Original Contribution

Redox proteomics analysis of oxidatively modified proteins in G93A-SOD1 transgenic mice—A model of familial amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron degenerative disease characterized by the loss of neuronal function in the motor cortex, brain stem, and spinal cord. Familial ALS cases, accounting for 10–15% of all ALS disease, are caused by a gain-of-function mutation in Cu,Zn-superoxide dismutase (SOD1). Two hypotheses have been proposed to explain the toxic gain of function of mutant SOD (mSOD). One is that mSOD can directly promote reactive oxygen species and reactive nitrogen species generation, whereas the other hypothesis suggests that mSODs are prone to aggregation due to instability or association with other proteins. However, the hypotheses of oxidative stress and protein aggregation are not mutually exclusive. G93A-SOD1 transgenic mice show significantly increased protein carbonyl levels in their spinal cord from 2 to 4 months and eventually develop ALS-like motor neuron disease and die within 5–6 months. Here, we used a parallel proteomics approach to investigate the effect of the G93A-SOD1 mutation on protein oxidation in the spinal cord of G93A-SOD1 transgenic mice. Four proteins in the spinal cord of G93A-SOD1 transgenic mice have higher specific carbonyl levels compared to those of nontransgenic mice. These proteins are SOD1, translationally controlled tumor protein (TCTP), ubiquitin carboxyl-terminal hydrolase-L1 (UCH-L1), and, possibly, αB-crystallin. Because oxidative modification can lead to structural alteration and activity decline, our current study suggests that oxidative modification of UCH-L1, TCTP, SOD1, and possibly αB-crystallin may play an important role in the neurodegeneration of ALS.

Keywords: Redox proteomics; ALS; Mechanisms of neurodegeneration; Oxidatively modified proteins; Enzyme activity decline; Free radicals

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron degenerative disease characterized by the loss of neuronal function in the motor cortex, brain stem, and spinal cord. ALS typically presents in middle age and progresses rapidly. Life expectancy of victims of ALS usually is 3–5 years after diagnosis [1,2]. Inherited ALS accounts for 10–15% of cases, and among all of the familial ALS (FALS) patients, 20–30% of them are caused by a gain-of-function mutation in Cu,Zn-superoxide dismutase (SOD1) [3,4]. SOD1 catalyzes the disproportionation of superoxide anion radical to hydrogen peroxide and oxygen. Over 100
different missense substitutions in the 153-amino-acid SOD1 have been described in individuals and kindreds affected by SOD1-linked FALS [5]. One of the most common mutations of SOD1 is the substitution of glycine by alanine at residue 93 (G93A) [1].

Two principal hypotheses have been proposed to explain the toxic gain of function of mutant SOD (mSOD) [6]. One is that mSOD can directly promote reactive oxygen species and reactive nitrogen species generation [7–12]. Evidence supporting this hypothesis showed that mSODs enhance oxidative activity by acting as peroxidases [9,11,13] or superoxide reductases [14] or by producing O$_2^-$ to form peroxynitrite [15,16]. The second hypothesis is that mSODs are prone to aggregation due to their instability or association with other proteins [17–20]. These aggregates facilitate toxicity [21,22] and deplete the mSOD-associated proteins and thus perturb the normal functions of cells [6]. The proteinaceous inclusions found in tissues from ALS patients [23–25] and mSOD transgenic mice [22,26] are rich in mSOD, ubiquitin, and neurofilament proteins. It is noteworthy that the hypotheses of oxidative stress and protein aggregation are not mutually exclusive [27], although the roles of oxidative stress and aggregation in ALS are highly controversial (recently reviewed in [18,28,29]).

