could potentially be harmful to cells, similarly to what has been reported in prion disease [49]. We previously reported that mutant SOD1 aberrantly binds to Bcl-2 in the mitochondrial outer membrane where it can trigger Bcl-2 mediated toxicity [40,50]. Perturbations from the wild type state unrelated to the presence of mutations, can also increase SOD1 affinity for Bcl-2. For instance, an iperoxidized form of SOD1 found in a cohort of sporadic patients with bulbar ALS onset shares toxic features with mutant SOD1-G93A as it leads to an aberrant interaction with Bcl-2 in mitochondria and a conformational change in Bcl-2 similar to the one induced by mutant SOD1 [48].

2.2. Possible mechanisms of mitochondrial damage induced by mutant SOD1

One of the interacting proteins of mutant SOD1 is Bcl-2, which mediates, to some extent, the mutant SOD1 toxicity to mitochondria [37,40,50]. Upon docking to mitochondria, mutant SOD1 aberrantly interacts with Bcl-2 [37], which is converted into a toxic protein featuring exposure of the BH3 death domain [40]. Bcl-2 family proteins have been extensively characterized for their regulatory mechanisms on programmed cell death through the conformational reorganization of their respective BH domains [51]. They also have a dual role in regulating mitochondrial bioenergetics. For example, anti-apoptotic Bcl-2 family proteins, such as Bcl-xL and N-terminal truncated Mcl-1, promote mitochondrial respiration and oxidative metabolism; while pro-apoptotic Bcl-2 family proteins such as Bad and Noxa, when phosphorylated, enhance glycolysis (non-oxidative metabolism) and pentose phosphate pathway [52]. By mediating BH3 domain exposure in Bcl-2, mutant SOD1 could initially switch the bioenergetics states in affected motor neurons by favoring glycolysis and limiting mitochondrial respiration. In the short term, this change in metabolism may not necessarily have negative consequences. However, on a more extended time scale, neurons have been shown to be particularly sensitive to dysregulated energy metabolism because oxidative metabolism supplies the additional ATP necessary to sustain neuronal activity [53].

Systematic analysis of mitochondrial respiration kinetics revealed a drop of outer membrane permeability in spinal cord mitochondria of mutant SOD1 mice starting at pre-symptomatic stage [50], suggesting a direct inhibition by the mutant SOD1/Bcl2 complex on VDAC1 channel, a mitochondrial porin located on the mitochondrial outer membrane (MOM), which regulates mitochondrial respiration and flow of ATP through the mitochondrial outer membrane (MOM) out into the cytosol and ADP into mitochondria for oxidative phosphorylation. VDAC1, a key player in mitochondria-mediated apoptosis and cell

survival, is an anion-selective channel at full open state, but becomes cation selective when closed to sub-conducting states, with the pore diameter narrowing from 3 to 1.8 nm [54]. These changes of both size and selectivity of the channel have profound functional implications for the permeability to ions. For small and mono-valent ions such as K^+, Cl^-, $H_2PO_4^-$, VDAC1 is a weak selective channel. In the closed (or sub-conducting) states, VDAC1 becomes a cation-selective channel with increased ion selectivity. For example, the permeability to Ca^{2+} ions increases dramatically at closed states [55]. In addition, the size change of the pore of the channel could introduce a physical barrier to large metabolites. As a result, the closed channel becomes virtually impermeable to large multi-valent anions such as ATP due to the electric barrier [56]. Thus, the change in gating of VDAC1 results in a mild reduction of conductance and a rather dramatic change in its selectivity [57] for multi-valent metabolites. Interestingly, reduced ATP production has been observed in motor neurons of mutant SOD1-G93A mice [25,58], as well as in neuroblastoma cells expressing mutant SOD1-G37R [59].

Additional disturbances in VDAC gating mediated by the toxic mutant SOD1/Bcl-2 can be predicted according to the known parameters of mitochondrial respiration. The single channel permeability to ATP drops from 0.7×10^5 ions/s to essentially zero at closure of VDAC, while the permeability to creatine^{3-}, HPO_4^{2-}, succinate^{2-} drops from 4–8 $\times 10^6$ ions/s to 3×10^5 ions/s [57]. Instead, for monovalent ions such as pyruvate and $H_2PO_4^-$, there is less permeability drop in the close states of VDAC [57]. Thus, in both open and closed states, ATP permeability is a limiting step for a functional mitochondrial respiration. As VDAC closes, perhaps through the toxic function of the mutant SOD1/Bcl-2 complex, ATP accumulates in the mitochondrial intermembrane space where it slows down ADP/ATP translocation through the adenine nucleotide translocator complex (ANT). However, since metabolites such as pyruvate can still permeate the MOM and proceed to the Kreb's cycle, the proton gradient through the inner membrane cannot be dissipated. We can summarize these events in the following simplified biophysical model for proton motive force (illustrated in Fig. 1).

