Amyotrophic lateral sclerosis: Protein chaperone dysfunction revealed by proteomic studies of animal models

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons and causes progressive muscle weakness and atrophy. The etiology and pathogenesis of ALS are largely unknown and no effective treatment is presently available. About 10% of patients have the familial or inherited form of the disease (fALS), among which 20% is linked to mutations with Cu^{2+}/Zn^{2+} superoxide dismutase (*mSOD1*). Transgenic animals expressing human *mSOD1* are excellent models for understanding not only fALS but sporadic ALS as well. Pathological features in both ALS patients and *mSOD1* transgenic animals' spinal cords share commonalties including the accumulation of misfolded protein inclusions. Recent proteomic investigations on ALS animal models have discovered alterations in protein expression, protein-protein interactions and post-translational modifications. These efforts have revealed aspects of potential pathogenic mechanisms and identified probable therapeutic targets. The present review summarizes the major findings of proteomics studies performed on the *mSOD1* mice with particular emphasis on the spinal cord proteome. These results are compared with those reported using cell cultures or specimens obtained from ALS patients. The convergence of pathogenic processes on protein chaperone function, and its relationship to protein degradation, metabolic dysfunction and oxidative signaling events is discussed.

1 Introduction

ALS, also known as Lou Gehrig's disease in the United States, is a motor neuron disease mainly affecting adults. It is a rare fatal disease that impacts 1–2 people out of 100 000 *per* year. The histopathology of post mortem ALS spinal cord includes motor neuron atrophy, swelling of perikarya and proximal axons, the appearance of Bunina bodies containing protein precipitates and ubiquitinated proteins. The cellular pathology extends beyond motor neurons, and includes

astrocytes and other glia [1]. The exact mechanism of disease onset in humans is currently unknown. The vast majority of ALS cases occur sporadically (>90%, sALS), while the remaining cases are inherited familial cases (fALS). Although genetic mutations have only been detected in a small number of patients, they have provided valuable insights into the possible mechanisms underlying ALS pathogenesis. More importantly, the discoveries of these genes have enabled the establishment of valuable transgenic animal models for both mechanistic studies and therapeutic discoveries. Multiple molecular pathways and a complex interplay among different cell types of the CNS may underlie the development of ALS.

The present review first outlines the strengths and limitations of existing proteomics technologies as applied to ALS studies and discusses the need for the proper interpretation of proteomics results, and secondly, compares information obtained from published proteomics studies on both animal models of fALS and specimens obtained from patients. Based on the combined analysis of protein expression, protein-protein interactions and PTM in diverse proteomic investigations of *mSOD1* models, we propose the convergence of pathogenic mechanisms on protein chaperones and related pathways. We will focus our discussions on the functions of Hsp70 and its co-chaperones, which assist Hsp70 during substrate binding, ATP/ADP nucleotide exchange, protein folding/unfolding and protein degradation processes.

2 The discovery of gene mutations in fALS leads to the establishment of animal models

2.1 Identification of gene mutations in fALS

Advancements in ALS genetics have enabled the discovery of specific gene mutations in sub-populations of fALS patients. This information has provided the foundation for the establishment of animal models mimicking the symptoms of ALS, which enabled the better understanding of the disease. To date, over 100 different mutations in Cu^{2+}/Zn^{2+} super-oxide dismutase (*mSOD1*) gene have been linked to fALS [2], representing up to 20% of fALS cases. Ongoing research on transgenic animals expressing different mutations in the *SOD1* gene revealed that a toxic gain of function, independent from the dismutase activity is responsible for the deleterious outcomes [3]. It has been well established that *SOD1*-knockout mice do not develop ALS symptoms, suggesting that mSOD1 acquires its toxicity within spinal cords. However, downstream targets to mSOD1 cytotoxicity have not been identified. Protein chaperones are likely candidates, since motor neurons in ALS models are defective at proper protein folding, leading to the accumulation of protein precipitates containing neurofilaments, mSOD1, and many other ubiquitinated proteins [4]. In addition to *mSOD1*, several other gene mutations have been implicated in the development of ALS. An autosomal recessive *alsin* mutant is known to cause juvenile onset ALS [5]. This protein contains several guanine exchange factor homology domains, and may be involved in endosomal vesicular trafficking. A mutation in *dynactin*, encoding for a key factor involved in axonal retrograde transport has been shown to cause an atypical adult onset ALS [6]. Another ALS-associated gene product, vesicle-associated membrane protein/synaptobrevin-associated membrane protein B is involved in diverse processes including protein folding, inositol metabolism and vesicular transport [7]. A mutant *senataxin*, a DNA/RNA helicase, which may be involved in RNA processing, has also been linked to an autosomal dominant adult onset ALS [8]. As these mutations have only been detected in a very small number of patients, it is difficult to generalize the significance of these findings in the onset of sALS. Nevertheless, these discoveries have enabled the creation of transgenic animals for the study of disease mechanisms, and have provided clues on ALS pathogenesis, which may involve many genes and pathways, that ultimately result in motor neuron death.

2.2 Mouse models of ALS

Earlier studies on motor neuron diseases including ALS involved *wobbler* mouse [9], which has a spontaneous mutation in VPS 54, a gene encoding a vacuolar-vesicular protein-sorting factor involved in vesicular trafficking [10]. The mutation, which is located on chromosome 11, is inherited in an autosomal recessive manner. *Wobbler* mice develop motor neuron degeneration in both spinal cord and brainstem, similar to human ALS. Since ALS is a consortium of diseases with subtle yet divergent pathological phenotypes [2], a wide range of molecular alterations may be leading to motor neuron death. In 1993, a mutation in the *SOD1* gene, in which glycine at position 93 has been replaced by alanine ($SOD1^{G93A}$), was identified as the genetic basis for a subset of fALS patients [11]. Subsequently, the transgenic mouse overexpressing human $SOD1^{G93A}$ was created and appeared to recapitulate many symptoms of motor neuron diseases [12]. This animal reproducibly exhibited both clinical and histological similarities to human ALS in an age-dependent manner. The pathological reproducibility of this model offers an effective means to study the early molecular events prior to the onset of the disease. Subsequently, many other mutations in the *SOD1* gene have been linked to ALS. Twenty percent of fALS [13] or 2% of all ALS cases are caused by mutations in the *SOD1* gene. Since both sALS and fALS, including ALS cases involving mutations other than *SOD1*, show similarities in symptoms and pathology, it is possible that different pathogenic pathways share commonalities or may converge downstream to mSOD1 function. The pathological features observed in *mSOD1*-transgenic mice include specific and progressive loss of motor neurons, formation of phosphorylated neurofilament inclusions [14], astrocytosis [15] and muscle atrophy, which are observed in human ALS patients [16].

