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MINI-SYMPOSIUM: AMYOTROPHIC LATERAL SCLEROSIS: AN UPDATE ON ITS COMPLEXITY

The Role of Skeletal Muscle in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset disease primarily characterized by upper and lower motor neuron degeneration, muscle wasting and paralysis. It is increasingly accepted that the pathological process leading to ALS is the result of multiple disease mechanisms that operate within motor neurons and other cell types both inside and outside the central nervous system. The implication of skeletal muscle has been the subject of a number of studies conducted on patients and related animal models. In this review, we describe the features of ALS muscle pathology and discuss on the contribution of muscle to the pathological process. We also give an overview of the therapeutic strategies proposed to alleviate muscle pathology or to deliver curative agents to motor neurons. ALS muscle mainly suffers from oxidative stress, mitochondrial dysfunction and bioenergetic disturbances. However, the way by which the disease affects different types of myofibers depends on their contractile and metabolic features. Although the implication of muscle in nourishing the degenerative process is still debated, there is compelling evidence suggesting that it may play a critical role. Detailed understanding of the muscle pathology in ALS could, therefore, lead to the identification of new therapeutic targets.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating condition primarily characterized by the selective loss of upper motor neurons in the motor cortex and lower motor neurons in the brainstem and the spinal cord. Clinical hallmarks include progressive muscle wasting, speech and swallowing difficulties, fasciculations, altered reflexes, and spasticity. Death usually occurs by respiratory complications within 2–5 years of diagnosis. The disease typically appears between 40 and 70 years of age, and affects about two in 100 000 people. Around 90% of cases are sporadic. The remaining 10% exhibit a Mendelian pattern of inheritance, mainly in an autosomal dominant manner. Both forms are clinically and pathologically undistinguishable, so that it is assumed that they share common pathogenic mechanisms. Riluzole, which provides neuroprotection against glutamate-induced excitotoxicity, is the only accepted medication for the treatment of ALS, although its benefit is limited (56).

Defects in a heterogeneous group of genes have been implicated in the pathogenesis of ALS (listed at http://alsod.iop.kcl.ac.uk/). Mutations in *SOD1*, which encodes the free radical-scavenging enzyme Cu/Zn superoxide dismutase, account for 20% of familial cases and 2%–7% of sporadic cases (96, 100). Transgenic mice with mutations in *sod1* have precipitous, age-related loss of motor neurons, and are a well-characterized animal model of human ALS (44, 98, 130). Most of the investigations presented in this article have been conducted on transgenic mouse lines overexpressing mutant forms of SOD1. Other major genes whose mutations cause ALS are *C9orf72* (40% of familial cases and 5–7% of sporadic cases), *FUS* (5% of familial cases and less than 1% of sporadic cases) and *TARDBP* (3% of familial cases and 1.5% of sporadic cases) (27, 64, 108).

Multiple pathogenic mechanisms have been proposed to contribute to motor neuron degeneration, including excitotoxicity, oxidative stress, aberrant protein aggregation, defective axonal transport, mitochondrial dysfunction and altered RNA metabolism (4, 6, 7, 19, 40, 68). However, the precise nature of the selective loss of motor neurons still remains obscure. The situation is even more complex than imagined, since growing evidence supports that ALS not only affects motor neurons but also other cells. In the spinal cord, astrocytes and microglial cells, as well as oligodendrocytes and interneurons, which have been more recently implicated, appear to contribute to the degenerative process (84, 93, 95, 120). Other neurons are also affected, such as serotonergic neurons in the brainstem and neurons in the frontal and temporal lobes (28, 118). Beyond the central nervous system, it is also commonly accepted that the dismantlement of

neuromuscular junctions is one of the earliest events occurring prior to motor neuron degeneration (81). In this context, it has been postulated that skeletal myocytes could play an active role, instead of merely suffering from motor neuronal loss. Here, we describe the features of ALS muscle pathology and discuss on the contribution of muscle to the pathological process. We also give an overview of the therapeutic strategies proposed to alleviate muscle pathology or to deliver curative agents to motor neurons.

