Mitochondria in Amyotrophic Lateral Sclerosis: A Trigger and a Target

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Abstract

Strong evidence shows that mitochondrial dysfunction is involved in amyotrophic lateral sclerosis (ALS), but despite the fact that mitochondria play a central role in excitotoxicity, oxidative stress and apoptosis, the intimate underlying mechanism linking mitochondrial defects to motor neuron degeneration in ALS still remains elusive. Morphological and functional abnormalities occur in mitochondria in ALS patients and related animal models, although their exact nature and extent are controversial. Recent studies postulate that the mislocalization in mitochondria of mutant forms of copper-zinc superoxide dismutase (SOD1), the only well-documented cause of familial ALS, may account for the toxic gain of function of the enzyme, and hence induce motor neuron death. On the other hand, mitochondrial dysfunction in ALS does not seem to be restricted only to motor neurons as it is also present in other tissues, particularly the skeletal muscle. The presence of this 'systemic' defect in energy metabolism associated with the disease is supported in skeletal muscle tissue by impaired mitochon-

drial respiration and overexpression of uncoupling protein 3. In addition, the lifespan of transgenic mutant SOD1 mice is increased by a highly energetic diet compensating both the metabolic defect and the motor-neuronal function. In this review, we will focus on the mitochondrial dysfunction linked to ALS and the cause-and-effect relationships between mitochondria and the pathological mechanisms thought to be involved in the disease.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the selective death of upper and lower motor neurons leading to profound muscle weakness and death, mostly by respiratory failure. While in most patients the disease appears sporadically, a subset of cases are inherited. Three causative genes, *sod1*, *als2* and *als4*, have been determined so far. *Sod1* encodes cytosolic Cu/Zn superoxide dismutase (SOD1), and since the discovery in the early 90s of several SOD1 mutants associated with the disease [1], more than 100 dominantly inherited mutations have been identified that lead to ALS (www.alsod.org). More recently, two genes mutated in juvenile atypical forms of ALS were cloned

and called *als2* [2, 3] and *als4* [4, 5]. *Als2* encodes a putative GTPase regulator [6], and *als4* encodes a putative DNA helicase [4, 5]. However, it is currently unknown how mutations in *als2* and *als4* trigger ALS. The identification of *sod1* as a causative gene in ALS allowed the generation of multiple lines of transgenic mice, which exhibit a transgene dose-dependent ALS-like pathology featuring the progressive motor neuron death and muscle atrophy characteristic of the disease [7–10]. Given the pathophysiological similarities between sporadic and familial forms of ALS, it is generally accepted that these transgenic mice are valuable animal models for the analysis of the pathogenic mechanisms leading to ALS [11, 12].

Many different mechanisms have been postulated to explain motor neuron death in ALS, including oxidative stress, glutamate excitotoxicity, disruption of axonal transport and apoptosis among others [for review, see 11, 12]. However, very recent data from mutant SOD1 mice showed that motor neurons do not die following a cell-autonomous insult [13]. The targeted expression of mutant SOD1 does not lead to an ALS-like phenotype either in motor neurons [14, 15] or in astrocytes [16]. More interestingly, while normal motor neurons surrounded by mutant SOD1-expressing cells are prone to die, those harbouring an ALS-linked SOD1 mutation placed in a wild-type background are not [13], which suggests that the cellular environment of motor neurons is a determining factor to activate the pathological process. On the other hand, it is becoming increasingly clear that mitochondria are key players in ALS pathogenesis. The occurrence of functional abnormalities in both human ALS and related animal models has been demonstrated by several research groups, including ours [17]. Surprisingly, these abnormalities are by far not restricted to the central nervous system, since they are also found in peripheral tissues such as skeletal muscle [18–20], liver [21] and lymphocytes [22]. The purpose of this review is to summarize these findings in an attempt to understand how mitochondrial dysfunction, not only in the central nervous system but also in skeletal muscle, may contribute to the early and late stages of motor neuron degeneration in ALS.

