

Review

Mitochondrial Dysfunction is a Converging Point of Multiple Pathological Pathways in Amyotrophic Lateral Sclerosis

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Abstract. A better understanding of the etiology of amyotrophic lateral sclerosis (ALS) is needed to develop effective therapies for the treatment of this fatal neurodegenerative disease. Extensive studies have produced a general agreement that ALS is likely to be a multifactorial and multisystem disease. Many mechanisms have been postulated to be involved in the pathology of ALS, such as oxidative stress, glutamate excitotoxicity, mitochondrial damage, defective axonal transport, glia cell pathology, and aberrant RNA metabolism. Mitochondria have shown to be an early target in ALS pathogenesis and contribute to the disease progression. Morphological and functional defects in mitochondria were found in both human patients and ALS mice overexpressing mutant SOD1. Mutant SOD1 was found to be preferentially associated with mitochondria and subsequently impair mitochondrial function. Recent studies suggest that axonal transport of mitochondria along microtubules is disrupted in ALS. Furthermore, new evidence suggests that mitochondrial fission and fusion as well as mitophagy clearance may also be affected by mutant SOD1. These results also illustrate the critical importance of maintaining proper mitochondrial function in axons and neuromuscular junctions, supporting the emerging “dying-back” axonopathy model of ALS. In this review, we will discuss findings supporting that mitochondrial dysfunction is likely to be a converging point of multiple pathways underlying the ALS pathogenesis and progression.

Keywords: Amyotrophic lateral sclerosis, autophagy, axonal transport, mitochondrial dynamics, mitochondrial function, mutant SOD1

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by preferential motor neuron death. Approximately 10–20% of the ALS cases are familial whereas the major-

ity of them are sporadic without family history. The copper-zinc superoxide dismutase (SOD1) gene was the first ALS gene discovered in 1993 and is the most prevailing gene accounting for approximately 20% of familial ALS cases (i.e., ~2% of all ALS cases) [1, 2]. Mutations in other genes have also been found to cause various subsets of familial ALS, including a potential G-protein exchange factor ALS2 [3,4], vesicle-associated membrane protein B (VAPB) [5], senataxin [6], the p150 subunit of dynactin (DCTN1) [7], angiogenin [8], and the recently discovered RNA pro-

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cessing proteins TDP-43 [9,10] and FUS/TLS [11,12]. Studying the familial ALS gene mutations will help understand the etiology of ALS, particularly the potential mechanisms underlying both sporadic and familial ALS. Among all ALS-causing genes being studied, mutant SOD1-mediated familial ALS has been studied most extensively in the past decade. The field has reached a general agreement that mutant SOD1 can cause disruption to multiple cellular processes, which may collectively contribute to the disease pathogenesis. Although new insights are likely to be revealed in coming years studying the newly discovered ALS-causing RNA metabolism proteins TDP-43 and FUS/TLS, this review is focused on the mitochondrial dysfunction in the mutant SOD1-mediated familial ALS.

The mitochondrion is a critical organelle in aerobic cells executing multiple functions. Mitochondria are the primary site of ATP production, maintain calcium homeostasis, participate in calcium signaling, and regulate intrinsic apoptosis. The structure, position, and function of the mitochondria are regulated by mitochondrial biogenesis, fission and fusion, transport, and clearance [13]. Therefore, mitochondrial malfunction confers pleiotropic effects on the cells, especially neurons with an elevated susceptibility to aging and stress. Mitochondrial pathology is a key player among multiple working hypotheses in the study of ALS [2,14]. Mutant SOD1 has been reported to be associated with mitochondria, and the morphology, membrane potential, and bioenergetic function of mitochondria can consequently be impacted. More recent studies also support that axonal transport of mitochondria is disrupted by mutant SOD1 in ALS. Furthermore, mitochondrial dynamics and function can be disrupted when the axonal transport is compromised by mutant SOD1 in motor neurons. The above mechanisms are not mutually exclusive, but instead may form a vicious cycle that deteriorates mitochondria in motor neurons especially in the distal nerve terminals. These observations suggest that mitochondrial dysfunction is likely to be a converging point of multiple pathways underlying ALS pathogenesis and progression.