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION CHARACTERIZE ALS MUSCLE PATHOLOGY

Early studies conducted on mutant SOD1 mice showed a progressive age-dependent aggregation of mutant enzyme in hindlimb muscle (116), leading to the belief that similar pathogenic events might affect motor neurons and myofibers. In addition, heat shock proteins were present in muscle at lower levels than in spinal cord (5, 126), suggesting that myofibers would be intrinsically susceptible to accumulation of malformed proteins. In parallel, increased amounts of reactive oxygen species were found in mutant SOD1 muscle even before motor impairment (46). As a result, superoxide dismutase and catalase activities were shown to increase in an attempt to counterbalance the disturbances in the normal redox state of myofibers (65, 72). The stimulation of these antioxidant defences, therefore, points to the presence of oxidative stress. Interestingly, this would account for the enhanced vulnerability of mutant SOD1 muscle to paraquat, which is an herbicide that generates high amounts of highly toxic radicals (92).

On the basis of proteomics studies, it was postulated that the accumulation of reactive oxygen species in mutant SOD1 muscle might be at least in part the result of an exacerbated oxidative metabolism (12). The increased expression of several genes involved in lipoprotein clearance and fatty acid transport, even during the presymptomatic stage (35, 39), would be related to such a boosted metabolism. Interestingly, high fat diets provided beneficial effects to mutant SOD1 mice, suggesting that a "hypermetabolic" condition could help to fight against the disease (35). This viewpoint is supported by recent studies that investigated the consequences of the genetic ablation of AMPK, an enzyme that typically stimulates the consumption of fatty acids in skeletal muscle via the β-oxidation pathway. AMPK knockout mice exhibited gait disturbances reminiscent of that observed in the mutant SOD1 model (121). However, it should be taken into account that an enhanced oxidative metabolism, together with an excess of reactive oxygen species, would eventually lead to dysfunction of the respiratory electron transport chain, which would generate in itself more oxidative strees. The altered expression of PGC-1α, a transcription coactivator that normally stimulates mitochondrial biogenesis (101, 113), would contribute to this vicious circle. In fact, increasing PGC-1α content in mutant SOD1 muscle by genetic means maintained mitochondrial biogenesis and improved muscle function even at end-stage disease (23), further reinforcing the relevance of mitochondrial dysfunction to ALS muscle.

Metabolic perturbations were also observed in other animal models of ALS, such as mice knockout for TDP-43 and VAPB (47, 109). In addition, several studies conducted on muscle biopsies obtained from patients pointed to mitochondrial dysfunction, as revealed by biochemical abnormalities (22, 106, 107, 123, 127), alterations of mitochondrial DNA (2, 122) and, in some cases, ultrastructural modifications (16). Contrasting with these observations, other studies showed that mitochondrial damage was only mild (1, 37, 62, 102) but increased with disease progression (36). Taken together, these findings support the notion that oxidative stress and bioenergetic alterations are essential features of ALS muscle pathology.

MOTOR NEURONS OR MYOFIBERS: WHO ARE FIRST?

Some studies mentioned above suggested that skeletal muscle can be precociously affected in ALS in a manner that is independent on the denervation process propelled by degenerating motor neurons. Several lines of research support this hypothesis. First reports provided evidence for the activation of an antioxidant response during the presymptomatic stage in muscle of two transgenic mouse lines expressing mutant SOD1 (60). Based on magnetic resonance imaging, other investigations revealed that muscle volume of mutant SOD1 mice was reduced from as early as 8 weeks of age, long before disease (75). By injecting oocytes with muscle membranes derived from ALS patients, it was shown that the affinity of acetycholine receptors for their ligand was lower than that of receptors coming from surgically denervated muscle (88). Additional studies reported electrophysiological postsynaptic alterations in diaphragm at 4–6 weeks of age (99), indicating that neuromuscular transmission could be intrinsically affected. Several other changes at the molecular level occurred presymptomatically, that is, between 27 and 40 days of age, including a decrease in the activity of CDK5, which has been involved in myogenesis (90), and an abnormal cytoplasmic accumulation of nNOS, which has been shown to stimulate mitochondrial oxidative phosphorylation (112). Finally, the expression of different panels of genes involved in muscle growth and development was reported to be up-regulated in gastrocnemius of presymptomatic mutant SOD1 mice (25, 41).