$$\frac{d[H^+]_o}{dt} = k_1\left[H^+\right]_i - k_2[ADP]\left[H^+\right]_o - P\left(\left[H^+\right]_o - \left[H^+\right]_i\right)$$

where $[H^+]_o$ and $[H^+]_i$ are the proton concentration respectively outside and inside the matrix, k_1 is the apparent kinetic constant of respiratory chain complex (assuming constant substrates and enzymes levels), k_2 is the kinetic constant for ATP synthase, P is the permeability of the inner membrane to proton.

At steady state, $k_1[H^+]_i - k_2[ADP][H^+]_o - P([H^+]_o - [H^+]_i) = 0$. Therefore,

$$\frac{[H^+]_o}{[H^+]_i} = \frac{k_1 + P}{k_2[ADP] - P}$$

$$\Delta_m = \Delta p + 59 \log \frac{k_1 + P}{k_2[ADP] - P}$$

where Δ_m is the mitochondrial membrane potential, Δp is the proton motive force. Closure of VDAC mediated by the mutant SOD1/Bcl-2 complex can, therefore, slow down the ADP/ATP permeation through the MOM, decreasing ADP levels available in the intermembrane space and matrix for ATP synthase to produce ATP and therefore dissipate the proton gradient, ultimately inducing mitochondrial hyperpolarization. In reality, mitochondrial substrate concentrations such as NAD/NADH ratio could affect the parameters above. This simplified model provides a quantitative approach to understand the effects of intermembrane space ADP concentration on the mitochondrial membrane potential and thus it is more related to the transient effects of VDAC closure on mitochondria.

We found that a specific SOD1-mimetic peptide can restore normal mitochondrial membrane potential and VDAC1 permeability by lessening the interaction between mutant SOD1 and Bcl-2. Studies in other pathological conditions would also seem consistent and supportive of this model. For example, the anti-cancer drug G3139, which closes VDAC channel and causes apoptosis in cancer cells, also induces mitochondrial hyperpolarization (Colombini M., personal communication). Hyperpolarization state can lead to reactive oxygen species (ROS) generation by Complex I [60]. These two important aspects of mitochondrial dysfunction have also been reported in ALS [50] and will be discussed in the next section.

Other lines of evidence pointing at defective mitochondrial bioenergetics as the leading mechanism in mitochondria-mediated cell damage have also been identified in ALS, including mutation in cytochrome c oxidase subunit I [61], mutations in mitochondrial tRNA gene [62], complex I deficiency and decreased ATP/ADP ratio [63].

2.3. Calcium signaling and mitochondria in ALS

Increased oxidative phosphorylation and ATP production is required during action potential firing in neurons. During each cycle, Ca^{2+} is released from the endoplasmic reticulum (ER) stores, enters mitochondria, buffers in the matrix, and finally is extruded from the mitochondria and taken up by the ER. Therefore, there is a resilient; tightly regulated ER-mitochondrial coupled Ca^{2+} signaling in neurons. Because neurons fire action potentials repeatedly in a millisecond time scale, they are also particularly susceptible to mitochondria and ER stress or impairment leading to calcium dysregulation.

VDAC actively participates in the mitochondrial-ER Ca^{2+} coupling. VDAC channels (mainly VDAC1) are expressed close to the mitochondrial-ER tether sites, where Ca^{2+} permeable channels such as inositol triphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) are expressed at the ER side [64,65]. There are direct local Ca^{2+} fluxes from ER to mitochondria, to expedite the whole process [64,65]. Calcium ions enter mitochondrial matrix via the mitochondrial calcium uniporter (MCU) with the support of mitochondrial calcium uptake 1 (MICU1) by following the electrochemical gradient [66,67]. Several lines of evidence showed that calcium uptake activity is impaired in ALS. For example, mutant SOD1 affects Ca^{2+} uptake in ALS-affected tissues such as spinal cord but not in ALS-spared tissues such as liver [27]. It has recently been shown that the activity of MCU and MICU1 is depressed in the SOD1-G93A mouse model, even though the expression