MITOCHONDRIAL LOCALIZATION OF SOD1

SOD1 is a soluble cytosolic protein that functions to dismutate reactive superoxide radicals into hydrogen peroxide and molecular oxygen. Besides the cytoplasm, wild-type (WT) SOD1 has been found in the nucleus [15], endoplasmic reticulum (ER) [16], and inter-

membrane space (IMS) of mitochondria [17,18]. Depending on the subcellular localization, mutant SOD1 protein may exert compartment-specific toxicity. For instance, mutant SOD1 was found to accumulate inside the ER, form insoluble high molecular weight species, interact with the ER chaperone proteins, and induce ER stress within spinal motor neurons [16]. Other studies also showed that mutant SOD1 caused dysfunction of ER-associated degradation machinery and triggered ER stress, which could influence the progressive manifestations of familial ALS [19–21].

More interestingly, the ALS-related mutant SOD1 proteins have also been found in the IMS, matrix, and outer membrane of mitochondria [22–25]. Copper chaperone for SOD1 (CCS) is also partially localized in mitochondria [18] and assists in the entry and retention of SOD1 in mitochondria [26]. Increased localization of mutant SOD1 in mitochondria by CCS overexpression in the CCS/G93A-SOD1 double transgenic mice caused early mitochondrial pathology and accelerated the disease course [27]. A separate study found that the ALS-linked SOD1 mutants were selectively associated with spinal mitochondria and a fraction of them aggregated on the cytoplasmic surface of mitochondria in transgenic mutant SOD1 mice [28]. The study also showed that SDS-resistant aggregates functionally damaged mitochondria through either clogging import channels or inhibiting outer mitochondrial membrane components. It is likely that mutant SOD1 fails to fold properly and perturbs the physiological regulation of mitochondrial import and retention [25]. However, it remains unclear how mutant SOD1 becomes aggregated on the outer membrane or in the matrix of mitochondria. It is also unclear how mutant SOD1 is selectively recruited to spinal, but not liver, mitochondria [28].

Once associated with mitochondria, the mutant SOD1 is believed to cause multiple damages to mitochondria. An early event could be damage to the mitochondrial membrane leading to loss of mitochondrial membrane potential and swelling of the important organelle [29,30]. Potential consequences include impaired respiratory complex [31–33], disrupted redox homeostasis, and decreased ATP production [34]. Furthermore, calcium homeostasis can be disrupted [33, 35–37] and apoptosis may be activated [38–40]. Details of the above interrelated mechanisms underlying mutant SOD1 induced mitochondrial dysfunction will be discussed in the sections in the first half of this review.

ABNORMAL MITOCHONDRIAL MORPHOLOGY AND BIOENERGETICS IN ALS

Early studies have shown degenerating mitochondrial vacuoles in axons and dendrites of motor neurons in presymptomatic mice expressing mutant SOD1 [29, 30,41,42]. In addition, dense conglomerates of mitochondria in the anterior horn of lumbar spinal cord and proximal axons have been found in sporadic ALS patients [43,44]. Abnormal clustering of mitochondria was recently reported in motor axons in mutant SOD1 transgenic mice [45]. Extensive fragmentation of mitochondria was also reported in cultured NSC34 cells overexpressing mutant SOD1 [46,47]. A further study found that vacuolation of mitochondria in the spinal motor neurons of G93A transgenic mice was caused by expansion of the IMS and the outer mitochondrial membrane. The same study found that the degenerative vacuoles were bounded by mutant SOD1 that colocalized with mitochondrial outer membrane markers [48]. Besides alteration of mitochondrial morphology, damage to the mitochondrial membrane caused by mutant SOD1 can yield loss of mitochondrial membrane potential, disruption of mitochondrial respiratory chain activity [31,32], and reduction in mitochondrial Ca^{2+} buffering capability [37]. The dysregulation in electron transfer chain complexes was observed in both G93A SOD1 transgenic mice and human ALS patients [31, 33].