In view of these findings, several studies aimed at answering to the question of whether skeletal muscle plays a critical role in ALS neurodegeneration. It was first reported that mutant SOD1 overexpression in muscle could be partially reduced by genetic means without affecting disease progression or survival. In addition, AAV-based delivery of follistatin to muscles of mutant SOD1 mice stimulated, as expected, their growth but had no effect on survival (79). Using a complementary experimental approach, it was shown that the muscle specific expression of mutant SOD1 was able to reduce muscle strength, induce atrophy and cause mitochondrial dysfunction, but it was not sufficient to trigger motor neuron degeneration consistent with ALS (32). These initial findings led to the conclusion that skeletal muscle do not seem to be a primary source of toxicity for killing motor neurons. Contrasting with these results, follow-up studies revealed that muscle overexpression of mutant SOD1 did reproduce ALS hallmarks, including muscle weakness, abnormal neuromuscular junctions, axonopathy and motor neuron degeneration (129). These findings provided evidence for a muscle-to-motor neuron dying-back process which, in fact, is not without

precedent. For instance, muscle specific overexpression of the axon regeneration inhibitor Nogo-A triggered shrinkage of the postsynaptic apparatus and retraction of the presynaptic motor ending (54). In as much as Nogo-A up-regulation was observed in atrophic myofibers of ALS patients at levels that correlated with the severity of the clinical symptoms (55), its expression could be related to neuromuscular junction dismantlement (10). Additional studies reported that transgenic mice overexpressing UCP1, as a means to generate muscle restricted mitochondrial uncoupling, suffered from a progressive deterioration of neuromuscular junctions associated with signs of denervation and mild late-onset motor neuron pathology (34). Although not treated in this review, it is noteworthy to mention that skeletal muscle has been shown to contribute to motor neuron degeneration in another motor neuron disease such as spinal and bulbar muscular atrophy (18). In all, these findings provide the proof of concept that specific muscle defects can destabilize motor nerve terminals and hence contribute to ALS.

Last but not least, a few studies focused on the effects of ALS on satellite cells, which are skeletal muscle stem cells that can convert into mature myofibers in response to regenerative stimuli. It was shown that the expression of the satellite cell marker Pax7 was up-regulated in presymptomatic but not end-stage mutant SOD1 mice (74). Most importantly, satellite cells isolated from presymptomatic animals exhibited less proliferative capacity *in vitro* than satellite cells isolated from wild-type littermates (73). This diminished capacity to develop normally was also observed in satellite cells derived from ALS patients (94, 103). As these cells neither contract nor receive direct motor neuronal input, it is speculated that their modifications could attest at least in part to an intrinsic muscle pathology.

DOES MUTANT SOD1 TOXICITY AFFECT ALL MUSCLES INDISTINCTLY?

Skeletal muscle is a heterogeneous tissue composed of several kinds of myofibers with distinctive ultrastructural, contractile and metabolic features. The orchestrated action of different types of myofibers enables muscles to adapt to changing functional requirements. Whether or not these myofibers, or whole muscles, are affected by ALS in the same manner has been the subject of a number of (conflicting) studies. First experiments performed on mutant SOD1 mice showed decreased maximal oxygen consumption in mitochondria of oxidative slow-twitch soleus, compared to that observed in glycolytic fast-twitch extensor digitorum longus (EDL), suggesting that the disease would affect in particular muscles displaying oxidative metabolism (65). Mass spectrometry studies of the wobbler mouse model of motor neuron disease revealed an increase in the amount of the glycolytic enzyme G3PDH, hence suggesting a shift from oxidative to glycolytic metabolism during the course of the disease (110). Contrasting studies established, however, that, despite a similar charge in mutant SOD1, fast-twitch fibers isolated from transgenic mice developed less force than slow-twitch fibers in response to calcium stimulation, when compared to control fibers isolated from wild-type littermates (3). Using mutant SOD1 mice expressing neuronal YFP, additional studies demonstrated that motor terminals from EDL and plantaris were more affected by ischaemia/reperfusion stress than motor terminals from soleus. Most importantly, this phenomenon was observed presymptomatically from as early as 31 days of age (24). Similarly, quantification of isometric forces in several fast-twitch muscles of mutant SOD1 mice revealed a decrease in the number of motor units from 40 days of age, whereas this number was found to decline in slow-twitch soleus only after the onset of symptoms (48, 49).