Mitochondria Are Key Players in the Postulated Etiological Mechanisms Leading to ALS

Three major mechanisms, namely oxidative stress, glutamate excitotoxicity and axonal transport disruption, have been extensively investigated as playing a central

role in the pathogenesis of ALS and, interestingly, all three are directly or indirectly linked to mitochondrial physiology. Mitochondria constitute a primary site of intracellular production of reactive oxygen species, and hence a major source of oxidative stress [23, 24]. In turn, oxidative damage to mitochondrial proteins, lipids or nucleic acids is able to impair the normal function of mitochondria [23]. Glutamate excitotoxicity causes, through calcium overload, mitochondrial damage [25] and modification of the bioenergetic status of mitochondria [26]. Conversely, defects in mitochondrial energy metabolism sensitize neurons to otherwise normal glutamate levels [27], and diminish glutamate uptake leading to its toxic accumulation [28]. Disruption of axonal transport by genetic means perturbs the normal transit of mitochondria throughout the axon [29], and recent data showed that the efficiency of the bioenergetic production of mitochondria correlates with mitochondrial trafficking [30]. Taken together, these observations provide evidence for the intriguing connections between many of the postulated etiological mechanisms working in ALS and mitochondria (fig. 1), and point out that such a mitochondrial dysfunction can be considered either as a cause or a consequence of the pathological process.

Mitochondria Are Morphologically Abnormal in ALS

The first evidence of mitochondrial abnormalities in ALS came from the ultrastructural studies of Afifi et al. [31], who reported the presence of aggregates of mitochondria in the subsarcolemmal region of muscles of ALS patients. Later studies by Sasaki and Iwata [32] showed the appearance of dense conglomerates of mitochondria in anterior horn neurons of the lumbar spinal cord of ALS patients. These alterations were also observed, although to a lesser extent, in nondegenerating neurons. Siklos et al. [33] found increased mitochondrial volume in motor nerve terminals of ALS patients as compared with patients with other denervating neuropathies and non-denervating control subjects. Consistent with these findings, the most striking histopathological abnormality in two lines of mutant SOD1 mice, the SOD1(G93A) and SOD1(G37R) mice, is the early appearance of vacuolated mitochondria [9, 34]. These vacuoles derive from expansion of the outer membrane of mitochondria [35], and are present in motor neurons devoid of any apoptotic feature [34]. It should be stressed, however, that these morphological defects are not observed in other transgenic ALS

Fig. 1. Mitochondria are a target and a trigger in ALS glutamate excitotoxicity, axonal transport impairment and oxidative stress; all three are involved in ALS, and may cause mitochondrial defects as those characteristically observed during the course of the disease. Alternatively, an as yet unidentified mitochondrial dysfunction could exacerbate glutamate excitotoxicity and activate a cascade of events leading to axonal transport impairment and oxidative stress. ETC = Electron transport chain.

models. SOD1(G85R) mice do not show any characteristic abnormality, and in SOD1(G86R) mice vacuolization of mitochondria is only noticeable at the ultrastructural level with the appearance of microvacuoles [36]. Taken as a whole, these reports demonstrate the existence of morphological alterations in motor neuron mitochondria of ALS patients and related animal models. Whether similar changes also occur outside the central nervous system is still elusive. Since the seminal observations by Afifi et al. [31], few studies have dealt with this question. Nakano et al. [21] found giant mitochondria with intramitochondrial inclusions in the liver of 21 ALS patients, these alterations being highly specific to the disease. More recently, Chung and Suh [37] noticed abnormalities in 5 out of 49 muscle biopsies from ALS patients with the occurrence of giant mitochondria, paracrystalline inclusions and abnormal cristae. To our knowledge, no similar studies exist in mutant SOD1 mice.

Mitochondria Are Functionally Abnormal in ALS: Facts and Controversies

It is generally accepted that cell respiration is affected in spinal cord mitochondria of mutant SOD1 mice. This assumption is supported by several studies showing a decrease in the activity of the complex I of the electron transport chain [38–40], even detected as early as 60 days in a transgenic line living more than 200 days [38, 39]. Furthermore, the respiratory control ratio and ATP synthesis decline in parallel in the same mitochondria isolated from mutant SOD1 mouse spinal cord [40]. However, this mitochondrial dysfunction does not seem to be motor neuron specific. The above-mentioned studies used mitochondrially enriched fractions from the whole spinal cord, thus making it unlikely that the decreased respiratory chain activity is the result of the sole affected, otherwise sparse, population of motor neurons. In this regard, mitochondrial dysfunction has also been described in other regions of the central nervous system of mutant SOD1 mice [40] and familial ALS patients [41]. Outside the central nervous system, Mattiazzi et al. [40] found basically the same defects in respiratory control ratio and ATP synthesis in mitochondria isolated from mutant SOD1 mouse liver. Our own findings [17] and those reported by Leclerc et al. [42] also showed a decreased respiratory control ratio in skeletal muscle mitochondria. In the same study, we also reported that the levels of ATP in muscle extracts from mutant SOD1 mice are decreased while those of uncoupling protein 3 are increased, therefore providing a putative mechanism of energy dissipation that could account for mitochondrial dysfunction in this tissue [17]. We can thus conclude that mitochondrial functional abnormalities in mutant SOD1 mice are widespread and appear early in the course of the pathology (fig. 2).