DISRUPTION OF CALCIUM HOMEOSTASIS IN ALS

An important function of mitochondria is to buffer intracellular surges of Ca^{2+} in excitable cells such as neurons. In motor neurons, mitochondria play an important role in short-term handling of rapid cytosolic Ca^{2+} transients. Ca^{2+} is a ubiquitous second messenger and participates in many signaling pathways that are crucial for cell survival. Increased Ca^{2+} concentration and mitochondrial damage were found in ALS patients as well as animal and cellular ALS models [36, 37,49–51]. A significant decrease in mitochondrial Ca^{2+} loading capacity in brain and spinal cord, but not in liver, was observed in presymptomatic G93A mutant transgenic mice [50]. Elevated Ca^{2+} can induce reactive oxygen species and oxidative stress in primary motor neurons isolated from G93A transgenic mice [36]. Alternatively, Ca^{2+} mediated glutamate excitotoxicity

might contribute to the mutant SOD1 toxicity in motor neurons. In addition, elevated local calcium levels can play a role in regulating axonal transport of mitochondria [52–54] (see details in the later section), providing a signaling circuit of the calcium buffering ability of mitochondria, calcium homeostasis, and mitochondrial distribution. Altogether, the mitochondrial dysfunction induced by mutant SOD1, disrupted calcium homeostasis, aberrant mitochondrial distribution, and excitotoxicity are likely to be interrelated mechanisms that collectively contribute to motor neuron degeneration in ALS.

INTRINSIC APOPTOTIC PATHWAY MEDIATED BY MITOCHONDRIA IN ALS

Mitochondria-mediated apoptosis was found to be involved in motor neuron degeneration in early studies of ALS. In G93A SOD1 transgenic mice, cytosolic release of cytochrome *c* was observed [38–40], and levels of pro-apoptotic proteins Bad and Bax were increased while those of anti-apoptotic proteins Bcl-2, Bcl-xL, and XIAP were decreased [55–57]. It has been proposed that mutant SOD1 can sequester anti-apoptotic protein Bcl-2 [38], reduce mitochondrial membrane potential, and trigger cytochrome *c* release from mitochondria [38–40]. Caspase-1 and caspase-3 were also found to be sequentially activated in motor neurons and astrocytes in G93A SOD1 mice as well as in G37R SOD1 and G85R SOD1 mice [58–60]. Strategies intervening in mitochondria-mediated apoptosis were demonstrated to be effective in G93A SOD1 mice: (a) intraventricular administration of minocycline, which inhibits cytochrome *c* release from mitochondria, was shown to delay disease onset and extend survival [61]; (b) overexpression of anti-apoptotic protein Bcl-2 could delay activation of the caspases, attenuate neuron degeneration and delay disease onset and mortality [56, 62]; (c) intraventricular administration of a broad spectrum caspase inhibitor zVAD-fmk could delay disease onset and mortality [58]; and (d) the deletion of cyclophilin D, a component of the mitochondrial permeability transition pore, significantly delayed the disease onset and extended the lifespan of ALS mice expressing high and low levels of G93A mutant SOD1 [63]. However, the clinical trials based on the above apoptosis-inhibiting approaches (e.g., minocycline) failed in human ALS patients [64].

Goold and colleagues crossed the mutant SOD1 transgenic mice with Bax knockout mice and showed

that neuromuscular denervation and mitochondrial vacuolization persisted in the absence of apoptotic death of motor neuron cell bodies in the double mutant mice [65]. Neuromuscular denervation was observed to begin long before the activation of apoptotic proteins, and Bax deficiency delayed the onset of neuromuscular denervation. Motor neurons exhibited mitochondrial abnormalities at the innervated neuromuscular junction at the onset of neuromuscular denervation. In addition, presynaptic terminals of motor neurons accumulated high levels of mutant SOD1 before the axons were withdrawn from the neuromuscular junction. A separate study showed denervation of 40% of neuromuscular junctions in pre-symptomatic G93A SOD1 transgenic mice (47 days), 60% loss of ventral root axons in 80 days old G93A mice (immediately prior to onset), and significant motor neuron death until 100 days old (post-symptomatic) [66]. The results from these studies support the “dying back” hypothesis that clinical symptoms in the G93A SOD1 mice result from damages to the distal motor axon rather than the activation of the cell death pathway in cell bodies. Furthermore, the results suggest that local mitochondrial changes in distal axons may represent a triggering mechanism for axonal degeneration and denervation. The findings inspired more careful studies of mitochondrial abnormalities in ALS with respect to the subcellular localization of such changes as well as the transport and dynamics of mitochondria. The findings and implications of mitochondrial transport and dynamics in the context of ALS are discussed below.