Significant variations exist in the pathological features among animal models expressing different *mSOD1*, resulting from differences in mRNA and protein stability, level, or function. For example, accumulation of vacuoles was observed in enlarged axons and dendrites of motor neurons in both $SOD1^{G93A}$ and $SOD1^{G37A}$ mice [17,18] and in $SOD1^{G93A}$ rats [19,20], which is not evident in several other *mSOD1* mouse models [21]. A mouse model with low SOD1^{G93A} expression does not have as many vacuoles as those observed in high SOD1^{G93A} expressing models [18], suggesting that vacuole formation may be the result of high levels of mSOD1 expression. Indeed, $SOD1^{G93A}$ mice have 40-fold higher levels of mSOD1 protein relative to the endogenous SOD1 levels, while $SOD1^{G85R}$, $SOD1^{D90A}$, $SOD1^{G93A}$ and $SOD1^{G127X}$ models have about 20-fold higher mSOD1 levels compared with endogenous SOD1 levels [20]. The disease onset in mice having low mSOD1 expression is delayed as compared to the $SOD1^{G93A}$ mice [20]. Currently, many potential therapeutic agents to treat ALS are tested initially on the $SOD1^{G93A}$ mouse models. Despite the disagreements in therapeutic outcome between animal tests and human clinical trials [22–24], the combination of information obtained by use of *mSOD1* has provided valuable insight into the disease process.

2.3 *In vitro* models of motor neuron degeneration in ALS

The pathogenesis of ALS may involve many complex pathways in different cell types. Simpler *in vitro* systems have been developed to complement animal studies. Cultures of motor neurons are important for studying molecular mechanisms of neurodegeneration or to identify triggers that induce motor neuron death. The advantage of *in vitro* investigations is that specific cell types can be studied under controlled and well-defined conditions. It is now possible to maintain primary mouse motor neurons [25], or even human motor neurons [26] in culture. More often, however, immortalized mouse motor neuron cell lines, like the NSC34 cell line [27], are used as *in vitro* models to study ALS. For example, P62, also called sequestosome 1, a polyubiquitin-binding protein has been implicated in the progressive accumulation of protein aggregates in the $SOD1^{G93A}$ mouse spinal cord. When P62 was co-expressed with SOD1 in NSC34 cells, it greatly enhanced the formation of aggregates with mSOD1, but not with wild-

type (wt) SOD1 [28]. In addition, glutathione levels were found reduced in $SOD1^{G93A}$ mouse spinal cord motor neurons, which was correlated with the activation of multiple apoptotic pathways; this observation was later confirmed in NSC34 cells with glutathione depletion [29]. There are additional models developed with cultured motor neurons or motor neuron-like cells expressing mSOD1. The relevance of these models to ALS is debatable, because the expression of mSOD1 solely in neurons does not lead to ALS-like disease [30,31]. Expression of mSOD1 in other cell types including astrocytes or microglia may be necessary to trigger ALS symptoms [32]. Additional evidence obtained by crossing different transgenic mice further supports this notion, as healthy motor neurons undergo degeneration when surrounded by mSOD1-expressing non-neuron cells and mSOD1-expressing motor neurons survive if surrounded by healthy non-neuron cells [33].

3 Application of proteomics technologies to studies on ALS

The exact mechanism by which *mSOD1* and other related gene products induce ALS is not well understood. Non-biased high-throughput discovery methods including genomics, proteomics and metabolomics have been utilized to uncover the mechanisms linking ALS gene mutations to disease development. To critically evaluate the existing proteomics studies on ALS, a broader understanding of both the strengths and limitations of the state-of-the-art technologies is essential. Proteomics is a technology-driven field that is rapidly evolving. Major affiliated approaches include MS, LC, 2-DE and bioinformatics. When used within the framework of proper experimental designs, proteomic technologies can be very powerful at providing clues or answers to many crucial biological questions, including changes in protein expression, PTM, protein-protein interactions and protein activities. Traditionally, alterations in protein expression have been analyzed primarily by 2-DE-based methods. More recently, LC/MS-based protein quantification methods have gained increasing attention due to their ability to quantify thousands of proteins, compared to hundreds using 2-DE. A comprehensive review of proteomics technologies is beyond the scope of this review, but interested readers would be able to obtain more in-depth information about these technologies from several excellent reviews [34,35]. In 2-DE, intact proteins are usually analyzed according to their charge and size. A change in protein spot intensity correlates with the change of this particular "version" of the protein. Since many proteins are present in a typical gel as several 2-DE spots, due to PTM, proteolysis and differential mRNA splicing, the increase of one version of a protein does not automatically indicate increase in overall protein expression or activities. Downstream biochemical analyses are usually required to verify that protein changes are indeed "functional".

For LC/MS-based quantitative analysis, the most common workflow involves (i) trypsin-digestion, which converts all proteins into peptides; (ii) identification of peptides and quantification of their relative levels in MS and (iii) bioinformatics compilation of peptide information into protein expression changes. Since functionally related proteins can often share homologous sequence domains, it is likely that the relative quantities of selected peptides are the sum of the same peptide derived from the proteolytic digestions of many different, yet homologous proteins. Therefore, when changes in protein expression are quantified on the basis of a small number of peptides, the rate of false positives is likely to be higher than that obtained from the analysis based on a large number of peptides. Moreover, the number of peptides identified is determined by protein length, abundance, and instrument sensitivity. However, due to the wide dynamic range in proteins abundance, low-abundant proteins, including important signaling proteins and transcriptional regulators are usually represented by only one or two peptides and may not be detected at all. Thus, to identify significant changes in expression of low abundant proteins, it is crucial to enrich such proteins and perform sufficient experimental repeats.

4 Proteomic changes in models of ALS

Large-scale proteomics studies on ALS models are relatively scarce. Investigations have mostly focused on proteins obtained from rodent tissues and cells and the reported changes in the proteome are likely to involve relatively abundant proteins. In terms of experimental design, many investigations focus on pre-symptomatic stages, aiming to discover mechanisms of disease onset rather than pathways of neurodegeneration. Since most of the proteomics studies described here are based on $SOD1^{G93A}$-transgenic rodent model, we will analyze their significance in terms of the identification of possible targets mediating mSOD1 cytotoxicity, which are currently unknown. The effects of mSOD1 on its downstream targets can be manifested by changes in protein expression, protein-protein interaction and PTM. The changes in protein expression in NSC34^{G93A} cells, a culture-based ALS model, were more extensive than those observed in the spinal cords of rodents with ALS-like diseases (Table 1), presumably due to mitochondrial sub-proteome enrichment and differences between the systems. A summary of changes in protein PTM is presented in Table 2. Furthermore, information on known protein binding partners to either wt SOD1 or various forms of mSOD1 is presented in Table 3. In this review, we will discuss proteomic changes in ALS models from a global point of view, in order to find patterns among candidate proteins involved in mSOD1 cytotoxicity in motor neurons. We will focus our discussion primarily on protein chaperone function and related metabolic dysfunction and oxidative signaling events, because both protein-folding deficiency and oxidative stress have long been observed in ALS patients and in animal models.

