the FEBS Journal
REVIEW ARTICLE

# Understanding ALS: new therapeutic approaches

Antonio Musarò

Institute Pasteur Cenci Bolognetti, Istituto Italiano di Tecnologia, Department of Anatomy, Histology, Forensic Medicine and Orthopedics - Unit of Histology and Medical Embryology, Istituto Interuniversitario di Miologia, Sapienza University of Rome, Italy

**Keywords**
ALS-associated genes; amyotrophic lateral sclerosis; complexity of ALS pathogenesis; growth factors; mutant SOD1 toxicity; neuromuscular disease; oxidative stress; skeletal muscle; stem cells; therapeutic approaches

**Correspondence**
A. Musarò, Unit of Histology and Medical Embryology, Via A. Scarpa 14, Rome 00161, Italy
Fax: +39 06 4462854
Tel.: +39 06 49766956
E-mail: antonio.musaro@uniroma1.it

(Received 5 November 2012, revised 30 November 2012, accepted 3 December 2012)

doi:10.1111/febs.12087

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease associated with motor neuron degeneration, muscle atrophy and paralysis. Although numerous pathological mechanisms have been elucidated, ALS remains an invariably fatal disease in the absence of any effective therapy. The heterogeneity of the disease and the failure to develop satisfactory therapeutic protocols reinforce the view that ALS is a multi-factorial and multi-systemic disease. Thus, a better understanding of the pathogenic mechanisms and study of the potential pathological relationship between the various cellular processes is required to ensure efficacious therapy. The pathogenic mechanisms associated with ALS are reviewed, and the strengths and limitations of some new therapeutic approaches are discussed.

## Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder with a typical disease course of 1–5 years [1]. Typical features of this progressive lethal disease are the degeneration of motor neurons, muscle weakness, fasciculations, muscle atrophy, speech and swallowing disabilities, progressive paralysis, and death caused by respiratory failure. Cortical, bulbar and spinal motor neurons are severely compromised, but, for unknown reasons, neurons that control the bladder and the oculomotor muscles are spared by the disease.

ALS is epidemiologically classified into two forms: sporadic (90–95%), i.e. without an obvious genetic component, and familial (5–10%), with a positive family history [2]. The familial form usually shows an autosomal-dominant pattern of inheritance. Autosomal-recessive forms have also been described, particularly from highly consanguineous populations [3]. Notably, sporadic and familial ALS have similar pathological hallmarks. Interestingly, recent experimental evidence has also indicated common alterations in gene expression in both sporadic and familial ALS, suggesting that the two forms of ALS may share similar pathogenic mechanisms.

## The complexity of ALS pathogenesis

### Genetic causes

Approximately 20% of familial cases of ALS are caused by dominantly inherited mutations in the

**Abbreviations**
ALS, amyotrophic lateral sclerosis; FUS or FUS/TLS, fused in sarcoma/translocated in liposarcoma; IGF-1, insulin-like growth factor 1; SOD1, superoxide dismutase1; TDP-43, Tar DNA binding protein 43.

Cu/Zn superoxide dismutase (SOD1) protein [4]. The physiological activity of the metalloenzyme SOD1 is to convert superoxide, a toxic by-product of mitochondrial oxidative phosphorylation, to water or hydrogen peroxide, thus protecting cells from the accumulation of free radicals. Initially, the 'loss of function' hypothesis proposed that a mutation in the SOD1 gene led to a decrease in protein enzymatic activity. However, the finding that over-expression of mutant SOD1 in transgenic mice induces several clinical features of ALS disease, even in the presence of endogenous mouse SOD1 gene, has led to the conclusion that the disease results from a 'toxic gain of function' [2,5–9].