As far as ALS patients are concerned, the occurrence of mitochondrial dysfunction remains controversial. A series of studies did show the presence of mitochondrial defects in lymphocytes [22] and skeletal muscle [18–20] of ALS patients. Vielhaber et al. [20] correlated such defects with the occurrence of mitochondrial DNA deletions and a decrease in mitochondrial manganese superoxide dismutase activity in this tissue. Similarly, a statistically significant association between the occurrence of sporadic ALS and the frequency of the most common mitochondrial DNA deletion in skeletal muscle has also been reported [43]. Interestingly, such a deletion does not accumulate in ALS motor neurons or platelets [44, 45]. It should be stressed, however, that the findings of Viel-

Fig. 2. Time course of the cellular and molecular events associated with mitochondrial dysfunction in mutant SOD1 mice. Functional abnormalities affecting the ETC in central nervous system (CNS) and non-CNS tissues are detected in the early phases of the clinical manifestations of ALS. In contrast, mitochondria-dependent apoptosis appears late during the end stage. However, metabolic alterations may represent the earliest modifications associated with the disease process.

haber et al. [20] are in discrepancy with more recent data showing no defect in the respiratory chain function as compared with age- and activity-matched controls [46]. Finally, a few cases of motor neuron disease reported in the literature turned out to be mitochondriopathies [47–49]. It is thus necessary to determine the degree of implication of mitochondrial dysfunction in human ALS, particularly at the skeletal muscle level.

Mitochondria Are Involved in Motor Neuron Apoptosis

A second set of observations links ALS pathology to mitochondrial physiology. Not only are mitochondria the powerhouse of the cell, they are also controlling cell survival and death through the commitment of apoptosis. A huge amount of literature deals with the involvement of the apoptotic machinery in motor neuron death [for review, see 50]. The executioner proteases of apoptosis, the caspases, are activated during ALS disease [51, 52], and their inhibition either pharmacologically [53] or genetically [54, 55] delays disease onset and extends survival. Furthermore, release of cytochrome *c* from the mitochon-

drial intermembrane space into the cytosol, which is the point of no return for the activation of caspases, is recruited in ALS [56]. Most importantly, inhibiting cytochrome *c* release by administration of minocycline delays disease onset and increases survival in SOD1(G93A) mice [57], thus supporting the notion that mitochondria-dependent apoptosis is a predominant pathway in motor neuron degeneration in ALS. Changes in the ratio between anti- and proapoptotic members of the Bcl-2 family have also been documented. Decreased levels of the anti-apoptotic factors Bcl-2 [58] and Bcl-x_L [59], and increased levels of the death-promoting factors Bax [59, 60], Bid [61] and Bcl-x_S [59] are found in ALS spinal cord, thus compromising the chance of motor neurons to survive. Increasing Bcl-2 by genetic means retards the onset of the disease [62], which further supports the importance of apoptosis, for the most part at the end of the neurodegenerative process, in ALS (fig. 2).

Mutant SOD1 Is in Part Located in Mitochondria

Although it has long been thought that SOD1 was exclusively cytosolic, recent data suggest that the enzyme is also located, at least in part, within the mitochondrial intermembrane space. This was first described in yeast [63, 64], and later in transgenic mice overexpressing wild-type SOD1 [65]. In mutant SOD1 mice, the presence of part of the enzyme in mitochondria was first described by Jaarsma et al. [66], and then confirmed by two other groups [40, 67]. Recent data showed that mutant SOD1 was associated with the cytoplasmic side of mitochondria specifically in spinal cord tissue from mutant SOD1 mice and in one patient bearing an ALS-linked SOD1 mutation [68]. However, mitochondrial accumulation of mutant SOD1 is not limited to the spinal cord but also occurs in unaffected tissues such as liver [40].