DISRUPTION OF AXONAL TRANSPORT IN ALS

Neurons have extensive dendritic arbors and axonal processes that can extend far from the soma. Transport of materials (proteins and organelles) between the cell body and neuronal processes is crucial to signal transduction and neuronal survival. Disruption of slow axonal transport of the cytoskeleton is one of the earliest pathological events in mutant SOD1 mice [67,68]. Fast axonal transport mediated by kinesin and dynein motor complexes is responsible for transporting membrane-bound organelles (e.g., mitochondria) to maintain axonal and synaptic function. Mutations in the anterograde transport motor protein kinesin (KIF5A) can cause spastic paraplegia [69] and mutations in the retrograde motor complex dynein-dynactin cause motor neuron degeneration in humans [70] and mice [71,72].

Mouse strains carrying various dynein or dynactin mutations also showed retrograde transport impairment and motor neuron degeneration [72–76]. Decreased kinesin-mediated (anterograde) and dynein-mediated (retrograde) axonal transport have been observed both in ALS patients and in transgenic animal models [67, 77–82]. More intriguingly, crossing of G93A SOD1 ALS mice with different mouse strains carrying dynein or dynactin mutations resulted in various degrees of protection in the double mutant mice [73,74,83,84]. Nevertheless, the results strongly support that axonal transport is likely a critical component in ALS disease etiology.

The precise mechanisms by which axonal transport is affected by mutant SOD1 in ALS have yet to be established. In the last few years, ALS-related mutant SOD1 proteins have been shown to associate with both the anterograde motor kinesin-2 complex via kinesin-associated protein 3 (KAP3) and the retrograde motor dynein-dynactin complex, providing potential molecular mechanisms how mutant SOD1 might interfere with axonal transport [85–87]. Most recently, a genome-wide single nucleotide polymorphism analysis in a set of 1821 sporadic ALS cases and 2258 controls from the United States and Europe revealed that a variant within the KAP3 gene was associated with decreased KAP3 expression and increased survival in sporadic ALS [88]. Examination of the literature revealed a report showing increased expression of KAP3 in mutant SOD1 transgenic mice [89]. These findings provide an emerging concept that alterations of axonal transport might be involved in both familial and sporadic ALS.

It also remains unclear how impairment of axonal transport cause motor neuron dysfunction and degeneration. Some transport cargos such as neurotrophic factors, signaling molecules, and mitochondria are obvious targets. Perlson and collaborators recently showed the inhibition of retrograde survival signaling (P-Trk and p-Erk1/2) and increase of stress signaling (P-JNK, caspase-8 and p75^{NTT}) in the G93A transgenic mice [90], indicating that suppression of dynein-mediated retrograde transport can produce cargo-dependent insults to motor neurons in ALS. Several studies also demonstrated that p38 stress-activated kinase was activated and phosphorylated neurofilaments (NFM and NFH) in G93A transgenic mice [91, 92]. In addition, p38 has been shown to be involved in regulating fast axonal transport including transport of mitochondria [93,94]. Additional studies are needed to elucidate how axonal transport is perturbed and how it subsequently contributes to the disease.