4.1 Protein folding and degradation

One of the most prominent features of proteomic changes in the spinal cord of animals affected by ALS-like diseases relates to the alteration of protein chaperones regulating protein folding and degradation pathways. Chaperones play important roles in facilitating the folding of proteins into their native conformations, preventing the formation of protein aggregates and promoting the degradation of misfolded proteins. Chaperone dysfunctions can result in the accumulation of misfolded protein aggregates. Indeed, cytosolic protein aggregates appear to accumulate in ALS affected motor neurons and some are even associated with mitochondria [36]. Most protein aggregates in *mSOD1* mice contain mSOD1 while non-SOD1-containing aggregates have also been observed in the spinal cord of human sALS patients [3].

4.1.1 Chaperone expression—Given the fact that many SOD1 mutants are unfolded and tend to form aggregates in motor neurons, proteomic changes in chaperones that regulate protein folding can be anticipated. What may be significant, however, is the selectivity of a decrease in specific classes of chaperones, including chaperonin subunits 5, 6Aζ, Hsp70, Hsp40 and the increase in Hsp25, Hsp27, protein disulfide isomerase (PDI), cycliphilin A and ERP57 (Table 1). Hsp are a family of proteins with diverse expression patterns and functions. Different Hsp subfamilies appear to perform specific chaperone function on select proteins. Mechanisms governing the specificities between Hsp and their cognate targets are not well understood. Therefore, there may be specific dysregulation of a sub-group of chaperones by mSOD1 in ALS animal models.

4.1.2 Chaperone interactions—In addition to altered Hsp expression, protein chaperone dysregulation in ALS animal models is also manifested in terms of aberrations in protein-protein interactions. Hsp70, its co-chaperone, carboxyl terminus of Hsc70-interacting protein (CHIP), as well as chaperones Hsp105, Hsp40, Hsp27, Hsp25 and αB-crystalline, have all been shown to form complexes with SOD1^{G93A} and several other SOD1 mutants implicated in ALS but not with wt SOD1 (Table 3). By comparison, PDI binds to both wt SOD1 and SOD1^{G93A}, and it has been found in mSOD1-containing aggregates in the spinal cord in later

phases of the disease [37]. Selective binding and protein complex formation among mSOD1 and a specific Hsp may represent a second mechanism for mediating mSOD1 cytotoxicity.

4.1.3 Chaperone PTM—Functional changes in αB-crystalline, Hsp 70, Hsc71, Hsp4, disulfide-associated protein 3 and stress-induced phosphoprotein 1 may be important for ALS development; they are found oxidized *via* either carbonylation, nitration or 4-hydroxy-2-nonenal (HNE) modification in SOD^{G93A} mouse spinal cords (Table 2). Oxidative modifications could result in changes of their chaperone function. Therefore, they serve as a third mechanism for transducing mSOD1 toxicity in motor neurons. Recently, oxidative PTM of specific amino acids in key proteins has been shown under certain conditions to serve as a signal-transduction mechanism [3]. For example, nitrosylation of catalytic cysteine residues in PDI has been shown to inhibit its protein disulfide isomerase function in SH-SY5Y neuroblastoma cells and links protein misfolding to neurodegeneration [38]. Since there are extensive observations of abnormal protein-oxidation events in ALS models, in-depth examination of changes in redox signal-transduction events, especially the identification of sites of oxidative modifications among protein chaperones may provide novel insights into ALS pathogenesis.

4.1.4 Hsp70 and its co-chaperones for protein folding—Given the array of proteomic changes observed among protein chaperones, it is reasonable to hypothesize that the accumulation of mSOD1-containing protein aggregates in motor neurons may be the result of specific Hsp/mSOD1 interactions and the consequent dysfunction of protein chaperones. Hsp70 family of proteins are molecular chaperones that can facilitate proper protein folding, trafficking, and prevent the formation of protein aggregates. Based on proteomics results, the dysfunction in ALS motor neurons could be centered on Hsp70, which plays important chaperone function in an ATP-dependent manner. Through a network of co-chaperones, Hsp70 can recruit and bind to specific target proteins, including many forms of mSOD1, but not wt SOD1 (Table 3). Hsp70 machinery relies on co-chaperones including Hsp40 for selectively recruiting target proteins to bind to Hsp70. Hsp40, a member of DnaJ family of proteins, can stimulate ATP hydrolysis on Hsp70 and promote ADP/ATP nucleotide exchange. Hsp40 binds to Hsp70 *via* its J domain and stabilizes Hsp70 binding to its substrates. The determinants that govern the specificity between Hsp70 and its targets have not been established. However, both Hsp70 and Hsp40 bind specifically with mSOD1, but not wt SOD1; and both are down-regulated in ALS models (Table 1), suggesting they may be targets for mSOD1 cytotoxicity. Their recruitment by mSOD1 renders them unavailable for proper protein folding of newly synthesized mSOD1 and other proteins within motor neurons, resulting in the buildup of protein aggregates. The ATP-dependent substrate folding cycles of Hsp70 chaperone function can also be coupled with other chaperones that include Hsp110 family members for solubilizing unfolded protein aggregates, including mSOD1 for proper refolding [39]. Hsp105 is down-regulated in $SOD1^{G93A}$ model [39], and this chaperone has been demonstrated to suppress mSOD1^{G93A} aggregation, even in the absence of ATP. Given the explicit protein-protein interactions among mSDO1, Hsp70, Hsp40 and Hsp105 (Table 3), it is likely that the neuroprotective function of Hsp70 machinery is inhibited by mSOD1 accumulation. Consequently, any dysregulation among Hsp70 and its accessory chaperone factors could result in poor protein folding quality control and clearance within motor neurons.

4.1.5 Hsp70-mediated protein degradation—Hsp70 is also involved in delivering misfolded proteins to proteasome for degradation, thus reducing the potential accumulation of protein aggregates. For example, CHIP, another Hsp70 co-chaperone, has been shown to promote proteasome-mediated degradation of mSOD1 by facilitating the ubiquitination of Hsp70-bound proteins [40]. It has a U-box motif in its C terminus and may function as an Hsp70 chaperone-dependent E3 ubiquitin ligase for ubiquitination of unfolded proteins. CHIP

can selectively bind to SOD1[G93A], thus resembling similar binding selectivity between mSOD1 and other Hsp70 chaperone/co-chaperone systems. Ubiquitin/proteasome system (UPS) is important for the degradation of cellular proteins. Proteomic analyses have revealed additional dysfunction within the UPS and related protein degradation system. Increased expression of NEDD8 conjugating E2 enzyme Ubc12 and increased carbonylation of ubiquitin carboxyl-terminal hydrolase isozyme L1 in rodent *SOD1*[G93A] spinal cords has been demonstrated (Tables 1 and 2). In addition, dorfin, a novel centrosomal ring-finger protein known to possess ubiquitin E3 ligase activity forms a complex with mSOD1, but not with wt SOD1. Interestingly, dorfin forms a complex with CHIP and rescue neuronal cell function after mSOD1 aggresome formation [41,42]. Similarly, neuronal homologous to E6AP carboxyl terminus type ubiquitin-protein isopeptide ligase (NEDL1) binds only to ubiquitinated SOD1 mutants but not to wt SOD1 [43]. Further studies are needed to dissect out the precise relationships among all of these UPS components in the context of motor neuron dysfunction.