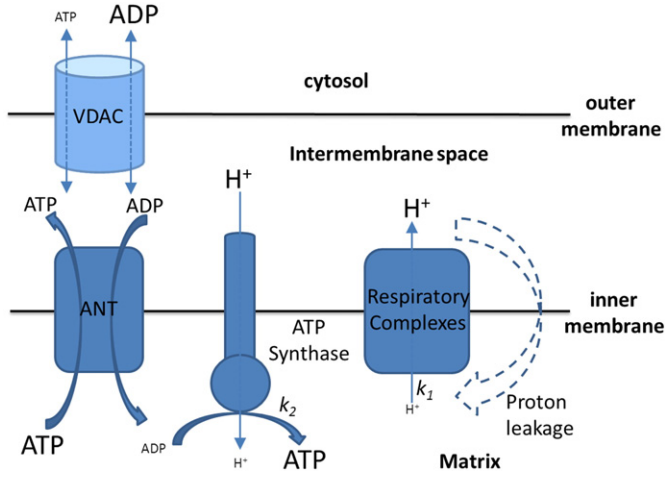

Fig. 1. Summarized impact of mutant SOD1/Bcl2 on mitochondria. Mutant SOD1 binds to Bcl2, exposes its BH3 domain, and alters VDAC conducting states, resulting in reduced ATP production, enhanced calcium signaling, increased mitochondrial potential and ROS production. We also propose that quinone from complex I is trapped in the oxidized form.

levels of these proteins were found to be higher than in control mice [68]. This may lead to a decreased Ca^{2+} buffering capacity in mitochondria of these mice. Indeed, evidence of defective Ca^{2+} buffering capacity has been reported prior to onset in neuronal mitochondria of SOD1-G93A mice [27]. In addition, SOD1-G93A motor neurons have higher intracellular calcium levels [69], leading to possible Ca^{2+} overload to mitochondria. It is well-established that Ca^{2+} overload results in mitochondrial permeability transition [70]. In line with this, multiple groups have examined how knockdown of CyPD, the only confirmed component of mitochondrial permeability transition pore, affects the survival of mutant SOD1 mice. Although the results are conflicting, it is the general consensus that Ca^{2+} overloads affect motor neuron survival [71–74].

2.4. Oxidative stress and mitochondria in ALS

Unrestrained production of ROS by mitochondria has been considered as one possible cause of neurodegeneration in ALS [75]. This belief is based on a number of circumstantial evidence: 1) Increased oxidative stress and ROS damage characterized by elevated protein carbonylation and tyrosine nitration have been observed in CNS tissues from ALS patients [76–80]; 2) A conformation specific antibody (B8H10) recognizes misfolded mutant SOD1 proteins in ALS spinal cord mitochondria, which displayed ROS accumulation [39], confirming a role for misfolded mutant SOD1 in mitochondrial dysfunction and elevated ROS production; 3) ROS actively participates in the disease progression in mutant SOD1 ALS mouse models. SOD1-G93A transgenic mice crossed with mice deficient in the mitochondrial matrix antioxidant enzyme MnSOD ($Sod2^{+/-}$ mice) caused a decrease in lifespan that was associated with a reduced disease duration [81]. However, in the same report, MnSOD1 deficiency does not affect the disease states of metal deficient H46R/H48Q mutant SOD1 ALS mice [97]. Therefore, different ALS mutations may affect mitochondria through different mechanisms. ROS production appeared to participate in motor neuron death pathways in some ALS models, although perhaps not a determining factor.

There are several types of ROS and reactive nitrogen species (RNS) that are formed in cells including superoxide anions, hydrogen peroxide, hydroxyl radicals, organic hydroperoxides, hypochlorous acid, peroxynitrite [82]. Endogenously, these compounds modulate different signaling pathways and their formation is tightly regulated [83,84]. However, when produced at higher levels, they can target and react with lipid membranes, proteins and nucleotides, thus accelerating cell senescence and eventually cause cell death. Most of the oxidative species are formed from superoxide anions generated by mitochondrial respiration. Therefore, superoxide anion generation in mitochondria is the most critical point that directly initiates all the oxidative stress signals. Superoxide anions can be generated from both complex I and complex III, depending on the organs [85]. In the CNS, most superoxide is generated by complex I [85], whose activity is decreased in lymphocytes of ALS patients [63,86–88]. The deficiency in complex I is associated with ROS production in the mitochondria [89–91]. Therefore, this defect may be the source of oxidative stress in ALS.