AXONAL TRANSPORT OF MITOCHONDRIA AND ITS REGULATION

Axonal transport of mitochondria is essential to neurons due to their extensive processes between the soma and the synaptic sites at the cell periphery [95]. Mitochondria are highly dynamic and respond to moment-to-moment changes in the energy demands of the cell [96]. In neurons, mitochondria frequently accumulate in axonal terminals for the high demand of ATP and Ca^{2+} influx at synapses [97,98]. In hippocampal neurons, mitochondria dynamically relocated into dendritic protrusions in response to synaptic excitation and facilitated synaptogenesis and spine formation [99]. The bidirectional transport of mitochondria mainly depends on two motor protein families: the kinesin superfamily proteins in the anterograde transport and the dynein protein complex in the retrograde transport. Disruption of kinesin heavy chain KIF5B causes perinuclear clustering of mitochondria in mouse neurons, indicating that KIF5B is essential for mitochondrial dispersion [100]. In addition, other kinesin superfamily members KIF1B and KLC3 are also implicated in anterograde transport of mitochondria [101,102]. Dynein is important not only for axonal retrograde transport of mitochondria but also for mitochondrial fission [103].

The precise mechanisms that regulate the kinesin and dynein motor complexes and their attachment to the organelles are yet to be fully understood [95]. One mechanism by which mitochondria are attached to kinesin has been described. Milton is an adaptor protein interacting with both kinesin heavy chain (KHC) and the mitochondrial Rho GTPase protein Miro and recruits mitochondria to the kinesin motor for transport [52,104–107]. The interaction between Milton and KHC is independent of kinesin light chain (KLC). Miro mutants in *Drosophila* cause enrichment of mitochondria in neuronal somata and reduction in neuropil [106]. GTPase defective mutants of Miro resulted in the aggregation of mitochondria and the constitutively active Miro induced apoptotic cell death [107]. The C-terminal transmembrane domain within Miro is required for mitochondrial outer membrane targeting. The N-terminus within Miro is responsible for binding with the kinesin-interacting protein GABA-A receptor-interacting factor 1 (GRIF-1) and O-linked N-acetylglucosamine transferase interacting protein 106 (OIP106), linking mitochondria to kinesin-mediated axonal transport [108–110].

Miro contains two Ca^{2+} -binding EF-hand motifs, providing a calcium-responsive mechanism to regulate

mitochondrial transport. Ca^{2+} binding to the EF-hand motif of Miro can promote direct binding of Miro to KHC rather than via Milton and prevent the attachment of KHC to microtubules [52]. In response to excitation, an influx of Ca^{2+} in both pre- and post-synaptic cytosol will consequently inhibit mitochondrial transport. The locally arrested mitochondria are then able to provide more ATP and to buffer high Ca^{2+} to avoid overexcitement. Mitochondria subsequently move away when the local Ca^{2+} concentration returns to normal and ATP is supplied [53,54]. This provides a mechanism for responding to local calcium homeostasis and energy demand.

Neurotrophic factors, which are critical to neuronal differentiation and survival and axonal growth and maintenance, can also regulate axonal transport of mitochondria. For instance, nerve growth factor has been shown to regulate the motility and distribution of axonal mitochondria and cause the local accumulation of mitochondria in cultured primary neurons [111,112].

IMPAIRED AXONAL TRANSPORT OF MITOCHONDRIA IN ALS

Impairment of mitochondrial transport has been reported in several neurodegenerative diseases including Huntington's disease [113] and Parkinson's disease [114] as well as ALS [95,115–118]. One study showed that mitochondrial transport was reduced in the anterograde direction in motor neurons and cortical neurons [115]. However, another study showed that both anterograde and retrograde transport of mitochondria were altered by mutant SOD1 in differentiated NSC34 cells expressing mitochondria-targeted SOD1 [116,117]. In addition, mutant SOD1 also caused mitochondrial fragmentation and impaired mitochondrial dynamics in the studies [116,117].

