Endoplasmic Reticulum Stress in Motor Neurons of the Spinal Cord in Sporadic Amyotrophic Lateral Sclerosis

Shoichi Sasaki, MD

Abstract

The accumulation of misfolded or unfolded proteins in the endoplasmic reticulum (ER) lumen causes a cellular stress response termed the unfolded protein response. Although ER stress has been implicated in various neurodegenerative diseases, the morphological features of aggregated proteins in ER lumina that may cause neurodegeneration have not been well characterized. We examined anterior horn neurons using immunohistochemistry and electron microscopy in 12 sporadic amyotrophic lateral sclerosis (ALS) patients and 12 controls. Approximately 2.6% of both normal-appearing and degenerated motor neurons in ALS cases were immunostained for the ER chaperone protein glucose-regulated protein 78, and approximately 0.1% of these neurons was glucose-regulated protein 78 positive in controls (p = 0.0004). Amyotrophic lateral sclerosis cases also tended to have glucose-regulated protein 78-positive motor neurons more frequently than control cases (p = 0.08). By electron microscopy, neurons in ALS patients showed accumulations of amorphous and granular material suggestive of misfolded or unfolded proteins in dilated predominantly normal-appearing ER. There were also wavy membranous structures extending from the ER membranes that lacked membrane-bound ribosomes, electron-dense material resembling Bunina bodies, Hirano bodies, honeycomb-like structures, and membrane-particle complexes associated with the ER in these neurons. Control sample neurons demonstrated none of these features. These ER alterations suggest that the unfolded protein response is activated in motor neurons in ALS patients and provide the first morphological evidence that ER stress may be involved in the neurodegeneration of motor neurons in early stages of sporadic ALS.

Key Words: Amyotrophic lateral sclerosis, Electron microscopy, Endoplasmic reticulum, ER stress, Motor neuron, Rough endoplasmic reticulum, Unfolded protein response.

INTRODUCTION

Newly synthesized proteins in the endoplasmic reticulum (ER) are properly folded and assembled in the ER lumen (membrane-bound cisternae); they then travel predominantly along this pathway to the Golgi apparatus and are transported to the plasma membranes or lysosomes. The proper conformational maturation of newly synthesized proteins in the ER is aided and monitored by numerous ER chaperones and folding enzymes in a complex process (termed ER quality control) in which cellular quality control networks play a key role in maintaining protein homeostasis (1, 2). Under physiological conditions, the most abundant ER chaperone, glucose-regulated protein 78/BiP (GRP-78 or BiP), is bound to 3 key ER stress sensors on the membranes of the ER: pancreatic ER kinase (PERK), inositol-requiring enzyme 1, and activating transcription factor 6. These proteins are regulated by GRP-78, which promotes proper protein folding in the ER lumen in an adenosine triphosphate-dependent manner. Misfolded or unfolded proteins are transported from the ER lumen to the cytoplasm and ubiquitinated and eventually degraded within the lysosomal 26S proteasome by ER-associated degradation (ERAD) (3). The conditions that impair the function of the ER or ERAD, designated ER stress, are caused by the accumulation of misfolded or unfolded proteins in ER lumina (4). The response to ER stress is initiated by activation of adaptive pathways, which is termed the unfolded protein response (UPR) (5–7). If ER stress is prolonged or severe, or if the UPR is overwhelmed by accumulated misfolded proteins, the persistent UPR causes an upregulation of the proapoptotic transcription factor C/EBP homologous protein, initiates the proapoptotic c-Jun N-terminal kinase signaling pathway, and activates caspase-12, leading to apoptosis (8, 9).