Overall, it is likely that within motor neurons, there is a finite supply of active Hsp70 and its affiliated co-chaperones. At later stage of ALS, the chaperone capacity may be reaching saturation due to increased unfolded mSOD1, oxidative down-regulation of chaperone activities and reduced ATP output from cellular metabolism. Consequently, Hsp70 function declines, affecting high-fidelity protein folding and targeting. In addition, its ability to guide misfolded proteins, including mSOD1 towards proteasome for degradation may also be compromised. *In vitro* studies have demonstrated that elevated Hsp70 expression can reduce mSOD1 aggregate formation and related neurotoxicity. In contrast, i*n vivo* investigations have yielded contradictory results. Liu *et al.* [44] concluded that elevation of Hsp70 is not sufficient to ameliorate mSODS1-mediated toxicity, while Kiaei *et al.* [45] showed that celastrol, a natural product extract capable of inducing Hsp70, could extend lifespan of *SOD1*[G93A] mice. The different outcome from Hsp70 activation could be due to the difference in the availability of the essential co-chaperones including CHIP, Hsp40 and other accessory factors needed for Hsp70 to carry out its function.

4.2 Metabolic function and redox regulation

Within motor neurons, there is high energetic demand for conducting cellular function. With mSOD1 overexpression in ALS models, the demand for ATP could be higher due to increased demand for Hsp70 and related protein chaperone functions.

4.2.1 Reduced energy output—Accumulation of mSOD1 protein aggregates has a significant impact on cellular metabolic function and energy production. Many protein levels are decreased in *SOD1*[G93A] mice, including those involved in metabolism, electron transport, ATP synthesis and mitochondrial membrane transport. Interestingly, some proteins have many isoforms, presumably due to PTM, and the expression of some PTM isoforms is increased while of others is decreased (*e.*g. aconitase 2 and pyruvate dehydrogenase, Table 1). It is therefore important to determine whether the changes in protein function and the identity of specific PTM sites are relevant to ALS pathogenesis. In ALS models, proteins involved in glycolysis, tricarboxylic acid (TCA) cycle and related energy metabolism show a pattern of down-regulation, suggesting a reduction in energy production available for the maintenance of normal motor neuron function. Both α-enolase and lactate dehydrogenase B have been shown to undergo oxidative modification *via* nitration and HNE-mediated lipid oxidation (Table 2). TCA-cycle proteins dihydrolipoamide S-succinyl transferase and isocitrate dehydrogenase 3β subunit are decreased in *SOD1*[G93A] models (Table 1). ATP synthesis also appears to be reduced in ALS models. Within the electron transport system complex I, NADH dehydrogenase (ubiquinone) 1β is decreased while NADH dehydrogenase (ubiquinone) Fe-S protein 8 is increased in NSC34 cell mitochondria (Table 1). Proteins in complex III (ubiquinol-cytochrome C reductase, Rieske iron sulfur polypeptide 1), complex IV (cytochrome c oxidase

subunit V a) and complex V (ATP synthase α and β) are also diminished (Table 1). The impact of likely metabolic down-regulation on ALS development in *mSOD1* mice is significant; lowered energy production and ATP output is likely to affect both normal motor neuron function and dampen Hsp70-related stress response, including, but not limited to protein folding and degradation. The consequence of reduced ATP levels in ALS has not been widely studied. Given the importance of ATP in essential cellular processes, including Hsp70-mediated protein folding and UPS, it is crucial to examine the significance of ATP reduction on ALS pathogenesis. A recent study by Browne *et al*. [46] has suggested that bio-energetic deficits are involved in the early stages of mSOD1-induced toxicity in *SOD1*^{G93A} mice. Another effect of metabolic dysfunction and mitochondrial impairment is likely to be the increase of oxidative stress.

4.2.2 Increased oxidative stress—Although it is well established that reactive oxygen/nitrogen species (ROS/RNS) are produced when energy metabolism pathways are affected, it remains unclear how mSOD1 specifically initiates the cascade of ROS/RNS-mediated damage in motor neurons but not in other cell types at certain developmental stages. Many proteins undergoing specific oxidative modifications have been discovered in several proteomics analyses (Table 2). The modifications include disulfide-bond formation, carbonyl oxidation, nitration, nitrosylation and HNE modification. In addition to the numerous protein chaperones discussed earlier, SOD1 is oxidized *via* carbonylation in *SOD1*^{G93A} mice. Similar SOD1 oxidation has also been reported in human tissues obtained from patients with Alzheimer's and Parkinson's diseases [47]. Although not yet directly discovered in ALS animal models, a variety of oxidative PTM events have been described for wt SOD1 (Table 2), suggesting that this molecule is very sensitive to redox modification and possibly functional regulation. In addition to carbonylation, other oxidative modifications of SOD1 have been shown to occur on cys-146 (to cysteic acid), cys-111 (to persulfide), his-46, 48, 80, 118 and 120 (to oxohistidine), trp-32 (oxidation or nitration), phe-20 (oxidation), val-116, 117 (oxidation), pro-60 (oxidation) and disulfide formation (Table 2). Although many of these modifications are induced *in vitro* (by oxidizing reagents, *e.g.* H_2O_2), increased oxidation of wt SOD1 has been demonstrated to trigger ALS-like symptoms in animal models [48].

Given the many changes observed in mitochondrial proteins, especially membrane proteins in *SOD1*^{G93A} models, including translocase of inner mitochondrial membrane 50 homolog, mitofilin, VDAC1 and VDAC2 (Table 1), it would be crucial to determine whether mitochondrial membrane integrity within motor neurons are compromised directly by mSOD1. Disruption of mitochondrial membrane structure is known to trigger oxidative stress and apoptosis.

4.2.3 Reduced antioxidant function—Normally, cellular antioxidant proteins, including SOD1 and mitochondrial manganese superoxide dismutase (SOD2) can counteract low levels of oxidative stress. Although SOD function for converting superoxide radicals to hydrogen peroxide does not seem to be affected in most *mSOD1* models, it is well known that increased oxidative stress is one of the hallmarks of ALS, as demonstrated by increased protein and lipid oxidation [3]. In addition, changes in cellular redox response in ALS models have been revealed from these proteomics studies. Alterations in peroxiredoxin 1, 3 and 6, the enzymes responsible for reducing hydrogen peroxide levels, and reduction in several isoforms of GST mu1, 2 and 5 and Pi B have been demonstrated in ALS rodent models, suggesting that cellular antioxidant response is compromised (Table 1). Furthermore, SOD2 undergoes nitration in ALS models, raising the possibility of increased mitochondrial superoxide radicals levels. Overall, it appears that the cellular antioxidant defense mechanisms are compromised in ALS models. However, ROS/RNS increase is not necessarily universally "bad" in terms of inhibition of normal protein function. At low levels, ROS/RNS are known to behave as signal transduction molecules that can modulate protein function including protein chaperones through oxidative PTM of specific

proteins [3]. When ROS/NOS levels elevate beyond the capacities of antioxidant proteins, oxidative stress may then disrupt cellular functions. It is therefore important to identify the specific oxidative PTM sites within Hsp70 and its co-chaperones that are known to be oxidized in ALS models and determine the role of such modifications on their chaperone functions.