The reasons for the differential vulnerability between myofibers are still obscure. The axon repellent semaphorin 3A was shown to be expressed by terminal Schwann cells only in fast-fatigable neuromuscular junctions of mutant SOD1 mice, thus suggesting a mechanism by which these synapses would exhibit less plasticity in response to ALS and hence would be affected earlier (26). It was also reported that the disease in mutant SOD1 mice progressed faster in the absence of microRNA-206, which has been involved in the regeneration of neuromuscular junctions in response to injury (117, 128). The expression of microRNA-206 was selectively up-regulated in fast-twitch muscle, likely as a compensatory mechanism to halt disease progression in this muscle (114). Notably, it has been observed that fast-twitch motor units become hyperactive in response to mild denervation. Thus, the exposure of mutant SOD1 mice to this challenge was able to prevent the reduction in the number of fast-twitch motor units in presymptomatic animals (43). Similarly, the recovery of muscle function after sciatic nerve crush was accelerated by repressing the expression of SCD1 or by reducing its enzymatic activity, both of which stimulate the β-oxidation of fatty acids (51). It seems, therefore, plausible that converting fast-fatigable fibers into slow fatigue-resistant ones render them more resistant to ALS. This phenomenon was observed to occur in mutant SOD1 mice during the course of the disease (104). However, the shift from glycolytic to oxidative metabolism is not without risk. Our more recent studies demonstrated that glycolytic muscle from presymptomatic mutant SOD1 mice switched in fuel preference toward fatty acids but this phenomenon was accompanied by mitochondrial dysfunction and oxidative stress (87), providing further evidence of the selective vulnerability of muscles in ALS.

MULTIPLE PATHWAYS LEAD TO ALS MUSCLE DEGENERATION

Multiple mechanisms have been implicated in the degeneration of skeletal muscle in ALS. The comparison of the effects of overexpressing mutant SOD1 only in muscle and in whole body showed that the atrophy process originated independently of denervation by way of inhibiting the PI3K/Akt pathway and stimulating FOXO3 (31). Down-regulation of the pro-survival Akt pathway was confirmed in muscle biopsies of ALS cases (66, 133). Afterward, atrophy progressed through caspase-dependent apoptosis in parallel to motor neuron degeneration (31). Although initial investigations reported accumulation of mutant SOD1 in skeletal muscle, more recent studies have cast doubts. Indeed, it was observed that mutant SOD1 activated the ubiquitin-proteasome and autophagy systems in muscular C2C12 cells to a greater extent than in the motor neuronal NSC34 cell line. This phenomenon would explain why mutant SOD1 accumulation could not be seen always *in vivo* (21). In the absence of aggregates of mutant enzyme, it was postulated that several as yet unidentified proteins

with aberrant conformation would be in fact responsible for triggering oxidative stress and mitochondrial dysfunction (125). Alternatively, the expression of mutant SOD1 in muscle would rather induce its presymptomatic accumulation inside mitochondria, subsequently causing loss of mitochondrial inner membrane potential and fragmentation of the mitochondrial network (71). Under these circumstances, an excess of calcium release from mitochondrial stores was shown to occur before the onset of symptoms, particularly in fiber segments near the neuromuscular junctions (135). Accompanying these changes, levels of several calcium buffering proteins, such as SERCA and parvalbumin, were shown to be reduced, further reinforcing the progression of the degenerative process (15).

Autophagy is a major intracellular pathway for degradation of misfolded proteins. The expression of several autophagic factors, including LC3, p62 and Beclin-1, increased in muscle of mice overexpressing mutant SOD1 ubiquitously (20, 85) or specifically in muscle (32). However, in contrast to that observed at the level of gene expression, the autophagy flux was unexpectedly low in mutant SOD1 muscle in response to stimulation by starvation. This deficiency was explained by the concomitant caspase-3 dependent cleavage of Beclin-1 that had been found under these conditions (131). An alternative explanation came from studies overexpressing in muscle mutant forms of VCP, a member of the ATPase family implicated in cellular protein homeostasis and degeneration affecting muscles and neurons. Tubular lysosomes in these mutants appeared disrupted, and were not able to fusion with autophagosomes (53). This phenomenon could represent another mechanism by which the activity of the autophagy degradation system would be altered in ALS muscle.

The abnormal accumulation of misfolded proteins in the endoplasmic reticulum activates the unfolded protein response, to restore the physiological equilibrium. However, if the stress persists, the response is aberrantly boosted, and eventually leads to cell death. The unfolded protein response was shown to be stimulated in ALS muscle, since the expression of several factors implicated in endoplasmic reticulum stress, including PERK, IRE1α, BiP and CHOP, was up-regulated in gastrocnemius of presymptomatic mutant SOD1 mice (14). Moreover, the IRE1α-dependent pathway was impaired in C2C12 cells transfected with mutant VAPB, which is a cause of familial ALS involved in vesicle trafficking. This occurred in association with a limited capacity to form myotubes, thus suggesting that the dysfunction of the unfolded protein response might interfere with the maintenance of muscle integrity in ALS (115).