Increased oxidative modification of macromolecules was demonstrated in neuronal tissues of SOD1-related FALS patients and transgenic mice [30–32]. Enhanced susceptibility of exogenous oxidative stress in mSOD1 cell cultures was also observed in in vitro studies [33–35]. Exogenous oxidative stress can even inhibit the rapid degradation of mSOD [36]. These studies are consistent with the notion that oxidative stress plays an important role in ALS development. G93A-SOD1 transgenic mice show significantly increased protein carbonyl levels in their spinal cord from 2 to 4 months [32] and eventually develop motor neuron disease and die within 5–6 months [37]. G93A-SOD1 catalyzes the oxidation of a model substrate by H$_2$O$_2$ at a higher rate [11] and has a higher capacity to generate free radicals [9] compared to wild-type SOD1. Also, elevation of inflammation-related genes (e.g., induced nitric oxide synthase, proinflammatory cytokines) occurs at 11 weeks of age in the presymptomatic stage before motor neuron death in G93A-SOD1 transgenic mice, suggesting that neuroinflammation-mediated oxidative stress is also present in G93A-SOD1 mice [38]. Therefore, the expression of G93A-SOD1 is believed to elevate the generation of oxygen radicals in vulnerable tissue, such as spinal cord [39], creating oxidative stress that may be responsible for the ALS-like syndrome observed in the G93A-SOD1 mice. One of the oxidatively modified proteins in G93A-SOD1 transgenic mice is SOD1 [32], indicating that oxidation of SOD1 is likely important to the development of this model of ALS. However, other oxidatively modified proteins were not identified. Recent quantitative proteomic studies enabled the identification of oxidized brain proteins in Alzheimer disease (AD) patients and models thereof [40–45] and provided important insights into the role of protein oxidation in AD. In order to better understand the role of oxidative modification of proteins in ALS, we employed quantitative redox proteomic analysis to identify the specific oxidized proteins in the spinal cord of G93A-SOD1 mice.

**Methods**

**Animals**

Transgenic mice expressing the human SOD1 gene with a G93A mutation, strain B6SJL/TgN (SOD1-G93A)-2Gur) [37], were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained as hemizygotes by mating transgenic males with B6/SJLF1 females as previously described [46].

All studies of live animals were authorized and overseen by the Institutional Animal Care and Use Committee of the Oklahoma Medical Research Foundation and conducted by trained and certified technical and veterinary staff. Every effort was made to avoid unnecessary discomfort to the experimental animals.

**Sample preparation**

Whole frozen mouse spinal cords ($n = 6$) were homogenized in 10 mM sodium acetate buffer, pH 7.2, containing 0.1% Triton X-100 and mammalian protease inhibitor cocktail (Sigma Chemical Co., St. Louis, MO, USA).

**Two-dimensional (2D) gel electrophoresis**

Samples of spinal cord proteins were prepared according to the procedure previously described [47]. Briefly, 300 μg of protein was incubated with 4 vol of 2 N HCl at room temperature (25°C) for 20 min. Proteins were then precipitated by addition of ice-cold 100% trichloroacetic acid (TCA) to obtain a final concentration of 15% TCA. The samples were then mixed with 185 μl of hydration buffer (8 M urea, 20 mM dithiothreitol, 2.0% (w/v) Chaps, 0.2% Biolytes, 2 M thiourea, and bromophenol blue).

In the first-dimension electrophoresis, 200 μl of sample solution was applied to a ReadyStrip IPG strip (Bio-Rad). The strip was then actively rehydrated in a protein isoelectric focusing (IEF) cell (Bio-Rad) for 16 h at 50 V. The focused IEF strip was stored at −80°C until second-dimension electrophoresis was performed. For the second-dimension electrophoresis, thawed IEF strips, pH 3–10, were equilibrated for 10 min in 50 mM Tris–HCl (pH 6.8) containing 6 M urea, 1% (w/v) sodium dodecyl sulfate, 30% (v/v) glycerol, and 0.5% dithiothreitol and then reequilibrated for 15 min in the same buffer containing 4.5% iodoacetamide in place of dithiothreitol. Linear gradient (8–
16%) precast Criterion Tris–HCl gels (Bio-Rad) were used to perform second-dimension electrophoresis. Precision protein standards (Bio-Rad) were run along with the sample at 200 V for 65 min. The protein levels on the 2D gels were detected by Bio-Safe Coomassie blue (Bio-Rad).

**Western blotting**

As previously described [47], 300 μg of protein was incubated with 2,4-dinitrophenyl hydrazine. The 2D gels were prepared in the same manner as for 2D electrophoresis. The proteins from the 2D gels were then transferred to nitrocellulose paper (Bio-Rad) and detected immunochemically.