Recently, ER stress and upregulation of the UPR have been observed in various neurological diseases, including Alzheimer disease, polyglutamine disease, Parkinson disease, prion disease, and amyotrophic lateral sclerosis (ALS) (8–17). Although there is evidence implicating the misfolded proteins in the pathogenesis of neurodegenerative diseases, the precise relationship between the accumulation of the misfolded protein aggregates and the neurodegenerative process remains unclear. Moreover, whether ER stress plays a central role in the pathogenetic mechanisms of neurodegeneration also remains controversial. Some researchers have suggested that ER stress may play only a minor role in the mechanisms of neurodegeneration (18). Thus far, the aggregation of misfolded proteins associated with most neurodegenerative diseases has not been observed within the ER lumen. In addition, to the best of our knowledge, no direct morphological evidence for ER stress caused by the accumulation of misfolded proteins has been shown at the ultrastructural level for any neurodegenerative disease. This is the first report describing various types of ER alterations,
including the accumulation of amorphous or granular materials within the ER lumen, in normal-appearing large motor neurons in patients with sporadic ALS.

**MATERIALS AND METHODS**

The lumbar levels (L1–L5) of the spinal cord of 12 autopsied patients with sporadic ALS were investigated. The patient ages at the onset of the disease ranged from 49 to 72 years (average, 63.0 ± 6.7 years) (Table 1). Age-matched patients without any neurological disease (n = 12) served as controls (aged 35–80 years; average, 67.9 ± 12.7 years) (Table 1). The spinal cords were fixed in formalin, and the lumbar spinal cords were embedded in paraffin. Paraffin sections (6-μm thick) were stained with hematoxylin and eosin and Klüver-Barrera.

**Immunohistochemical Study**

Lumbar spinal cord sections were immunostained for ubiquitin (polyclonal, rabbit, 1:100; DAKO, Glostrup, Denmark) and for GRP-78 (rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA). The anti–GRP-78 antibody was diluted 1:50 with PBS at pH 7.2 containing 3% bovine serum albumin. Incubation was done overnight at 4°C. Bound primary antibody was detected with the appropriate Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). The chromogen was 3,3′-diaminobenzidine tetrahydrochloride. Sections from which the primary antibody was omitted served as negative-reaction controls.

**Semiquantitative Analysis**

Total large motor neurons and GRP-78-positive neurons in both anterior horns of L1 to L5 spinal cord levels

![FIGURE 1. Large motor neurons are not immunostained with an anti–glucose-regulated protein 78 antibody in a control case.](http://jnen.oxfordjournals.org/)

### TABLE 1. Case Material for Immunohistochemistry

<table>
<thead>
<tr>
<th>Controls</th>
<th>Age, Years</th>
<th>Sex</th>
<th>Postmortem Delay</th>
<th>Pathological Diagnosis</th>
<th>No. GRP-78−Positive Neurons/No. Neurons Examined in L1−L5 (rt, lt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS Cases</td>
<td>Illness Duration, months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>49</td>
<td>Male</td>
<td>3 hours</td>
<td>Ruptured abdominal aneurysm</td>
<td>0/199, 0/177</td>
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<tr>
<td>2</td>
<td>71</td>
<td>Female</td>
<td>2 hours</td>
<td>Esophageal carcinoma</td>
<td>0/184, 1/152</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>Male</td>
<td>3 hours</td>
<td>Ruptured abdominal aneurysm</td>
<td>0/235, 1/193</td>
</tr>
<tr>
<td>4</td>
<td>63</td>
<td>Male</td>
<td>3 hours</td>
<td>Malignant lymphoma</td>
<td>0/179, 0/158</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>Female</td>
<td>3 hours</td>
<td>Myocardial infarction</td>
<td>0/129 0/131</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>Male</td>
<td>3 hours</td>
<td>Renal carcinoma</td>
<td>0/179, 0/158</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>Male</td>
<td>3 hours</td>
<td>Congestive heart failure</td>
<td>1/151, 0/167</td>
</tr>
<tr>
<td>8</td>
<td>63</td>
<td>Male</td>
<td>3 hours</td>
<td>Multiple myeloma</td>
<td>0/165, 0/166</td>
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<tr>
<td>9</td>
<td>65</td>
<td>Male</td>
<td>3 hours</td>
<td>Large intestinal carcinoma</td>
<td>0/186, 1/213</td>
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<tr>
<td>10</td>
<td>61</td>
<td>Male</td>
<td>3 hours</td>
<td>Lung carcinoma</td>
<td>0/119, 0/127</td>
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<tr>
<td>11</td>
<td>63</td>
<td>Male</td>
<td>3 hours</td>
<td>Thyroid carcinoma</td>
<td>0/223, 0/235</td>
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<tr>
<td>12</td>
<td>73</td>
<td>Male</td>
<td>3 hours</td>
<td>MRSA sepsis</td>
<td>1/170, 0/213</td>
</tr>
<tr>
<td>Total GRP-78−positive neurons/total neurons examined</td>
<td>5/4,209 (0.12%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALS, amyotrophic lateral sclerosis; lt, left; MRSA, methicillin-resistant *Staphylococcus aureus*; rt, right.