4.3 Other proteomic changes

4.3.1 Cellular structure and trafficking—Abnormalities in axonal structure and trafficking have long been implicated in ALS [10]. Proteomics analysis has provided additional candidates involved in this process. These new participants include peripherin, septin 5, similar to septin 6 type II, γ-actin, tubulin α2 and β5, Rho GDP dissociation inhibitor 1 (GDI), chromogranin A and B and several 14-3-3 isoforms (Tables 1–4). These proteins can be broadly classified into two categories: structural proteins and proteins regulating trafficking. An ALS gene product involved in trafficking, *alsin* mutant is known to cause juvenile onset ALS [5]. A long form of alsin (1–1657 amino acids), but not the short form (1–396 amino acids) has been found to bind to several mSOD1 but not to wt SOD1 and inhibit the toxic function of mSOD1 (Table 3). Selective mSOD1 binding property was also reported for dynein complex, which contains dynactin, a protein known to be the underlying cause of a small number of fALS cases [6]. The proteomics studies have therefore provided direct evidence on how various ALS gene products may serve as the targets for mSOD1 cytotoxicity.

As an unbiased discovery method, proteomics have also revealed novel participants in ALS pathogenesis. Chromogranins are calcium-binding proteins, and are precursors to the synthesis of neuropeptide-like molecules important for protein sorting in the secretory pathways. One peptide product derived from chromogranin, 7B2, has been recently found to specifically bind several versions of mSOD1 but not wt SOD1 in astrocytes [49], and facilitate mSOD1 secretion from glia and subsequent targeting of motor neurons death [49]. This discovery may open a new direction for ALS research beyond motor neurons.

4.3.2 Cell death signal transduction—A novel protein antibody array screening technique was utilized to reveal changes in a number of kinases and phosphatases in $SOD1^{G93A}$ mouse spinal cord [50]. Overall, there appear to be a delicate balance between pro- and anti-apoptotic events. Interestingly, both SAPK(beta) and P38 were activated while JNK was down-regulated, suggesting that a specific stress signal transduction pathway is induced to trigger motor neuron death (Tables 1 and 2). In agreement with these results, inhibition of P38 MAPK by SB203580 prevented mSOD1-mediated motor neuron death [51]. In addition to the activation of cell death pathways, signal-transduction events for blockade of apoptosis have been mobilized to counter proapoptotic signals. Raf1, MEK ½ and ERK ½ signal-transduction pathways have been found to be activated (Tables 1 and 2), possibly for rescuing motor neuron from apoptosis.

4.3.3 Protein-nucleic acid interactions in motor neuron diseases—Recently, an RNA hypothesis for motor neuron disease development has emerged that may be useful for explaining some aspects of ALS pathogenesis. Observation of neurofilament (NF) aggregation in ALS affected motor neurons suggests a pathogenic role for NF in ALS [52]. In addition, the expression of an NF light chain (NF-L) mRNA mutant containing *c-myc* insertion in the 3'-untranslated region (UTR) was found to have neuropathic effects on motor neurons function [53]. It was later proposed that proteins that normally bind to NF-L mRNA might possess biological functions that are regulated by NF-L mRNA. Several NF-L 3'-UTR-binding proteins have been identified and proposed as the candidate pathogenic factors for ALS (Table 4). They include p190 Rho GEF [54], which was later reported to also bind to BC1 RNA, an important regulatory short RNA, abundant in rodent neurons [55]; aldolases A and C [56], which compete with poly(A)-binding protein to bind NF-L mRNA [57]; and 14-3-3 proteins, a family of

conserved regulatory proteins that regulates phosphoprotein function [58]. Recently, TAR DNA-binding protein (TDP43), a highly conserved heterogeneous nuclear ribonucleoprotein, was observed to bind to and stabilize NF-L mRNA [59]; ubiquitinated TDP43 has been shown to be present in ALS inclusions [60]. Interestingly, wt *SOD1* mRNA 3'-UTR forms specific riboucleoprotein complex exclusively with neuronal tissue extracts [61]. Selected *mSOD1* mRNA bearing ALS-associated mutations cannot form such complexes [61]. This *SOD1* mRNA riboucleoprotein complex appears to be essential for maintaining neuronal cell function [61].

5 Correlation of ALS model with the human disease

Since most proteomics studies have been conducted on the animal or *in vitro* models, it is crucial that the observed proteomic changes are relevant to the human disease. Unfortunately, there are limited number of proteomics investigations on human tissues and cerebral spinal fluid of ALS patients (Table 5). A recent proteomics study on the spinal cord of ALS patients, reported select proteins that are found only in ALS [62]. Since no quantitative comparison between control and ALS proteome was offered, interpretation of such proteomics changes here is only tentative at best. It is important to emphasize that the changes in protein expression observed in the human disease and animal models do not always correlate. For example, increased transthyretin and a fragment of 7B2 along with decreased cystatin C and a fragment of VGF have been reported in the cerebrospinal fluid (CSF) of ALS patients, none of which has been found changed in the spinal cord of animal models (Table 5). Interestingly, the decrease in cystatin C in CSF has been independently reported by two different groups [63, 64], suggesting the possibility of using these proteins as ALS biomarkers. Cystatin C has been also reported to be altered in multiple sclerosis patients CSF and suggested as specific biomarker for multiple sclerosis [65]. However, later it turned out to be an artifact of sample conservation [66,67]. Therefore, the use of these proteins as biomarkers needs to be taken with some skepticism. There are a few commonalties between studies on human and animal or cell models. Increase of enolase expression is found in both ALS patient and *SOD^{G93A}* mouse spinal cords [62,68]. As mentioned earlier, 7B2 has been shown to be important for the secretion of mSOD1 in astrocytes [49]. The increase in 7B2 found in human CSF may indicate the accumulation of extracellular 7B2. Another common feature between human and mouse studies is the down-regulation of antioxidant proteins. Peroxiredoxin 2 and mitochondrial thioredoxin are found decreased in spinal cord of ALS patients (Table 5), while peroxiredoxin 3 and many GST isoforms are lower in the animal and cell culture models (Table 1). These results indicate that depleted cellular antioxidant function may play a role in ALS development.