**Image analysis**

The gels and nitrocellulose blots were scanned and saved in TIFF format using a Scanjet 3300C (Hewlett-Packard). An Investigator HT analyzer (Genomic Solutions, Inc., Ann Arbor, MI, USA) was used for matching and analysis of visualized protein spots among differential gels and oxyblots. The average mode of background subtraction was used to normalize intensity values, which represents the amount of protein (total protein on gel and oxidized protein on oxyblot) per spot. After completion of spot matching, the normalized intensity of each protein spot from individual gels (or oxyblots) was compared between groups using ANOVA.

**Trypsin digestion**

Samples were prepared using the techniques described previously [47]. The selected protein spots were excised with a clean blade and transferred into clean microcentrifuge tubes. After being washed with ammonium bicarbonate (NH₄HCO₃) and acetonitrile, the protein spots were treated with dithiothreitol and iodoacetamide separately. Then the gel pieces were washed with NH₄HCO₃ and acetonitrile, the protein spots were treated with dithiothreitol and iodoacetamide separately. After rehydration with modified trypsin (Promega, Madison, WI, USA). The gel pieces were chopped into smaller pieces and incubated at 37°C overnight in a shaking incubator.

**Mass spectrometry**

Mass spectra reported in this study were acquired from both the University of Kentucky Mass Spectrometry Facility (UKMSF) and the Department of Pharmacology at the University of Louisville School of Medicine and VAMC. A Bruker Autoflex MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) mass spectrometer (Bruker Daltonics, Billerica, MA, USA) at the UKMSF or a TOF Spec 2E (Micromass, UK) MALDI-TOF mass spectrometer at the University of Louisville operated in the reflectron mode was used to generate peptide mass fingerprints. Peptides resulting from in-gel digestion with trypsin were analyzed on a 384-position, 600-μm AnchorChip Target (Bruker Daltonics) and prepared according to AnchorChip recommendations (AnchorChip Technology, rev. 2; Bruker Daltonics). Briefly, 1 µl of digestate was mixed with 1 µl of α-cyano-4-hydroxy-cinnamic acid (0.3 mg/ml in ethanol:acetic acid:2:1 ratio) directly on the target and allowed to dry at room temperature. The sample spot was washed with 1 µl of a 1% TFA solution for approximately 60 s. The TFA droplet was gently blown off the sample spot with compressed air. The resulting diffuse sample spot was recrystallized (refocused) using 1 µl of a solution of ethanol:acetonitrile:0.1% TFA (6:3:1 ratio). Reported spectra are a summation of 100 laser shots. External calibration of the mass axis was used for acquisition and internal calibration using either trypsin autolysis ions or matrix clusters was applied postacquisition for accurate mass determination.

The MALDI spectra used for protein identification from tryptic fragments were searched against the NCBI protein databases using the MASCOT search engine (http://www.matrixscience.com). Peptide mass fingerprinting used the assumption that peptides are monoisotopic, oxidized at methionine residues, and carbamidomethylated at cysteine residues [41–45]. Up to one missed trypsin cleavage was allowed. Mass tolerance of 150 ppm was the window of error allowed for matching the peptide mass values.

**Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) assay**

The activities of UCH-L1 in the spinal cord were measured by determining the rate of conversion of ubiquitin-C-terminal 7-amido-4-methylcoumarin (Ub-AMC) (Calbiochem) to ubiquitin and free AMC [48]. In this assay, 39 µl of buffer (50 mM Hepes, pH 7.0, 10 mM DTT, and 0.1 mg/ml ovalbumin) was mixed with 10 µl of 2 mg/ml spinal cord homogenate of six individual transgenic animals and six individual control animals for 1 h. Then 10 µl of 200 nM Ub-AMC was added to the enzyme solution, and cleavage of AMC from Ub-AMC was monitored at 460 nm over time. The average UCH-L1 activities of six transgenic animals were compared to those of six control animals using Student’s t test.