Defects in other genes, including alsin [10,11], synaptobrevin/vesicle-associated membrane protein-associated protein B [12], senataxin [13,14], dynactin [15] and the DNA/RNA-binding proteins TDP-43 and FUS/TLS [16–19] have been also reported to cause ALS. Interestingly, FUS inclusions are common in both sporadic and familial ALS, but not in cases caused by SOD1 mutations [20,21]. These observations suggest that FUS-related ALS represents a different spectrum of disease compared to SOD1-related ALS. However, a clear correlation between the genetic defect and the pathophysiology of the disease has not yet been determined. More recently, exome capture followed by deep sequencing allowed the identification of novel mutations in the profilin gene that cause familial ALS [22]. The new mutations, which inhibit axonal outgrowth, suggest cytoskeletal defects.

## Pathways implicated in ALS pathogenesis

Several pathogenic mechanisms have been proposed to account for ALS, including glutamate-induced excitotoxicity, oxidative stress, protein aggregation and mitochondrial dysfunction (Fig. 1).

### Excitotoxicity and glutamate transport

Glutamatergic toxicity, exerted by abnormal stimulation of the glutamate receptor, represents a pivotal contributory factor in the neuronal injury in ALS [23–25]. Selective loss of the astroglial glutamate transporter EAAT2 (type 2 excitatory amino acid transporter) in the motor cortex and spinal cord of both sporadic and familial ALS patients interferes with normal clearance of glutamate, which remains in the environment and continuously activates its receptors. A consequence of this abnormal receptor activity is induction of massive calcium influxes that trigger apoptotic pathways and eventually motor neuron death and degeneration. Nevertheless, it remains to be determined whether glutamate-induced excitotoxicity represents a primary defect responsible for motor neuron degeneration, or whether, alternatively, it is the result of ALS.

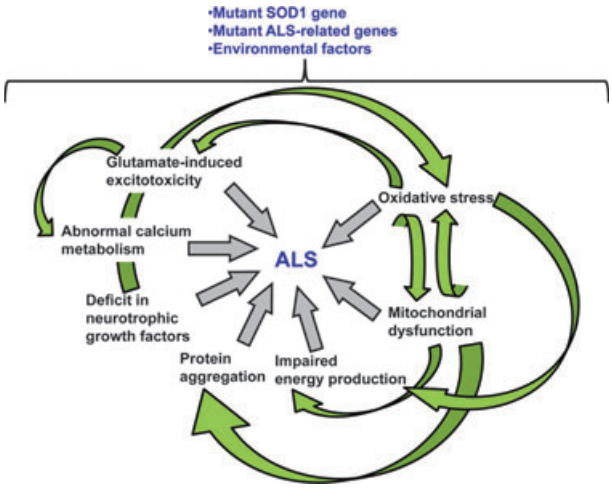


**Fig. 1.** Schematic model depicting potential mechanisms associated with the pathogenesis of ALS. Mutations in several genes, including SOD1, and environmental factors are responsible for the activation of pathogenic mechanisms, leading to ALS. Several pathogenic mechanisms have been proposed. Each of the toxic mechanisms depicted is able to induce signs of ALS; however, none of them is able to promote the severity of the diseases.

### Oxidative stress and ALS

The mutation in the antioxidant enzyme SOD1 in approximately 20% of familial ALS patients indicates that oxidative stress is one of the potential pathogenic events associated with the disease. Elevated levels of reactive oxygen species, and therefore accumulation of oxidative stress, damage proteins, lipids, DNA and RNA [26–29], leading to an alteration in cell and tissue homeostasis. Moreover, oxidative stress may exacerbate the disease by interacting with other pathophysiological processes that contribute to motor neuron degeneration, including excitotoxicity, mitochondrial impairment and protein aggregation (Fig. 1) [30–32].

### Mitochondrial dysfunction and ALS

Oxidative stress and mitochondrial dysfunction are recognized as a pathogenic events associated with ALS [33]. Mitochondria represent a primary site of intracellular production of reactive oxygen species, and hence a major source of oxidative stress that, in turn,

impairs the normal function of mitochondria [34]. A proposed mechanism suggests that mutant SOD1 is imported into mitochondria [35–37], causing direct damage of the organelle and activation of cell death [26]. A particularly severe consequence of defective mitochondrial function is impaired calcium handling, which in turn promotes activation of cell-death pathways.