Although the mechanism by which mutant SOD1 disrupts axonal transport of mitochondria is not completely understood, several possible scenarios are illustrated in Fig. 1. First, mitochondrial transport and membrane potential are correlated [119]. As discussed earlier, mutant SOD1 can cause a partial loss of mitochondrial membrane potential, and thus can change axonal transport of mitochondria. Second, the elevated local Ca^{2+} concentration induced by decreased mitochondrial buffering capability can promote detachment of KHC from microtubule [52–54]. Third, various kinases have been reported to be activated by pathogenic proteins implicated in different neurodegenerative dis-

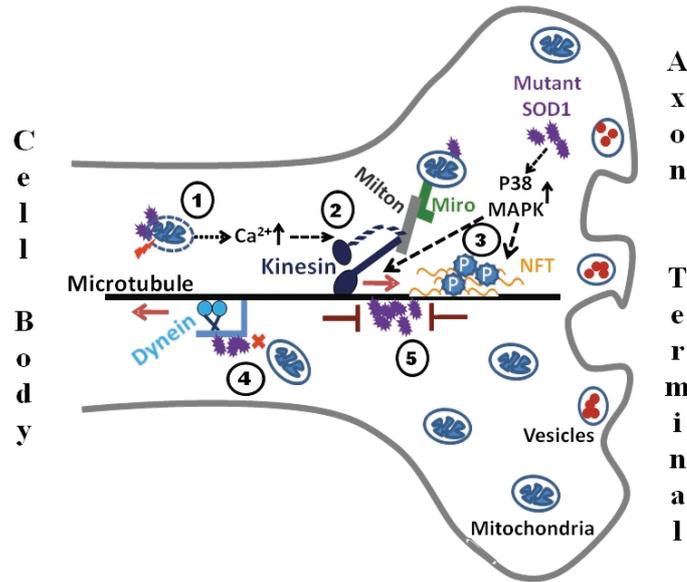


Fig. 1. Potential mechanisms for the involvement of mitochondrial dysfunction in ALS. Several possible mechanisms by which mutant SOD1 can cause impairment of axonal transport and abnormalities in mitochondrial function and dynamics are illustrated. (1) Disturbed mitochondrial membrane potential can impact mitochondrial transport. (2) Elevated Ca^{2+} levels can prevent kinesin from binding to microtubules and lead to decreased anterograde axonal transport. (3) The stress-responsive p38 MAP kinase pathway is activated and results in phosphorylated neurofilaments and their accumulation along axons. In addition, kinesin heavy chain and kinesin light chain can be phosphorylated by various kinases and anterograde transport could be affected. (4) Mutant SOD1 can interact with the dynein-dynactin complex and may result in impairment of retrograde transport. (5) Aggregation of mutant SOD1 along microtubules may interfere with both anterograde and retrograde transport like a blockade. Impairment of axonal transport of mitochondria would likely perturb mitochondrial dynamics.)

eases. The stress-responsive p38 MAP kinase pathway was reported to be activated in mutant SOD1 transgenic mice [91,120]. The activated kinases can phosphorylate KHC or KLC and affect anterograde transport [93,121–124]. Alternatively, activated MAP kinase can also phosphorylate neurofilaments and lead to their accumulation in axons [91,92]. The activation of MAP kinase potentially provides a connection between mutant SOD1 and kinesin-mediated axonal transport. Fourth, the aberrant interaction between mutant SOD1 and the dynein-dynactin complex could also disrupt retrograde transport of mitochondria [85,86]. Fifth, mutant SOD1 aggregation along microtubules may interfere with both anterograde and retrograde transport like blockades along highways. In addition, anterograde and retrograde transport are interrelated and highly regulated, thus it is not surprising that disruption of the transport in one direction would affect the transport in the other direction.

It is noted that the effect of mutant SOD1 on the axonal transport of different cargos could be distinct. It was recently reported that the retrograde survival signaling was suppressed while the transport of stress signaling was increased in the G93A transgenic mice [90]. The axonal transport of mitochondria was shown to be

impaired in either the anterograde direction [115] or in both anterograde and retrograde directions [117]. Another study showed that mutant SOD1 interacted with the kinesin-2 complex via kinesin-associated protein 3 (KAP3) and impaired anterograde transport of choline acetyltransferase [87]. Will the axonal transport of mitochondria and of choline acetyltransferase be affected in the same fashion in the presence of mutant SOD1? It is conceivable that transport of different organelles, cargos, or signals can be differentially altered in the presence of the ALS-linked SOD1 mutants. Future studies are needed to clarify how axonal transport of mitochondria is altered in ALS and to understand the mechanisms.