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were counted in the controls and sporadic ALS cases. Large motor neurons were defined as those with an average diameter of more than 22.5 µm, that is, the mean of the shortest (20 µm) and the largest (25 µm) diameters of an anterior horn neuron. The frequency of cases showing GRP-78 immunoreactivity was analyzed by the χ² test using a computerized statistical program. Whether GRP-78 immunopositivity was affected by differences in total numbers of motor neurons examined in the controls and ALS group was also assessed. Statistical analyses were performed using the GLM procedure, SAS system version 9 (SAS Institute, Cary, NC). A value of p < 0.05 was considered significant.

Electron Microscopy
Cases in which postmortem intervals were within 6 hours of death were selected for electron microscopy to minimize postmortem artifacts. There were 15 controls (aged 44–80 years; mean age, 62.7 ± 11.2 years) who died without having any known neurological disease and 15 sporadic ALS patients (aged 49–83 years; mean, 68.2 ± 8.5 years), including 5 patients (Cases 1–5) used in the immunohistochemical analysis. The lumbar spinal cords (L1–L5) were fixed in 2% glutaraldehyde with phosphate buffer (pH 7.40) at the time of autopsy. After fixation, the anterior horns at each level of the lumbar spinal cord were sectioned transversely, postfixed in 1% osmium tetroxide for several hours, dehydrated, and embedded in epoxy resin. Each block was cut into serial semithin sections approximately 1-µm thick. These sections were stained with toluidine blue. Appropriate portions of the sections were cut into ultrathin sections that were then stained with uranyl acetate and lead citrate for electron microscopy.

RESULTS

Light Microscopy
Bunina bodies and Lewy body-like hyaline inclusions (LBHIs) (round bodies) were observed in the anterior horn neurons in 10 of 12 ALS patients. The perikarya of these neurons usually contained a single LBHI or Bunina body, but occasionally, there were 2 or more. The LBHIs were usually eosinophilic but occasionally somewhat basophilic, round or oval, and appeared to consist of a hyalinized substance.

Immunohistochemistry
Other than a few motor neurons, neither small nor large neurons in control cases showed GRP-78 immunoreactivity (Fig. 1). The numbers of GRP-78–positive large motor neurons from the first to the fifth lumbar levels were 1 of 360, 2 of 751, 1 of 870, 1 of 1,038, and 0 of 1,190, respectively. Thus, a total of only 5 of 4,209 large motor neurons (~0.1%) were GRP-78 positive, and all of these appeared normal. Ubiquitin-positive inclusion bodies were not detected in control cases.

In the ALS patients, skeinlike inclusions and LBHIs were ubiquitin positive. Both normal-appearing (Fig. 2) and degenerated anterior horn neurons (Fig. 3) were GRP-78 positive in 6 cases. The neuron somata were focally (Fig. 3) or diffusely (Fig. 2) immunostained. The numbers of GRP-78–positive large motor neurons from the first to the fifth lumbar levels were 2 of 111, 6 of 183, 15 of 325, 11 of 403, and 5 of 454, respectively. Thus, a total of 39 of 1,476 large motor neurons (~2.6%) were GRP-78 positive. Most (i.e. 25) of these GRP-78–positive motor neurons appeared normal, whereas 14 appeared to be undergoing degeneration. Patients with both a short clinical course and relatively well-preserved motor neurons (e.g. Cases 3 and 4) showed relatively abundant GRP-78–positive neurons. Bunina bodies, LBHIs, and skeinlike inclusions were not GRP-78 positive (Fig. 4). The numbers of large motor neurons examined and the number of GRP-78–positive neurons in each control case and ALS case are shown in Table 1.