2.5. Fission and fusion of mitochondria in ALS

Mitochondria are very dynamic and intensively interconnected organelles that form tubular networks across the whole cell [92]. They constantly undergo fission and fusion in order to meet different cellular demands. This mitochondrial network remodels under stress conditions [93], changing in energy demand and Ca^{2+} levels [94]. Remodeling the mitochondrial network also serves the purpose of repairing damaged mitochondria and coping with increases in Ca^{2+} waves [95]. Any alteration in this process of fission and fusion in the nervous system can cause cell damage and eventually lead to neurodegeneration [96]. For example, mutations in PINK1 and Parkin, two important proteins that promote mitochondrial fission and inhibit fusion [97], cause alterations in mitochondrial dynamics and cause Parkinson's disease (PD), thus suggesting an active role for dysfunctional mitochondrial fission and fusion dynamics in PD pathogenesis. Similar alterations have been reported in ALS, Pro-fusion OPA1 protein levels are decreased, while pro-fission phosphor-DRP1 protein levels are increased in mutant SOD1 mice [98]. Overexpression of Glutaredoxin 2, which regulates mitochondrial fragmentation, preserves mitochondrial function and dynamics by restoring DRP1 and OPA1 levels and strongly protects neuronal cells from apoptosis [98]. Increased mitochondrial fragmentation (fission) was recently reported in differentiated NSC34 cells transfected with different mutant SOD1 proteins [24,99]. It is worth noting that the processes of mitochondrial fission/fusion and apoptosis/mitophagy share common mechanisms. For example, Bcl-xL increases both fission and fusion of mitochondria, which ultimately lead to a rise in mitochondrial biomass as net outcome [100]. Exposure of the BH3 domain in Bcl-2 by mutant SOD1 [40] could potentially affect the fission and fusion machinery in mitochondria as BH3 domain only proteins like Bax and Bak also increase mitochondrial fission. Changes in mitochondrial dynamics also involve both anterograde and retrograde transport of mitochondria in neurons that ultimately die in ALS [101] and are specific to mutant SOD1 motor neuron mitochondria, since they are absent in wild-type SOD1 motor neurons, they do not involve other organelles, and they are not found in neurons not affected by ALS, like cortical neurons [102].

2.6. Consequences of mitochondrial damage

ALS-altered mitochondria may trigger apoptosis and ultimately cell death by inducing mitochondrial release of cytochrome c [103]. However, whether mitochondrial initiated apoptosis plays an essential role in neurodegeration is still a matter of dispute. Knocking out the pro-apoptotic protein Bax in mutant SOD1-G93A mice prevents death of motor neurons and delays disease onset and progression, but mitochondrial degeneration still occurs [104], suggesting that mitochondria-initiated apoptosis in motor neurons could only be a secondary effect [105,106]. Interestingly, knockout of both Bax and Bak (two pro-apoptotic Bcl-2 family proteins) significantly extends survival of SOD1-G93A mice [107]. As Bax and Bak have similar function in forming pores in the mitochondrial outer membrane through which cytochrome c can be released [108], these results suggest a redundant mechanism of mitochondria-initiated toxicity between these two proteins, and point at the importance of mitochondria altered permeabilization in the pathogenesis of ALS.

Mitochondria are very abundant in nerve terminals where they serve the purpose of providing energy and regulate Ca^{2+} waves. Therefore, there must be robust coordination between synaptic activity, mitochondrial transport/distribution, and ATP generation. However, in mutant SOD1 mice, both anterograde and retrograde axonal transport are slowed down due to disruption of the neurofilament network [109,110] and accumulation of insoluble proteins [111]. Therefore, deficiency in mitochondrial transport [112], hence inability to replace uncoupled mitochondria at the neuromuscular terminals, can trigger the disease through a “dying back” mechanism. For example, evidence of motor axon degeneration occurring prior to motor neuron death has been reported in mutant SOD1-linked ALS [111]. In the mutant SOD1 mice, quantitative analysis reveals the weakening of NMJ early during the presymptomatic stage of disease, followed by loss of motor axons at symptomatic stage [106]. This “dying back” mechanism has been observed in other neurodegenerative diseases as well [113] and is compatible with “mitochondrial-mediated” mechanism of neurodegeneration as enlarged mitochondria were first observed in the distal part of the nerve terminal at the pre-symptomatic stage [114]. These mitochondrial damages are also not limited to motor neurons, e.g., NMJ deterioration and axonal degeneration occurs when the efficiency of mitochondria dependent oxidative phosphorylation is inhibited by overexpression of uncoupling protein 1 (UCP1) in the muscle cells [115]. Also,