**Immunoprecipitation**

Immunoprecipitation was performed essentially as described previously [49]. A rabbit anti-αB-crystallin antibody (5 µl) (Chemicon) was added directly to the spinal cord homogenate. Antibody/lysat solutions were incubated on a rotary mixer overnight at 4°C. The αB-crystallin/antibody complexes were precipitated with protein A-conjugated agarose beads. Protein A beads were added in 50-µl aliquots from a stock of 300 mg/ml in PBS and mixed on a rotary mixer for 1 h at room temperature. Beads were
then centrifuged and 2D electrophoresis was performed on the supernatant.

**Results**

We used a parallel approach to investigate the effect of G93A-SOD1 on protein oxidation [40–45]. The specific carbonyl levels were obtained by dividing the carbonyl level of a protein spot on the nitrocellulose membrane by the protein level of its corresponding protein spot on the gel. Such numbers give the carbonyl level per unit of protein. We found that in comparison to nontransgenic mice, four proteins in the spinal cords of G93A-SOD1 transgenic mice have significantly higher specific carbonyl levels than those in nontransgenic littermate controls. These proteins were identified as SOD1, translationally controlled tumor protein (TCTP), UCH-L1, and αB-crystallin. The specific carbonyl levels of the proteins that are significantly different are summarized in Table 1. The summary of the mass spectrometry results for the proteins is presented in Table 2.

Fig. 1 shows representative 2D-electrophoresis gels of G93A-SOD1 transgenic mice (Fig. 1A) and nontransgenic mice (1B) after Coomassie blue staining. Fig. 2 shows the representative 2D Western blots of the spinal cord of a G93A-SOD1 transgenic mouse (Fig. 2A) and a nontransgenic mouse (2B). We report here that the specific carbonyl levels of human SOD1, TCTP, UCH-L1, and, possibly, αB-crystallin are significantly increased in the spinal cord of G93A-SOD1 transgenic mice compared to that of nontransgenic mice. Spots close to human SOD1 are modified SOD1, possibly phosphorylated SOD1. However, these modifications cannot be resolved by MALDI mass spectrometry.

Although our mass spectra do not significantly match the mass spectra of αB-crystallin, the molecular weight and pI value of αB-crystallin agree with the location of the gel spots on the 2D gel map. In order to ensure the identification of αB-crystallin, we identified the protein spot immunologically. The spot was absent in the 2D gels upon immunoprecipitation (Fig. 3). Therefore, the protein identification of αB-crystallin is ensured.

In order to confirm that oxidative modification inactivated protein activity, we compared the activity of UCH-L1 in the G93A-SOD1 transgenic mice to that in the control mice. Consistent with our prior studies that demonstrate loss of activity of oxidatively modified proteins [49–51], UCH-L1 activity was significantly decreased (29%) in the G93A-SOD1 transgenic mice compared to that of nontransgenic control (Fig. 4).

**Discussion**

The G93A-SOD1 transgenic mouse is frequently used as an animal model of human FALS due to the neuropathological similarity of this mouse model to the human disease [52,53]. Moreover, this mouse model can provide insight into the mechanisms of the neurotoxicity of mutant SOD in vivo. It is well established that mutant SOD1 enhances oxidative activity by acting as a peroxidase [9,11,13] or a superoxide reductase [14]. Protein carbonyl levels in the spinal cord of 3- to 4-month-old G93A-SOD1 transgenic mice show a 557% increase compared to nontransgenic animals at the same age [32]. Such protein oxidation in the spinal cord of G93A-SOD1 transgenic mice followed an alteration in cytokine expression [46]. In the current study, we identified the proteins that demonstrate increased carbonyl levels compared to those of the nontransgenic mice as SOD1, TCTP, UCH-L1, and αB-crystallin.

SOD1 previously was identified immunochemically as one of the oxidatively modified proteins in G93A-SOD1 transgenic mouse spinal cord [32]. Here, we used a parallel proteomics approach to confirm that the specific carbonyl level of SOD1 is increased in the spinal cords of G93A-SOD1 transgenic mice. Although G93A-SOD1 shows dismutation activity identical to that of wild-type SOD1, the activity of SOD1 in FALS patients with mutations is decreased 50% in motor cortex, parietal cortex, and cerebellum [54]. Moreover, free radical production in the G93A-SOD1 transgenic animals is induced by SOD1 mutation [32], alteration of tumor necrosis factor α (TNF-α), and TNF-α-modulating cytokines [38,46]. Although the issue of whether oxidative stress plays an early role in ALS remains unclear, our current study is consistent with the notion that oxidative modification of SOD1 plays a role in the neurotoxicity of mutant SOD1 in the disease.