6 Future directions

Proteomics studies in animal models indicate that changes in protein chaperones, cellular metabolic function and oxidative response may participate in the pathogenesis of ALS. It is crucial to confirm these proteomic alterations in specimens obtained from ALS patients. So far, large-scale human investigations are rare, mainly due to the lack of easy access to high-quality samples. This problem is unlikely to be resolved soon. However, it is still possible to confirm select proteomic changes through analysis of post-mortem tissues. Recent development of advanced proteomics techniques enabled quantitative investigations on proteomic changes in formalin-fixed and paraffin-embedded tissues [69]. Even studies with the animal models require improvements. The pathogenesis of ALS may involve multicellular events [1] and dysfunctions of different neuronal subtypes may result in variations of the diseases that are collectively called ALS [36]. Given this diversity, it is essential to design proteomics investigations in a cell and phenotype-specific manner, whenever possible. The cells involved may include but are not limited to neurons, astrocytes, microglia cells and even myocytes [6]. It has been reported that expression of mSOD1 in astrocytes, but not in motor

neurons is detrimental to the development of ALS [1]. More recently, mSOD1 or other unidentified soluble factor(s) secreted from the astrocytes of *mSOD1*-transgenic mice have been demonstrated to specifically cause motor neuron death [49]. Depending on the questions asked, isolation of specific cellular organelles and groups of proteins (*e.g.* phosphoproteins, secreted proteins) prior to quantitative proteomics analysis may be necessary.

7 Conclusions

Proteomics studies on ALS models have expanded our knowledge of this disease. One of the many consequences of mSOD1 expression is the accumulation of misfolded proteins. This is likely an indication of the inactivation of specific chaperones, including Hsp70, its co-chaperones CHIP, Hsp40, Hsp105 and others. Further investigations are necessary to determine whether and how this selective group of chaperones is more sensitive to mSOD1-mediated cytotoxicity in motor neurons. There is growing evidence indicating that up-regulation of Hsp70 and related chaperone system may offer cytoprotective function in other neurodegenerative diseases including, Alzheimer's, Huntington's and Parkinson's disorders [70]. In addition, since chaperones and their co-chaperones regulate the passage of unfolded proteins to UPS for degradation, combined Hsp70 and UPS dysfunction may contribute to the buildup of unfolded and ubiquitinated protein inclusions. Future studies focusing on cell-type and organelle-specific proteomic changes may reveal a more precise relationship among different pathways. Both confirmatory and exploratory proteomics studies with human samples will likely provide more relevant information for therapeutic strategies to treat ALS.

Acknowledgments

Abbreviations

ALS
> amyotrophic lateral sclerosis

CHIP
> carboxyl terminus of Hsc70-interacting protein

CSF
> cerebrospinal fluid

fALS
> familial (or inherited) form of ALS

sALS
> sporadic ALS

UPS
> ubiquitin/proteasome system

wt
> wild type

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Table 1

Reported protein expression changes in *SOD1*[*G93A*] model

Protein name	Accession[a]	System	Change	Ref.
α Enolase	13637776	Mouse spinal cord	+	[71]
Aconitase 2	18079339	NSC34 mitochondria	+	[68]
Aldolase 1, A isoform	6671539	NSC34 mitochondria	+	[68]
Apolipoprotein E	6753102	Mouse spinal cord	+	[72]
Arginosuccinate synthase	114290	NSC34 cells	+	[73]
Calmodulin-dependent kinase 4 p64	50367	Mouse spinal cord	+	[50]
Casein kinase 1 delta p39	123289512	Mouse spinal cord	+	[50]
Cyclophilin A	118105	Mouse spinal cord	+	[71,73]
Electron transfer flavoprotein α subunit	21759113	Mouse spinal cord	+	[71]
Enoyl-co a hydratase	62900285	Mouse spinal cord	+	[71]
Erp57	62868455	Rodent spinal cord	+	[37]
Extracellular regulated kinase 1 p42	52001483	Mouse spinal cord	+	[50]
Extracellular regulated kinase 3 p56	52001482	Mouse spinal cord	+	[50]
Ferritin heavy chain	120517	Mouse spinal cord	+	[71,72]
Heat shock 27 kDa protein	547679	Mouse spinal cord	+	[72]
Heat shock protein 25 kDa	20342499	Mouse spinal cord	+	[72]
NADH dehydrogenase (ubiquinone) Fe-S protein 8	46195430	NSC34 mitochondria	+	[68]
NEDD8 conjugating enzyme Ubc12	46577656	Mouse spinal cord	+	[71]
P38 Hog MAP kinase p38	1346566	Mouse spinal cord	+	[50]
Peripherin	2253159	NSC34 mitochondria	+	[68]
Peroxiredoxin 1	547923	NSC34 cells	+	[73]
Peroxiredoxin 6	6671549	Mouse spinal cord	+	[72]
Phosphatidylinositol transfer protein alpha	21465804	Mouse spinal cord	+	[72]
Phosphoglycerate mutase 1	20178035	Mouse spinal cord	+	[71]
Protein disulfide isomerase	129729	Mouse spinal cord	+	[37,71]
Protein kinase C delta p80	45330876	Mouse spinal cord	+	[50]
Protein kinase C mu p124	6679351	Mouse spinal cord	+	[50]
Pyrroline-5-carboxylate reductase I	13879494	NSC34 mitochondria	+	[68]
Pyruvate dehydrogenase E1	548409	Mouse spinal cord	+	[71]
Raf1 p73	62024525	Mouse spinal cord	+	[50]
Retinal pigment	30691136	NSC34 mitochondria	+	[68]
Sepiapterin reductase	2498952	Mouse spinal cord	+	[71]
Septin 5	83305642	NSC34 mitochondria	+	[68]
Similar to septin6 type II	26324430	NSC34 mitochondria	+	[68]
Superoxide dismutase mutant (k136e, c6a, c111s)	5822065	Mouse spinal cord	+	[72]
Translocase of inner mitochondrial membrane 50	22094989	NSC34 mitochondria	+	[68]
γ Actin	809561	NSC34 mitochondria	−	[68]
Aconitase 2	18079339	NSC34 mitochondria	−	[68]
Adenylate kinase 2	34328230	NSC34 mitochondria	−	[68]
ATP synthase, mitochondrial F_1 complex, α subunit	6680748	NSC34 mitochondria	−	[68]