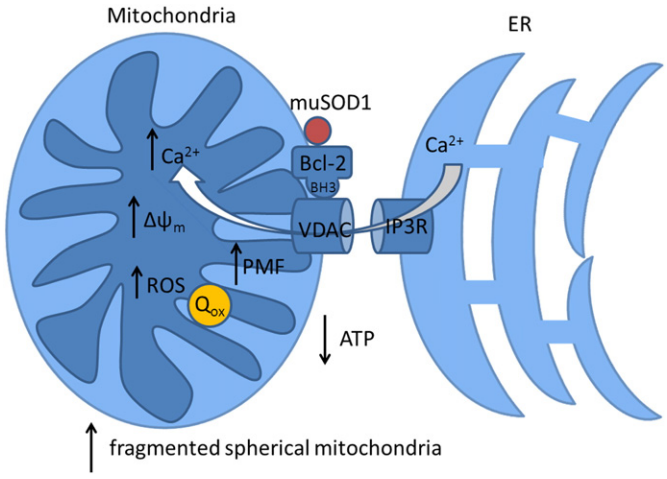

Fig. 2. Kinetic model of generation of proton gradient (not drawn to scale). ADP permeates through VDAC channels in the outer membrane, translocates through ANT in the inner membrane, where it is phosphorylated into ATP inside the matrix by ATP synthase through dissipation of proton gradient, with apparent kinetic constant of k2. The proton gradient is generated through respiratory complexes with apparent kinetic constant of k1 and subjected to leakage through the inner membrane.

mitochondrial hyperpolarization, as predicted by the quantitative model, was observed in muscle cells prior to NMJ destruction [116], further proving evidence for mitochondrial dysfunction as one of the early mechanism of the disease.

2.7. Perspectives and conclusions

Since the first reports on mitochondrial abnormalities in tissues from ALS patients, several lines of evidence gathered from studies subsequently done on the mutant SOD1 mouse and cell models expressing mutant SOD1 proteins have pointed at mitochondrial dysfunction as cause of motor neuron death in ALS. One example is the recent observation that VDAC1 channel can enter less conductive (closed) states by directly interacting with mutant SOD1 [117]. Bcl-2 is a mitochondrial outer membrane protein that directly regulates VDAC activity and these two proteins are very tightly associated. In this respect, we showed that at the mitochondria, mutSOD1 forms a toxic complex with Bcl-2, which is then converted into a toxic protein via a structural rearrangement that exposes its toxic BH3 domain (Pedrini et al., 2010). The formation of this toxic complex with Bcl-2 is the primary event in mutSOD1-induced mitochondrial dysfunction, inhibiting mitochondrial permeability to ADP and inducing mitochondrial hyperpolarization, calcium stress and ROS production (Fig. 2). In mutSOD1-G93A cells and mice, the newly exposed BH3 domain in Bcl-2 alters the normal interaction between Bcl-2 and VDAC1 thus reducing permeability of the outer mitochondrial membrane. In motor neuronal cells, the mutSOD1/Bcl-2 complex causes mitochondrial hyperpolarization leading to cell loss [50]. In addition, alterations in mitochondrial metabolism could be one of the disease mechanisms, as knocking down VDAC1 in mutant SOD1 ALS mice, accelerates the onset of the disease [117], suggesting that mitochondrial bioenergetics is critical for the good health of motor neurons. Increased mitochondrial Ca^{2+} storage capacity reduces aggregation of misfolded SOD1 and motor neuron cell death. Surprisingly, this does not seem to extend survival in mouse models of inherited ALS [72]. At minimum, motor neuron death is, however, an essential measurement of disease progression, but it is possible that the final muscle paralysis event is independent from motor neuron loss. Nevertheless, this result is puzzling and needs further cross-examination, since at least in multiple sclerosis, a similar approach does protect motor axons and enhances recovery [118].

Mitochondrial dysfunction is not only observed in SOD1 ALS but has also been seen in familial cases involved with FUS, TDP43 and sporadic cases [119]. In summary, the wealth of evidence published thus far leads to the conclusion that mitochondrial dysfunction occurs early in disease and it is one key mechanism responsible for the degeneration of motor neurons in ALS.

Acknowledgments

This work was supported by the National Institute of Health, grants RO1-NS044292 and RO1-NS064488 to DT, and RO1-NS051488 to PP. The Weinberg Unit for ALS research is also supported by the Farber Family Foundation.

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