### Protein aggregation and ALS

Protein aggregation represents one of the pathological features of ALS [38,39]. Under physiological conditions, cells are capable of handling mutant proteins sufficiently well to prevent them from exerting toxic effects and/or being sequestered into inclusions [40]. However, under circumstances of increased physiological or environmental stress, the ubiquitin–proteasome system, which is activated to maintain protein quality control, may become overloaded and impaired. This results in engulfment of cells, which become defective in disposal of altered macromolecules and more prone to damage.

Of note, each of the toxic mechanisms discussed above is able to induce signs of ALS (Fig. 1). However, none of them is able to promote the severity of the diseases when activated individually, and the primary causes of ALS are therefore still unknown.

## New therapeutic approaches for ALS

### Riluzole: an old friend with limited efficacy

Riluzole belongs to the chemical class of benzothiazoles; it acts by blocking voltage-gated sodium channels, with a consequent decrease in the pre-synaptic release of glutamate. Riluzole is the only therapeutic drug approved for ALS with regard to prolonging survival and delaying the use of surrogates approaches, such as tracheotomy and mechanical ventilation. Adverse effects of riluzole are relatively minor, and for the most part are reversible after stopping the treatment. However, questions persist about its clinical utility because of high cost and modest efficacy.

Recently, Miller *et al*. [41] reviewed several registers of controlled trials to examine the true efficacy of riluzole. The first conclusion of the study was that 100 mg riluzole daily is reasonably safe, and probably prolongs the median survival of ALS patients by ~ 2–3 months. Nevertheless, the beneficial effects are very modest (a small beneficial effect on both bulbar and limb function, but not on muscle strength), thus additional treatments are needed.

### Growth factors, IGF-1 and ALS

Several growth factors, such as insulin-like growth factor 1 (IGF-1), glial cell line-derived growth factor, brain-derived growth factor, vascular endothelial growth factor and ciliary neurotrophic factor, have been evaluated in experimental models of ALS [42], and have been shown to have positive effects in nearly all cases. However, human trials showed modest or absent effects of neurotrophic factor on ALS [42a,42b].

Among these growth factors, IGF-1 promotes muscle hypertrophy and nerve survival [43,44]. Moreover, it has been demonstrated that muscle IGF-1 expression counteracts the symptoms of ALS and reduces components of catabolism in $SOD1^{G93A}$ mice, carrying a G→A mutation in the SOD1 protein [45–47]. However, use of IGF-1 in ALS patients has previously produced conflicting results [48,49], and a new study reported no benefit in either survival or functional scales [50].

### Why is there discrepancy between animal studies and human trials?

First it should be noted that the experimental approaches used with the SOD1 transgenic mice were different from those used in human trials. To my knowledge, there are no studies on animal models in which the IGF-1 was delivered subcutaneously as in humans.

There are two hypotheses as to why IGF-1 did not work in human trials. First, it is possible that the subcutaneous delivery approach may be a critical issue. The amount of IGF-1 injected subcutaneously that reaches both muscle and cervical cord may be insufficient to trigger a positive result. Second, IGF-1 alone may be insufficient to counteract motor neuron degeneration.

It is worth considering that the human IGF-1 recombinant protein used in all of the human studies is related to the circulating form of IGF-1, whereas the beneficial effects reported in mouse models of ALS were obtained using the local form of IGF-1, which differs from the circulating one at the molecular and functional levels [44,51,52]. Thus, further studies are necessary to better define the potential therapeutic effects of various IGF-1 isoforms and to determine the best approach to deliver genes, by gene therapy approach, with therapeutic potential.

### Stem cells and ALS

Stem-cell technologies represent a promising approach for treating ALS [53]. Cell-based therapy may be used

to supply trophic factors to support and preserve endogenous cells and/or for replacement of damaged cells. However, despite promising results in animal models, the potential restorative mechanisms of stem-cell treatment are still uncertain.