HDAC4 is known to play an important role in muscle development and maturation, via the suppression of the stimulatory effect of MEF2 on the transcription of structural and contractile genes. MEF2-dependent gene expression was inhibited by abnormally high levels of HDAC4 observed in muscle of mutant SOD1 mice, a mechanism that would contribute to muscle degeneration (17). In support of this notion, HDAC4 up-regulation in patients' muscle samples negatively correlated with reinnervation and functional outcome (11). Finally, a perturbed metabolism of iron, together with its subsequent accumulation, was also envisaged to play a role in muscle pathology, as deduced from increased amounts of ferritin H that correlated with the progression of disease in mutant SOD1 rats (45).

MUSCLES ARE A PRIMARY SITE FOR THERAPEUTIC INTERVENTION

Beyond the question of whether or not skeletal muscle contributes to ALS, numerous studies have attempted to palliate muscle pathology in itself as a means to counterbalance motor neuron degeneration (Table 1). Based on the presence of mitochondrial dysfunction, early investigations showed that oral supplementation with creatine, given as an energy source, was beneficial to mutant SOD1 mice (59). Follow-up studies, however, did not find any effect, except that the degree of atrophy in EDL was partly diminished (29). Similarly, muscle-restricted expression of PGC-1α in mutant SOD1 mice was able to increase mitochondrial ATP production and muscle endurance but did not affect lifespan (23). In contrast, stimulation of the β-oxidation of fatty acids by L-carnitine ameliorated motor function and extended survival (57). Highly energetic diets, mainly in the form of elevated lipid content, also prolonged life expectancy, and improved muscle function and motor neuron survival (35, 76, 86).

Some studies performed on muscle biopsies obtained from patients revealed a decrease in the amount of Igf-1, which is a well-known stimulator of growth and development (70). Although this finding was not confirmed in other cohorts (38), interfering with the process of muscle atrophy using growth factors has been another way to fight against ALS. Thus, preclinical investigations conducted on mutant SOD1 mice aimed at increasing the muscle content of Igf-1. This growth factor ameliorated muscle function and increased motor neuron survival in most cases (30, 33, 97), but not always (77). Similar effects were observed with subcutaneous implants of dihydrotestosterone which, indeed, induced muscle expression of Igf-1 (134). Another anabolic steroid derivative called nandrolone also increased muscle mass but only slightly sustained muscle innervation (13). Blocking the activity of the muscle growth inhibitor myostatin promoted muscle mass and strength but did not affect survival (50, 82). More recent studies evaluated the influence of manupulating myogenic factors as a means to keep muscles in health. Strinkingly, gene transfer of myogenin into muscle ameliorated motor neuron survival and improved innervation but, in contrast, gene transfer of MyoD aggravated the condition (89). Additional studies also demonstrated beneficial effects on ALS muscle by targeting the response to stress (42) and oxidative damage (119), the stimulation of the contractile apparatus (52, 105) or the inhibition of several cell death pathways (9, 83).

The high levels of several neurotrophins found in muscle samples of ALS patients were interpreted as a compensatory mechanism to prevent motor neuron degeneration (63). Therefore, some therapeutic strategies have proposed that muscles may serve to deliver protective molecules to motor neurons in a retrogade manner. We cannot rule out, however, the possibility that these neuroprotective strategies, although designed in principle to target specifically motor neurons, could, in some cases, exert beneficial actions at the muscle level. The most significant results were achieved by providing motor neurons with GDNF, which is a potent survival factor for these cells. Delivery approaches included the use of retroviral vectors (69, 80, 124), electroporation (132), transgenic muscle-restricted overexpression (67) and intramuscular transplantation of stem cells (61, 91, 111). Neuroprotective effects were also obtained by delivering other neurotrophic factors, such as

Table 1. Experimental approaches with therapeutic potential targeting ALS muscle pathology.