Protein name	Accession[a]	System	Change	Ref.
ATP synthase, mitochondrial F$_1$ complex, β subunit	25052136	NSC34 mitochondria	−	[68]
ATPase β chain	20455479	Mouse spinal cord	−	[71]
Calmodulin-dependent kinase 4 p62		Mouse spinal cord	−	[50]
Chaperonin subunit 5 (ε)	6671702	NSC34 mitochondria	−	[68]
Chaperonin subunit 6A(ζ)	6753324	NSC34 mitochondria	−	[68]
Coiled-coil-helix-coiled-coil-helix domain	21313618	NSC34 mitochondria	−	[68]
Cytochrome c oxidase, subunit Va	21707954	NSC34 mitochondria	−	[68]
Dihydrolipoamide S-succinyl transferase	21313536	NSC34 mitochondria	−	[68]
Dihydropteridine reductase	62510641	Mouse spinal cord	−	[71]
DnaJ (Hsp 40) homolog subfamily B member 11	110625998	NSC34 mitochondria	−	[68]
Extracellular regulated kinase 6 p47	3023716	Mouse spinal cord	−	[50]
Glutamic pyruvate transaminase 2	21707978	NSC34 mitochondria	−	[68]
Glutathione S-transferase Mu 1	121716	NSC34 cells	−	[73]
Glutathione S-transferase Mu 2	121718	NSC34 cells	−	[73]
Glutathione S-transferase Mu 5	1346207	NSC 34 cells	−	[73]
Glutathione S-transferase Pi B	121747	NSC34 cells	−	[73]
Glycogen synthase kinase 3 alpha p45	134034134	Mouse spinal cord	−	[50]
Heat shock 70-kda protein 5	31981722	NSC34 mitochondria	−	[68]
Iron regulatory protein 1 (IRP 1)	46577686	Mouse spinal cord	−	[71]
Isocitrate dehydrogenase 3, β subunit	18700024	NSC34 mitochondria	−	[68]
(JNK;SAPK) beta p40	30578161	Mouse spinal cord	−	[50]
Mitochondrial ATP-dependent protease Lon	26984237	NSC34 mitochondria	−	[68]
Mitochondrial inner membrane protein (Mitofilin)	29427692	NSC34 mitochondria	−	[68]
NADH dehydrogenase (Ubiquinone) 1β subcomplex	58037109	NSC34 mitochondria	−	[68]
NADP-dependent leukotriene b4	73621182	NSC34 cells	−	[73]
NPC derived proline rich protein 1 (NDPP-1)	220500	NSC34 mitochondria	−	[68]
Ornithine aminotransferase	8393866	NSC34 mitochondria	−	[68]
Peroxiredoxin 3	6680690	NSC34 mitochondria	−	[68]
Phosphatidylinositol 3′-phosphatase p54	6679523	Mouse spinal cord	−	[50]
Profilin 1	51702779	Mouse spinal cord	−	[71]
Proteasome subunit beta type 8 precursor	1172603	NSC34 cells	−	[73]
Protein phosphatase 1-C subunit-gamma p30	1280027	Mouse spinal cord	−	[50]
Protein phosphatase 5-C subunit-p48	2407637	Mouse spinal cord	−	[50]
Pyruvate dehydrogenase E1α 1	6679261	NSC34 mitochondria	−	[68]
S6 kinase p70 (S6K) p83	54036143	Mouse spinal cord	−	[50]
Serine (or cysteine) proteinase inhibitor, clade B	114158675	Mouse spinal cord	−	[71]
Serine hydroxymethyl transferase2	21312298	NSC34 mitochondria	−	[68]
Stathmin	14625464	Mouse spinal cord	−	[72]
Stomatin-like protein 2	12963591	NSC34 mitochondria	−	[68]
Ts translation elongation factor, mitochondrial	21313468	NSC34 mitochondria	−	[68]
Tubulin α 2 chain	55977764	NSC34 mitochondria	−	[68]

Protein name	Accession[a]	System	Change	Ref.
Tubulin β 5	7106439	NSC34 mitochondria	−	[68]
Tyrosyl-t RNA synthetase	15488844	NSC34 mitochondria	−	[68]
Ubiquinol-cytochrome c reductase	13385168	NSC34 mitochondria	−	[68]
Voltage-dependent anion channel 1 (VDAC1)	6755963	NSC34 mitochondria	−	[68]
Voltage-dependent anion channel 2 (VDAC2)	6755965	NSC34 mitochondria	−	[68]

[a]NCBI accession number.

Table 2

Increased PTM of selected proteins implicated in ALS

Proteins	Accession[a]	Modification	Induction	Ref
Transcriptionally controlled tumor protein 1	6678437	Carbonylation	$SOD1^{G93A}$	[74]
Ubiquitin carboxyl-terminal hydrolase isozyme L1	18203410	Carbonylation	$SOD1^{G93A}$	[74]
α B crystalline	6753530	Carbonylation	$SOD1^{G93A}$	[74]
Dihydropyrimidinase- related protein (DRP-2)	94730376	HNE[b]	$SOD1^{G93A}$	[75]
Hsp-70	124339826	HNE[b]	$SOD1^{G93A}$	[75]
α Enolase	12963491	HNE[b]	$SOD1^{G93A}$	[75]
Aconitase 2, mitochondrial	60391212	Nitration	$SOD1^{G93A}$	[76]
Actin	46397334	Nitration	$SOD1^{G93A}$	[76]
Adenylate kinase isoenzyme 1	13959400	Nitration	$SOD1^{G93A}$	[76]
ATP synthase subunit β, mitochondrial precursor	20455479	Nitration	$SOD1^{G93A}$	[76]
Carbonic anhydrase II	146345383	Nitration	$SOD1^{G93A}$	[76]
Creatine kinase, B chain	417208	Nitration	$SOD1^{G93A}$	[76]
Dihydropyrimidinase-related-2	94730376	Nitration	$SOD1^{G93A}$	[76]
Disulfide isomerase associated 3	112293264	Nitration	$SOD1^{G93A}$	[76]
α Enolase	13637776	Nitration	$SOD1^{G93A}$	[76]
Heat shock cognate 71 kDa	51702275	Nitration	$SOD1^{G93A}$	[76]
Heat shock protein 4	81903134	Nitration	$SOD1^{G93A}$	[76]
L-Lactate dehydrogenase B chain	126042	Nitration	$SOD1^{G93A}$	[76]
Phosphoglycerate mutase 1	20178035	Nitration	$SOD1^{G93A}$	[76]
Rho GDP dissociation inhibitor 1	21759130	Nitration	$SOD1^{G93A}$	[76]
Stress-induced phosphoprotein 1	54036445	Nitration	$SOD1^{G93A}$	[76]
Superoxide dismutase (Mn)	3041732	Nitration	$SOD1^{G93A}$	[76]
Adducin p120	66792810	Phosphorylation	$SOD1^{G93A}$	[77]
Adducin p80	31542111	Phosphorylation	$SOD1^{G93A}$	[77]
Glycogen synthase kinase 3 alpha p44	72384361	Phosphorylation	$SOD1^{G93A}$	[77]
Glycogen synthase kinase 3 beta p40	9790077	Phosphorylation	$SOD1^{G93A}$	[77]
Protein kinase C alpha/beta p83	6755078	Phosphorylation	$SOD1^{G93A}$	[77]
Protein kinase R p68	6755160	Phosphorylation	$SOD1^{G93A}$	[77]
S6 kinase p64	54036143	Phosphorylation	$SOD1^{G93A}$	[77]
Cu, Zn-superoxide dismutase	45597447	Carbonylation	$SOD1^{G93A}$	[74]
		Cys-146 to cysteic acid	Parkinson's Disease, Alzheimer's Disease	[47]
		Cys-111 to persulfide	Na_2S	[78]
		Trp-32 oxidation	H_2O_2/HCO_3^-	[79]
		Nitration and/or Trp-32oxidation	Peroxynitrite/NaHCO3	[80,81]
		SOD1 cross-linked via disulfide bonds	fALS mice	[82]

Proteins	Accession[a]	Modification	Induction	Ref
		Oxidation of His48, Phe-20	Ascorbic acid/CuCl$_2$	[4]
		Oxidation of His-80 and 120	Ascorbic acid/CuCl$_2$	[83]
		Oxidation of His118	H$_2$O$_2$	[84]
		Dioxygenized His-46, Oxidized Pro-60 & Val-116, Val-117	H$_2$O$_2$	[85]

[a]NCBI accession number.