Their developmental plasticity, i.e. the ability of stem cells to change their fate in response to extracellular signals, further stimulated the performance of stem-cell trials in patients. It has been reported that transplantation of autologous mesenchymal stem cells into the spinal cord of ALS patients is safe and well tolerated [54]. However, preliminary stem-cell transplantation trials performed in ALS patients produced conflicting results [55], including with regard to efficacy of treatment. The demonstration that a new pluripotent stem-cell type, namely induced pluripotent stem cells, may be generated from somatic cells, such as fibroblasts, by introducing the transcription factors Oct3/4, Sox2, Klf4 and c-Myc, attracted a great deal of attention [56]. Moreover, the observation that induced pluripotent stem cells may be induced to differentiate into motor neurons [57–59] brought new hope for ALS. Nevertheless, whether these converted motor neurons can successfully rescue ALS awaits further validation.

In addition to cellular replacement, transplanted stem cells may be used as a source of growth factors, providing support for endogenous cells and contributing to a more hospitable microenvironment in the spinal cord. However, a major concern with any cellular therapy is the potential for tumour formation.

In any case, the use of autologous stem cells as an effective tool for curing diseases that currently have no cure, for correcting defective genes, and for replacing genetically abnormal tissues by genetically corrected stem cells that replenish the differentiated cell compartment in the relevant tissues requires significant modulation and customization when specific diseases are tackled. Thus, in the context of cell therapy, use of innovative strategies for gene correction in autologous progenitor cells and the design of strategies to modulate the hostile local environment of damaged tissues are imperative.

## Critical issues in ALS therapies

One of the questions that remains to be addressed is the following: why is ALS an incurable disease? The simple answer is that, despite intensive investigation, the dominant pathogenic mechanisms underlying ALS have not been defined. In fact, the situation is more complicated because many mechanisms are involved in the pathogenesis of ALS (Fig. 1), each of which generate many uncertainties, and different tissues are targets of the mutant gene products (Fig. 2). In the last 15 years, more than 70 drugs have been tested in ALS animal models, and more than 30 large clinical trials have been designed and performed to treat ALS patients, based on 'solid' pre-clinical data on animal models. However, none of them has proved successful, suggesting that the therapy and biological targets are incorrect and/or the animals used to study ALS are not valid models.

### Targeting non-neuronal cells

To date, any attempt to cure ALS mouse models by acting specifically on motor neurons has failed miserably. Increasing evidence suggests that damage to motor neurons is enhanced by alterations in the neighboring non-neuronal cells [60–62]. For example, it has been demonstrated that mutant SOD1 in motor neurons

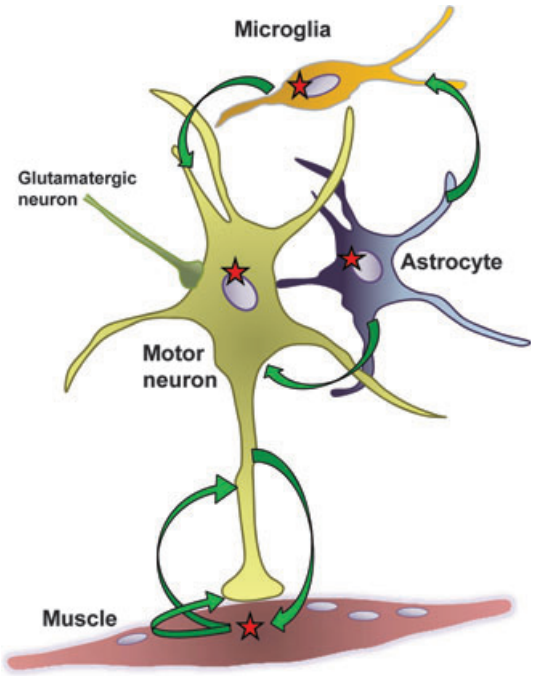


**Fig. 2.** Schematic model depicting the cells and tissues that can be affected by the non-cell-autonomous toxicity of mutant SOD1 protein. Mutant SOD1 (red stars) causes morphological and functional alterations in the tissue in which it is expressed. Nevertheless, non-cell-autonomous toxicity of mutant SOD1 is not sufficient to trigger and propagate ALS pathogenesis. It is likely that a damaged cell/tissue may negatively affect other cell types (green arrows); thus a combination of damage to various cell types (i.e. motor neurons, glia and muscle) may act synergistically to propagate and exacerbate the disease.