Target	Approach	Model	Survival	Effects	Ref.
Creatine	Oral	SOD1(G93A)	Yes	∨ oxidative stress ∨ mitochondrial dysfunction ∨ motor neuron loss ∧ motor performance	(45)
Creatine	Oral	SOD1(G93A)	No	∨ EDL atrophy	(59)
PGC-1α	Muscle expression	SOD1(G37R) x MCK/PGC-1α	No	∧ mitochondrial ATP production ∧ muscle endurance ∨ muscle degeneration	(23)
L-carnitine	Injection (s.c.)	SOD1(G93A)	Yes	∨ myofiber apoptosis ∨ motor function deterioration	(29)
HF diet	Oral	SOD1(G86R)	Yes	∨ muscle denervation ∧ motor neuron survival	(35)
HF/HC diet	Oral	SOD1(G93A)	Yes	Delayed onset	(76)
Olive oil	Oral	SOD1(G93A)	Yes	∧ MyoD and MyoG expression ∧ LC3 and Beclin-1 expression ∨ Atf6 and Grp78 expression ∧ myofiber area ∧ motor performance	(86)
Igf-1	Muscle expression	SOD1(G93A) x MLC/mIgf-1	Yes	∧ satellite cell activation ∧ NMJ stabilization ∧ motor neuron survival ∨ muscle atrophy ∨ spinal cord inflammation	(33)
Igf-1	Muscle expression	SOD1(G93A) × SαA/hIgf-1	No	No effect	(77)
Igf-1	Muscle expression	SOD1(G93A) × MLC/mIgf-1	N/A	∨ ubiquitin expression ∨ caspase activity ∨ p25 accumulation ∧ CDK5 expression	(30)
MGF	Plasmid	SOD1(G93A)	N/A	∧ muscle strength ∧ motor neuron survival	(97)
DHT	Implant	SOD1(G93A)	Yes	∧ Igf-1 expression ∨ muscle atrophy ∨ motor neuron loss ∧ muscle strength ∧ motor function	(134)
Nandrolone	Injection (s.c.)	SOD1(G93A)	N/A	∧ muscle mass ∧ pre-synaptic activity	(13)
Myostatin	Antibody	SOD1(G93A)	No	∧ muscle mass ∧ muscle strength ∨ motor neuron loss	(50)
Myostatin	ActRIIB injection (i.p.)	SOD1(G93A)	No	∧ muscle mass ∧ muscle strength	(82)
Myogenin	AAV	SOD1(G93A)	N/A	∧ muscle innervation ∧ motor neuron survival	(89)
MyoD	AAV	SOD1(G93A)	No	Aggravated phenotype	(89)
Hsp70	Injection (i.p.)	SOD1(G93A)	Yes	∧ innervated NMJ number ∧ motor neuron survival ∧ motor function	(42)
Nrf2	Muscle expression	SOD1(G93A) x MLC/Nrf2	No	Delayed onset	(119)
Tirasemtiv	Oral	SOD1(G93A)	N/A	∧ forelimb strength ∧ rotarod performance	(52)
Tweak	Antibody	SOD1(G93A)	No	∨ muscle atrophy	(9)
GPNMB	Plasmid	SOD1(G93A)	N/A	∧ myofiber number ∨ myofiber atrophy	(83)

"Yes" means an increase in survival while "No" means lack of effect. (∧) = increased effect; (∨) = decreased effect; AAV = adeno-associated virus; ActRIIB = soluble activin receptor type IIB; DHT = dihydrotestosterone; GPNMB = glycoprotein nonmetastatic melanoma protein B (osteoactivin); HF = high fat; HF/HC = high fat/high carbohydrate; i.p. = intraperitoneal; MGF = mechano-growth factor (Igf-1 splice variant); MLC = myosin light chain; N/A = not applicable; NMJ = neuromuscular junction; SαA = skeletal alpha actin; s.c. = subcutaneous; Tirasemtiv = fast skeletal troponin activator; Tweak = tumor necrosis factor-like weak inducer of apoptosis.

VEGF (61, 58) and cardiotrophin-1 (8), or by supressing mutant SOD1 overexpression with RNAi (78).

CONCLUSION

As a matter of conclusion, it is recognized that oxidative stress, mitochondrial dysfunction and bioenergetic disturbances are hallmarks of the pathology of ALS muscle. However, the way by which the disease affects myofibers depends on their contractile and metabolic features. The implication of muscle in nourishing the degenerative process is still debated but there exists compelling evidence suggesting that it may play a critical role. Detailed understanding of this contribution could, therefore, lead to the identification of new therapeutic avenues.

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