MITOCHONDRIAL DYNAMICS (FISSION, FUSION, AND MITOPHAGY) IN ALS

Mitochondria maintain their proper morphology and function through fusion, fission, and clearance by autophagy (mitophagy). Disrupting the balance of the processes would result in mitochondrial fragmentation, elongation, or aggregation [125]. It is logical to spec-

ulate that mitochondrial morphology, metabolic function, membrane potential, axonal transport, and fission and fusion are highly inter-dependent.

In mammalian cells, mitochondria migrate along microtubules, encounter each other, and form a large network of long interconnected tubules. The fusion machinery includes Mfn1, Mfn2, and OPA1. Mfn1 and Mfn2 belong to the large GTPase family and localize to the mitochondrial outer membrane. OPA1 is a dynamin family GTPase and localizes within the mitochondrial intermembrane space. Mfn1 and Mfn2 deletion leads to loss of mitochondrial fusion, high fragmentation, and correspondingly no mitochondrial tubules. In addition, Mfn1 and Mfn2 deletion results in severe cellular defects, including widespread heterogeneity of mitochondrial membrane potential and decreased cellular respiration [126]. In humans, mutations in Mfn2 cause Charcot-Marie-Tooth neuropathy type 2A and mutations in OPA1 cause the most common form of hereditary optic atrophy [127,128]. On the other hand, the components of mitochondrial fission machinery in mammals include Drp1 and Fis1. Dominant-negative mutants of Drp1 inhibit mitochondrial division and result in highly interconnected mitochondrial tubules [129]. Overexpression of Fis1 leads to mitochondrial fragmentation, release of cytochrome *c*, and ultimately apoptosis [130].

Abnormal mitochondrial clustering and fragmentation [43,44,46,47,131] and altered mitochondrial transport [115,117,132] are highly suggestive that mitochondrial dynamics may be influenced in the presence of mutant SOD1. A recent study showed mitochondrial fragmentation, impaired mitochondrial dynamics, and decreased density of mitochondria along neurites in differentiated NSC34 cells expressing mutant SOD1 [117]. Another study also showed that mutant SOD1 induced an extensive fragmentation of the mitochondrial network in NSC34 cells but not in other non-motor neuron cell lines [46]. Changes in mitochondrial dynamics have also been found in other neurodegenerative diseases such as Alzheimer's disease (AD) [133–135]. The results provided evidence that A β PP mutant can cause an imbalance of mitochondrial fission and fusion likely through Dlp1, OPA1, and Fis1 proteins. Unlike in AD, little is known regarding the molecular mechanism by which mutant SOD1 induces such defects in mitochondrial dynamics in ALS.

Mitochondrial dynamics is also closely related to apoptosis regulation and calcium homeostasis. During apoptosis, the dynamic balance of mitochondrial fission and fission is altered, which leads to an extensive

fragmentation of the mitochondria and release of proapoptotic factors [136,137]. Compared to normal interconnected mitochondrial network, fragmented mitochondria have lower Ca²⁺ buffering capacity and therefore probably cause selective death of motor neurons in ALS [138]. Thus, defects in mitochondrial fission and fusion can result in the malfunction of mitochondria.

Mitochondria clearance is believed to be accomplished by the autophagic degradation pathway, which is called mitophagy. Recent evidence showed that the mitochondrial protein Nix was a selective autophagy receptor by binding to LC3/GABARAP proteins that are required for autophagosome formation. Ablation of the Nix:LC3/GABARAP interaction suppressed mitochondrial clearance in maturing murine reticulocytes [139]. Mitophagy has been implicated in Parkinson's disease mediated by PINK1 and Parkin [13,140,141]. This decreased mitochondrial clearance could cause the accumulation of defective and damaged mitochondria and ultimately lead to neurodegenerative diseases.