Thus, the exact location of mutant SOD1 in ALS mitochondria and its contribution to the pathology still need further research.

Functional Consequences of Mitochondrial Localization of Mutant SOD1

We will propose in this section three putative mechanisms that may lend clues to further improve our knowledge about the implication of the presence of mitochondrially targeted mutant SOD1 in ALS pathology (fig. 3).

Fig. 3. Three potential mechanisms may explain the toxicity of mitochondrially targeted mutant SOD1. Accumulation of mutant SOD1 into the mitochondrial intermembrane space may (1) induce local oxidative stress, especially in the form of peroxynitrite, whose generation is facilitated by an aberrant Zn-deficient SOD1 activity, (2) impair the energetic metabolism by affecting the ETC, or (3) sequester the anti-apoptotic protein Bcl-2, thus leading to apoptosis.

Local Generation of Peroxynitrite

One of the etiological hypotheses of ALS postulates that the toxicity of mutant SOD1 is linked to increased production of the oxidant peroxynitrite [69–72]. SOD1 mutations have been proposed to facilitate the loss of Zn from the enzyme. Zn-deficient SOD1 exerts an aberrant redox chemistry involving production of superoxide, which can combine with nitric oxide by a diffusion-limited reaction to form the stronger oxidant peroxynitrite [73]. In turn, the toxicity of peroxynitrite is in part dependent of the formation of peroxynitrite-derived carbonate, nitrogen dioxide and hydroxyl radicals. Peroxynitrite reacts with protein tyrosine residues to form nitrotyrosine, a stable posttranslational modification that has been utilized as a footprint for nitrative stress [74, 75]. However, alternative pathways for nitrotyrosine formation have been recently proposed [76]. Although the mechanisms of peroxynitrite formation in mitochondria have been described in detail [77], there is no direct evidence in support of the intramitochondrial formation of peroxynitrite in ALS or related cellular or animal models. Nitrotyrosine staining by specific antibodies was reported in cultured motor neurons undergoing apoptosis [71, 78–80], mutant SOD1 mice and sporadic and familial cases of ALS [81–84], but the pattern of staining did not correspond to the characteristic patchy distribution of mitochondria. These results do not discard the occurrence of tyrosine nitration in mitochondria, but rather suggest that

the more abundant cytoskeletal proteins hinder the visualization of nitrotyrosine immunoreactivity in mitochondria. Peroxynitrite formation in mitochondria may account for much of the metabolic defects initially attributed to direct actions of nitric oxide. Radi et al. [85] first proposed a role for peroxynitrite to explain the long-term inhibitory effects of nitric oxide on cell respiration. Nitric oxide may diffuse to mitochondria to combine with mitochondrion-derived superoxide to form peroxynitrite. Superoxide is considered to be largely formed towards the matrix, but it may also be produced in the intermembrane space [86]. To counterbalance these actions, superoxide diffusion is limited by mitochondrial SODs, including Mn-SOD in the matrix and SOD1 in the intermembrane space. Alternatively, peroxynitrite formed in the cytoplasm might diffuse into the mitochondria, although the exact mechanisms remain to be elucidated. In mitochondria, peroxynitrite is able to promote mitochondrial oxidative damage by primarily causing oxidation, but also by inducing nitration and nitrosation of mitochondrial components [77]. Peroxynitrite formation in mitochondria might also lead to mitochondrial DNA mutations, alterations in energy and calcium homeostasis, and the opening of the permeability transition pore. In particular, peroxynitrite indirectly nitrates tyrosine residues in mitochondrial proteins *in vitro* and *in vivo*, including Mn-SOD, aconitase, cytochrome *c*, voltage-dependent anion channel and ATPase [77, 87]. Interestingly, the nitration

of Mn-SOD by peroxynitrite leads to enzyme inactivation in damaged cells [88], and increased expression of the active enzyme in mitochondria prevents mutant SOD1-mediated neuron cell death [89]. The fact that the expression of Mn-SOD is increased in the spinal cord of SOD1(G93A) transgenic mice at presymptomatic and symptomatic stages [90] may indicate a protective role of the enzyme in its nonnitrated form. In addition, nitrated cytochrome *c* is also found in the cytosol [91] suggesting a role for this species during motor neuron apoptosis, which would be in accord with cytochrome *c* release from the mitochondria in the spinal cord of SOD1(G93A) mice [56].