Another oxidatively modified protein in G93A-SOD1 transgenic mice identified by proteomics was TCTP. TCTP processes calcium-binding activity (reviewed in [55]) and has a tubulin binding region [56]. Overexpression of TCTP stabilizes microtubules and alters cell morphology [57]. Other molecular interactions of TCTP include self-interaction [58] and the interaction with myeloid cell leu-

### Table 1

<table>
<thead>
<tr>
<th>Proteomics identified protein</th>
<th>Nontransgenic mice (AU ± SD)</th>
<th>G93A-SOD1 transgenic mice (AU ± SD)</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcriptionally controlled tumor protein 1</td>
<td>15.1 ± 10.1</td>
<td>30.6 ± 14.1</td>
<td>5.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cu,Zn-superoxide dismutase</td>
<td>2.88 ± 1.78</td>
<td>18.2</td>
<td>&lt;0.005</td>
<td></td>
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<tr>
<td>Ubiquitin carboxyl-terminal hydrolase isozyme L1</td>
<td>0.74 ± 0.23</td>
<td>2.31 ± 1.27</td>
<td>10.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>αB-crystallin</td>
<td>Not detected</td>
<td>20.1 ± 8.4</td>
<td>39.7</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

*Because specific carbonyl levels are not detected in nontransgenic mice, a value of 0 was used to calculate the p value.*

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kemia 1 protein [59]. TCTP levels are highly regulated in response to various stress conditions and extracellular signals, including growth signal [55], cytokines [60,61], starvation [55,62], heat shock, heavy metals, calcium stress [63], and proapoptotic/cytotoxic signals [64,65]. Along with the structural similarity to chaperones [66] and characterization as an antiapoptotic protein [67], these observations suggest that TCTP may exert a cytoprotective function for cells. The current study showed that TCTP was oxidatively modified in the spinal cord of G93A-SOD1 mice, suggesting that the putative cytoprotective function and the calcium binding affinity of TCTP are impaired in G93A-SOD1 mice.

<table>
<thead>
<tr>
<th>Identified protein</th>
<th>gi accession number</th>
<th>No. peptides matched</th>
<th>% coverage matched peptides</th>
<th>pI, M_r (kDa)</th>
<th>Mowse score</th>
<th>Probability of a random identification hit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcriptionally controlled tumor protein 1</td>
<td>gi</td>
<td>6678437</td>
<td>5</td>
<td>28</td>
<td>4.76, 19.5</td>
<td>79</td>
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<tr>
<td>Cu,Zn-superoxide dismutase</td>
<td>gi</td>
<td>2982081</td>
<td>8</td>
<td>42</td>
<td>5.73, 16.1</td>
<td>85</td>
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<tr>
<td>Ubiquitin carboxyl-terminal hydrolase isozyme L1</td>
<td>gi</td>
<td>18203410</td>
<td>8</td>
<td>39</td>
<td>5.29, 24.6</td>
<td>86</td>
</tr>
<tr>
<td>αB crystallin</td>
<td>gi</td>
<td>6753530</td>
<td>5</td>
<td>26</td>
<td>7.05, 20.1</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 2
Summary of proteins identified by mass spectrometry

Fig. 1. (A) Representative gel of proteins from the spinal cords of G93A-SOD1 transgenic mice after 2D electrophoresis. (B) Proteins from the spinal cords of nontransgenic mice after 2D electrophoresis. Insets: (Left) Expansion of the region of the 2D gel of G93A-SOD1 transgenic mouse spinal cord outlined by the box. (Right) Expansion of the region of the 2D gel of nontransgenic mouse spinal cord outlined by the box.
because oxidative modification alters the structure and function of proteins [49–51,68,69]. Consistent with this notion, free cytosolic calcium was increased in lymphocytes from ALS patients [70], suggesting that oxidative modification of TCTP may also play an important role in neurotoxicity of G93A-SOD1 and thus neurodegeneration in the spinal cords of G93A-SOD1 transgenic mice.