affects disease onset, whereas mutant SOD1 in microglia and astroglia contributes to propagation of the disease at a late stage [61]. This evidence has been substantiated by co-culture experiments, demonstrating that motor neurons are less likely to survive when they are co-cultured with astrocytes expressing mutant SOD1, or are exposed to astrocyte-conditioned medium, than on astrocytes expressing wild-type SOD1 [62]. This suggests that the mutant toxic SOD1 protein operates through a dominant paracrine activity that originates from non-neuronal cells (Fig. 2).

### Targeting skeletal muscle

Is skeletal muscle a primary target of mutant SOD1 toxicity? Several studies have suggested that morpho-functional alterations in skeletal muscle precede motor neuron degeneration [63–66]. We recently demonstrated that muscle-selective expression of the SOD1 mutation causes pathological alterations and induces pre-symptomatic signs of ALS [63]. Another study reported that muscle-restricted expression of the human mutant SOD1 gene causes motor neuron degeneration in old transgenic mice [64]. Moreover, muscle-selective alterations in mitochondrial function occur in young $SOD1^{G93A}$ mice prior to disease onset, and may initiate destruction of the neuromuscular junction, followed by distal axonopathy, astrocytosis in the spinal cord, and mild motor neuron loss [65,66]. This suggests that muscle cells suffer direct toxicity from mutant SOD1 (Fig. 2). Skeletal muscle is also a source of anabolic signals that influence neuron survival, axonal growth and maintenance of synaptic connections. Thus, skeletal muscle is probably an important target for therapeutic intervention. Nevertheless, similar to motor neurons, targeting muscle tissue either pharmacologically or genetically is not sufficient to reduce progression of the disease. It has been reported that a partial reduction of mutant SOD1 within muscle, using either a lentivirus that encodes a siRNA directed against mutant SOD1 or muscle-selective SOD1 mutant gene excision, does not affect the disease [67], suggesting that residual expression of the $SOD1^{G93A}$ mutant gene is able to maintain a pathological muscle phenotype. Moreover, it has been recently demonstrated in a mouse model of amyotrophic lateral sclerosis that keeping muscles strong and active by over-expressing the mitochondrial biogenesis-signaling factor, namely peroxisome proliferator-activated receptor alpha/PPAR$\gamma$ coactivator 1$\alpha$ (PGC-1$\alpha$) in skeletal muscle allows the animals to perform better in the face of dwindling motor neuron input, but ultimately cannot slow their demise [68].

## Conclusions

The many therapeutic failures have reinforced the idea that ALS is a multi-factorial and multi-systemic disease in which alterations in structural, physiological and metabolic parameters in motor neurons, glia and muscle act synergistically to exacerbate disease (Fig. 2). Thus, a single treatment method may be not successful. Thus, in order to be effective, therapeutic approaches should target multiple mechanisms and various cells/tissues.

Moreover, the failure to translate the positive results obtained in animal models into successful trials in human has cooled previous enthusiasm and raised important questions regarding the validity of the animal models and methodological approaches. Recently, a useful standard operating procedures for pre-clinical animal research in ALS was proposed [69] with the aim of providing guidelines to improve the validity of animal models and the design of pre-clinical experimental therapeutic approaches.

Thus, use of robust criteria and guidelines for pre-clinical animal studies and the design of multiple approaches will provide new and hopefully more efficacious strategies for the treatment of ALS.

## Acknowledgements

Work in the author's laboratory has been supported by Seventh Framework Programme Myoage (grant number 223576), the Fondation Thierry Latran, the Fondazione Roma, Ministero dell'Istruzione, dell' Università e della Ricerca (MIUR) and Association Française contre Myopathie (AFM).

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