Activation of Apoptosis

A recent study by Takeuchi et al. [92] demonstrated that expression of mitochondrially-targeted mutant SOD1 triggers neuronal death accompanied by cytochrome *c* release and caspase cascade activation. On the contrary, neither nuclear nor endoplasmic reticulum overexpression induces cell death, thus suggesting that a small amount of mutant SOD1 accumulated in mitochondria is sufficient to induce toxicity. A clue towards understanding this phenomenon was provided by Pasinelli et al. [93], who showed that mutant SOD1 in spinal cord mitochondria interacts with Bcl-2, suggesting that entrapment of Bcl-2 by large aggregates of mutant SOD1 might deplete motor neurons of this anti-apoptotic protein.

Impairment of Energy Metabolism

Overexpression of mutant SOD1 in cultured motor neuron-like cells causes defects in two complexes of the electron transport chain, and renders the cells highly sensitive to inhibition of the glycolytic pathway [94–96]. Whether this toxic effect on mitochondrial metabolism is mediated by mutant SOD1 accumulation in mitochondria is currently unknown. Since in human and mouse ALS mutant SOD1 is ubiquitously expressed, one can expect alterations in metabolism and energy homeostasis on a large scale that may accompany the disease process. SOD1(G86R) and SOD1(G93A) mice are leaner than their wild-type counterparts, and this trait appears long before any detectable sign of motor neuron death [97] (fig. 2). This is not due to reduced food intake, but rather the result of depleted fat stores and alterations in the endocrine status indicative of increased energy expenditure. Furthermore, such an increase in energy expenditure appears associated with a hypermetabolic rate particularly noticeable in skeletal muscle tissue. Compensating this characteristic phenotype in SOD1(G86R) mice with a

highly energetic diet reverses the expression of markers of muscle denervation, prevents the loss of large motor neurons in the ventral horns of the lumbar spinal cord and extends survival [97]. Studies on human ALS have shown that at least a subset of patients harbors a hypermetabolic phenotype reminiscent of that found in mutant SOD1 mice [98]. Therefore, findings emerging from mutant SOD1 mice can be extrapolated to the sporadic forms of ALS, which are the most frequent forms. It has also been proposed that the nutritional status may be a determining factor in the course of the disease [99–101], which prompts us to suggest that mitochondrial dysfunction and its physiological consequences may represent an additional driving force for motor neuron vulnerability in ALS. Through which mechanisms the impairment of energy homeostasis settles clearly deserves further investigation.

Mitochondrially Targeted Therapy in ALS

Different therapeutic strategies have focused on mitochondria as a target to stop the progression of ALS. A first group of compounds were thought to cure ALS by blocking the apoptotic process responsible for motor neuron death. Inhibition of cytochrome *c* release by minocycline [57] or the opening of the transition pore by cyclosporin A delays disease onset and increases survival in SOD1(G93A) mice [102, 103]. However, it should be mentioned that these two drugs are likely to have additional effects independent of mitochondrial pathways of apoptosis. Overexpression of the anti-apoptotic factor Bcl-2 also retards the beginning of the disease [62]. Another group of treatments aimed at improving the bioenergetic status of the affected mitochondria. Administration of coenzyme Q10 [104] or lipoic acid [105] have little effect on survival, whereas creatine, an intracellular energy shuttle between mitochondria and sites of energy consumption, protects motor neurons and extends the lifespan of SOD1(G93A) mice by 20% [106]. Whether the effect of creatine is addressed to motor neurons or skeletal muscle remains elusive. Unfortunately, clinical trials failed to show a beneficial effect of creatine in ALS patients [107]. Indeed, none of the therapeutic strategies proved useful in mutant SOD1 mice has succeeded in slowing the course of the disease in man. Additive results were obtained with the concomitant administration of minocycline and creatine [108], which suggests that combinations of two drugs, and maybe three with the up-to-date prescribed riluzole, could be more effective than a

single compound. It should be stressed that our recent findings of the protective action of a high-fat diet on SOD1(G86R) mice [97] also provides new avenues for targeting mitochondrial dysfunction in ALS.

Conclusion

Mitochondria are currently a major topic in the field of ALS research. A consensus is rising on the occurrence of mitochondrial dysfunction in ALS and the implication of such a metabolically important organelle in motor neuron death. Future studies should aim at deciphering the

precise nature of mitochondrial dysfunction and why motor neurons are irrevocably prone to die when the function of mitochondria is handicapped.

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