UCH-L1 belongs to a family of ubiquitin carboxyl-terminal hydrolases that play important roles in the ubiquitin–proteasome system [71]. The ubiquitin–proteasome system is a major pathway for selective protein degradation [72]. Ubiquitinated proteins form polyubiquitin chains that are eventually degraded by the 26S proteasome [73]. UCH-Ls then recycle ubiquitin from ubiquitinated protein complexes or polyubiquitin chains by cleaving the amide linkage next to the C-terminal glycine of ubiquitin [72]. Loss of UCH-L1 function causes neuroaxonal dystrophy [74–76], significant protein oxidation [45], and accumulation of synuclein protein in gracile axonal dystrophy mice [77]. Similarly, decreased UCH-L1 activity by mutation also enhances protein aggregation in Escherichia coli [78]. Therefore, based on the prior literature, oxidative inactivation of UCH-L1 presented in the current study possibly contributes to both the protein aggregation and the oxidative stress observed in G93A-SOD1 transgenic mice and ALS patients. Consistent with this notion and consistent with our finding (Fig. 4) that UCH-L1 activity is decreased in G93A-SOD1 mouse spinal cord, the inclusions of human ALS and mSOD1 (including G93A) mice are excessively ubiquitinated [79–82].

αB-Crystallin belongs to the small heat shock protein (sHSP) class of the heat shock protein family. HSPs are
cellular constituents synthesized by living organisms under stress conditions as well as normal conditions. The major function of sHSP is to stabilize other proteins under stress conditions, whereas the high-molecular-weight HSPs normally play roles in protein folding during biosynthesis [83,84]. Incorporation of αB-crystallin into red cell ghosts protects ATPase against oxidative stress [85], and overexpression of αB-crystallin protects cells against apoptosis and necrosis during myocardial ischemia and reperfusion [86] by acting as a chaperone [87]. Therefore, one can speculate that αB-crystallin contributes to neuroprotection when the cells are under oxidative stress. Moreover, α-crystallins were recruited to aggregates when cells were treated with proteasome inhibitor [88], and the degradation of αB-crystallin, along with ubiquitin conjugation, was decreased in bovine lens epithelial cells when αB-crystallin was oxidized [89]. Therefore, these studies suggest that oxidized αB-crystallin has a higher tendency toward aggregation than degradation. Therefore, oxidative modification of αB-crystallin observed in our current study may impair neuronal protection and increase aggregation of αB-crystallin in cells. Consistent with this notion, inclusions in ALS patients contain αB-crystallin, metallothionein, glutamine synthetase, and tubulin immunoreactivities [90].

The remarkable feature of the oxidatively modified proteins (except for TCTP) in G93A-SOD1 transgenic mice is that they are involved in the formation of inclusions in ALS patients or ALS models. Ubiquitin protein epitopes and αB-crystallin are found in fibrillar neuronal inclusions in the cortex of sporadic ALS patients [79–81,91]. Aberrant accumulation of mutant SOD1 is demonstrated in Caenorhabditis elegans expressing human mutant SOD1 [36]. Based on our current observations, the increased oxidative modification of SOD1, UCH-L1, and αB-crystallin plays a significant role in the protein aggregation in the spinal cords of G93A-SOD1 transgenic mice. Our current study provides insight into the mechanism of G93A-SOD1 neurotoxicity in vivo, which involves oxidative modification of a Ca2+ regulating protein (TCTP) and proteins involved in inclusion formation (SOD1, UCH-L1, and αB-crystallin), suggesting a potential relationship between protein oxidation, protein aggregation, and Ca2+ regulation in ALS. Moreover, one can speculate that the oxidative modification of these proteins impairs protein stability (αB-crystallin), Ca2+ binding (TCTP), protein degradation (UCH-L1), and antioxidant capacity (SOD1). It should be noted that other oxidative modifications could also play a role in the pathogenesis of ALS involving other proteins [92]. Moreover, because only symptomatic mice were compared to the nontransgenic mice in this study, it is possible that the oxidative modification of the proteins described is related to the consequences of the degenerative process. Therefore, future studies will address whether similar changes are observed in presymptomatic G93A-SOD1 transgenic mice.

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References