[b]4-Hydroxy-2-nonenal.

Table 3

Proteins interacting with wild-type SOD1 and its mutants

Binding partner	Accession[a]	SOD1 genotype	Ref.
α B-crystalline	6753530	G93A, G41S	[86]
Alsin (long form consisting 1657 amino acids)	17505210	A4T, G85R, G93R	[5]
Alsin (short form consisting 396 amino acids)	17505210	WT, A4T, G85R, G93R	[5]
B-cell leukemia/lymphoma 2 (Bcl-2)	133892547	WT, G37R, G41D, G58R	[87]
Calcineurin	42415473	WT	[88]
Carboxyl terminus of Hsc70-interacting protein (CHIP)	4928066	G93A	[40]
Chromogranin A	6680932	A4V, G85R, G93A	[49]
Chromogranin B	6680934	A4V, G85R, G93A	[49]
Copper chaperone for superoxide dismutase (CCS)	8393066	WT, A4V, H46R, H48Q	[89,90]
Dorfin	7305437	G37R, H46R, G85R, G93A	[41]
Dynein complex	6753656	A4V, G85R, G93A	[91]
Heat-shock protein 105 (Hsp105)	114145505	G93A	[39]
Heat-shock protein 25 (Hsp25)	194012	G93A	[92]
Heat-shock protein 27 (Hsp27)	424145	G93A	[92]
Heat-shock protein 40 (Hsp40)	9055242	G93A, G41S	[86]
Heat-shock protein 70 (Hsp70)	124339826	G93A, G41S	[86]
Homeo box B2 (Hoxb2)	94158904	G93A, G86R, G37R, C6F, D90A, L126S	[93]
Lysyl-tRNA synthetase (KARS)	16716381	G85R, G93A	[94]
Neuronal homologous to E6AP carboxyl terminus (HECT)- type ubiquitin-protein isopeptide ligase (NEDL1)	126215717	C6F, A4V, E132Dstop, G93A, L106V, I149T, E100G, L84F	[43]
Protein disulfide isomerase (PDI)	129729	WT, G93A	[37]
Translocon-associated protein delta	6678145	G85R, G93A	[94]

[a]NCBI accession number.

Table 4

Proteins interacting with NF-L mRNA

Protein	Accession[a]	Ref.
14-3-3 eta	6756037	[58]
14-3-3 gamma	31543976	[58]
14-3-3 protein beta/alpha	31543974	[58]
14-3-3 protein sigma	134023662	[58]
14-3-3 protein zeta/delta	6756041	[58]
14-3-3 theta	6756039	[58]
Aldolase 1, A isoform	6671539	[56]
Aldolase 3, C isoform	60687506	[56]
Junction plakoglobin	28395018	[58]
Rho-guanine nucleotide exchange factor	123702010	[54]
TAR DNA binding protein (TDP93)	6678271	[59]

[a]NCBI accession number.

Table 5

Proteomic changes identified from ALS patients

Protein	Accession[a]	Sample	Change	Ref.
α Enolase	2023	Spinal cord	+	[62]
ALS2CR14 protein	65068	Spinal cord	+	[62]
Calmodulin-dependent kinase p52 (CaMKK p52)	14150045	Spinal cord	+	[77]
Calmodulin-dependent kinase p56 (CaMKKp56)		Spinal cord	+	[77]
Carbohydrate sulfo transferase 9	61211793	Spinal cord	+	[62]
Cholineacetyltransferase	74736657	Spinal cord	+	[62]
Cyclin-L2	74752124	Spinal cord	+	[62]
DnaJ homolog subfamily B member 14	74713900	Spinal cord	+	[62]
Extracellular regulated kinase 2 p37 (ERK2 p37)	6754632	Spinal cord	+	[77]
Extracellular regulated kinase 2 p39 (ERK2p39)		Spinal cord	+	[77]
Gap junction epsilon-1 protein	349149	Spinal cord	+	[62]
G-protein coupled receptor kinase2 p80 (GRK2 p80)	4501971	Spinal cord	+	[77]
Neuregulin1	9297048	Spinal cord	+	[62]
Neuroendocrine protein 7B2 precursor (Secretogranin-5)	23830842	CSF	+	[64]
Neuropeptide B precursor	33301344	Spinal cord	+	[62]
Protein kinase B alpha p62 (PKB alpha p62)	62241011	Spinal cord	+	[77]
Protein kinase C alpha p83 (PKC alpha p83)	4506067	Spinal cord	+	[77]
Protein kinase C zeta p83 (PKC zeta p83)	52486327	Spinal cord	+	[77]
Protein kinase G p70 (PKG p70)	10835242	Spinal cord	+	[77]
Protein kinase G p75 (PKG p75)		Spinal cord	+	[77]
Protein Tyrosine Phosphatase 1 delta p68 (PTP1 delta p68)	33356177	Spinal cord	+	[77]
Rap1 GTPase-activating protein 1	1350590	Spinal cord	+	[62]
Ribosomal S6 Kinase 1 p82 (RSK1 p82)	20149547	Spinal cord	+	[77]
Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 12	24211443	Spinal cord	−	[62]
Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyl-transferase	74713891	Spinal cord	−	[62]
Cyclin-dependent kinase (cdk) associated phosphatase p31	17981704	Spinal cord	−	[77]
Cystatin C	118183	CSF	−	[63,64]
Extracellular regulated kinase 1 p40 (ERK1 p40)	91718899	Spinal cord	−	[77]
Gamma-taxilin	74733483	Spinal cord	−	[62]
Myelin basic protein (MBP)	17378805	Spinal cord	−	[62]
Nuclear receptor coactivator 7	74713912	Spinal cord	−	[62]
Peroxiredoxin 2	2507169	Spinal cord	−	[62]
Small EDRK-rich factor1a	23396841	Spinal cord	−	[62]
Thioredoxin.mitochondrial precursor	20455529	Spinal cord	−	[62]
Transthyretin	136464	CSF	−	[64]
VGF nerve growth factor inducible	17136078	CSF	−	[63]

[a]NCBI accession number.