AXONOPATHY MODEL OF ALS

A “dying-back” axonopathy hypothesis of ALS is emerging in the field. Recent studies provide convincing evidence that denervation of motor neurons from muscles occurs in early stage of the disease pathogenesis prior to clinical symptoms in mutant SOD1 transgenic animals [65,66,82,142–144]. Interestingly, transgenic mice with muscular overexpression of uncoupling protein 1 (UCP1), which only caused mitochondrial defects in muscles, displayed age-dependent deterioration of neuromuscular junctions and subsequent motor neuron pathology [145]. The results are supportive of the notion that the distal pathology at neuromuscular junction can contribute to motor neuron degeneration in ALS. The results also illustrate the critical importance of proper function of mitochondria at both the presynaptic and postsynaptic compartments of the neuromuscular junction. As discussed earlier, mutant SOD1 may interfere with the mitochondrial function via multiple mechanisms, particularly via impairment of mitochondrial transport and dynamics. Thus, maintaining appropriate population of properly functioning mitochondria in distal axons could provide a critical therapeutic avenue for potential ALS treatment.

CELL AUTONOMOUS OR NOT?

Another critical notion is that multiple cell types may contribute to the disease although motor neurons are the primarily affected cells in ALS (see review [146]). Multiple studies showed that neuron specific expression of mutant SOD1 was insufficient to produce ALS phenotypes in transgenic mice [147,148]. Further studies using chimeric mice with a mixture of normal and mutant SOD1-expressing cells [149] or transgenic mice carrying a cell-specific deletable mutant SOD1 gene [150–153] suggest that disease onset and progression may be influenced by mutant SOD1 expression in different cell types including motor neurons, microglia, astrocytes, and Schwann cells. Similar non-cell autonomous effects were also observed with primary and embryonic stem cell-derived motor neurons [154–156]. However, another study suggested that high expression of mutant SOD1 in neurons was sufficient to cause late onset disease [157]. Moreover, recent studies showed that expression of mutant SOD1 in muscles could also produce phenotypes reminiscent of ALS in transgenic mice [158,159]. These studies, which may appear conflicting, suggest that both cell-autonomous and non-cell-autonomous processes may contribute to mutant SOD1 mediated motor neuron degeneration. It is likely that these two types of processes may differentially contribute to the disease onset and progression. The contribution of mitochondria in the cell-autonomous and non-cell-autonomous processes has yet to be better understood.

CONCLUDING REMARKS

It is believed that alterations in multiple pathways in multiple cell types can contribute to ALS pathogenesis and progression. Mitochondrial dysfunction plays a critical role in the pathogenesis of mutant SOD1 mediated familial ALS. Various aspects of the underlying mechanisms as well as functional consequences of mitochondrial dysfunction are discussed in this review. They include association of mutant SOD1 aggregates with mitochondria, abnormal mitochondrial morphology, impaired mitochondrial bioenergetics and degradation through autophagy, loss of mitochondrial membrane potential, reduced mitochondrial calcium buffering capacity and disrupted calcium homeostasis, impaired axonal transport of mitochondria, and potential imbalance of mitochondrial fission and fusion. In fact, many of the events can be cause for and consequence of

each other and they create a vicious cycle that results in motor axon denervation and ultimately motor neuron degeneration in ALS.

It is evidently critical to determine the very first event induced by mutant SOD1 and devise a strategy to stop or delay it before the cycle becomes unstoppable. Misfolding of mutant SOD1 due to the intrinsic structural properties of the mutation is probably the first event. The impairment of axonal transport by misfolded mutant SOD1 is likely the immediate next event. The mitochondrial abnormalities can be secondary effects caused by compromised axonal transport as discussed in the review. This hypothesis would explain the accumulation of dysfunctional mitochondria in distal axon terminals and the axon degeneration that were observed in very early stage of presymptomatic ALS mice. This speculation is supportive of the “dying back” axonopathy model that is emerging in the ALS field. It is also essential to study the mechanisms in the context of each other to understand the crosstalk among the events so that potential therapeutic strategies may be designed to tackle multiple pathways simultaneously.

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