Semiquantitative Analysis
In controls, 2 of 12 cases had GRP-78–positive motor neurons, whereas 9 of 12 ALS cases had GRP-78 positivity. Thus, the frequency of the cases showing GRP-78–positive
neurons was significantly greater in the ALS group ($\chi^2$ test, $\chi^2 = 8.22; p = 0.004$). The GRP-78 immunopositivity was not affected by differences in numbers of motor neurons examined between the controls and the ALS group ($p = 0.35$) but was caused by differences between the groups ($p = 0.08$) based on statistical analysis (GLM Procedure, SAS system) (Table 1).

Electron Microscopy

The rough ER (rER) in controls consisted of stacks of flattened membrane-bounded cisternae (ER lumina), the surfaces of which were studded with ribosomes (Fig. 5). The rER was disposed in orderly arrays of nearly parallel and broad cisternae that focally accumulated and formed Nissl substances or Nissl bodies (Fig. 5). The rER was frequently close to the nucleus and proximal portion of the dendrites but not in the axon hillock, the axonal initial segment, or the axons. The lumen of the rER in all controls was almost al-

translucent, not dilated or distended and not stacked with any other material. Central chromatolytic neurons with eccentric nuclei were only occasionally observed. These neurons showed fragmentation and paucity of rER (Fig. 6), and the rER was frequently very close to the nucleus in them. Lamellar bodies were observed in 1 case and intracytoplasmic hyaline (colloid) inclusions containing granular material in another, both of these appeared to be derived from rER or Nissl substance.

In ALS patients, the rER was disposed in orderly arrays of nearly parallel and broad cisternae, and most of the ER lumina were translucent, not dilated or distended, and not stacked with any other material, as in the controls. There were, however, various types of rER alterations observed. First, focally dilated or distended ER lumina containing amorphous or granular materials were occasionally seen in the somata of normal-appearing large anterior horn neurons in Cases 8, 11, and 14 (Fig. 7). In Case 8 (in which there was a very short clinical course of ~10 months), the anterior horn neurons were relatively well-preserved but showed this alteration frequently, that is, in 2 of 9 neurons in 1 grid and in 2 of 6 in another. Large motor neurons showing accumulations of amorphous

FIGURE 4. A Lewy body-like hyaline inclusion (arrow) is not immunostained for glucose-regulated protein 78 in an anterior horn cell of an amyotrophic lateral sclerosis patient.

FIGURE 5. In a control anterior horn cell, the rough endoplasmic reticulum (rER) consists of stacks of flattened membrane-bound cisternae (ER lumina); the surfaces of these are studded with ribosomes. Scale bars = 0.5 μm.

FIGURE 6. A central chromatolytic neuron in a control. (A) The neuron has an eccentrically located nucleus. Scale bars = 5 μm. (B) High magnification of (A) demonstrates fragmentation and paucity of rough endoplasmic reticulum in the cytoplasm. Scale bars = 1 μm.
or granular material within the ER lumina were almost always normal appearing, with or without inclusion bodies such as Bunina bodies, or only occasionally showing pigmentary atrophy. The ER with dilated lumina containing amorphous or granular material coexisted with normal-appearing ER within Nissl bodies in a single neuron.
Second, there were proliferated and extended membra-
nous structures, the surfaces of which were not studded
with membrane-bound ribosomes, resulting from the mem-
branes forming the ER cisterna space; this was frequent in Case 8. The membranous structures were usually waving, not flat-
tened (Fig. 8) and nonuniform, and no accumulation of
electron-dense material was seen in the intermembranous
spaces. They occasionally formed membrane bundles and were almost always seen in normal-appearing large motor
neurons.

In Cases 1, 3, and 8, there were lamellar bodies of dif-
f erent stages, that is, from small ones in the initial stage to
large mature ones (Fig. 9). The electron-dense material of the
rER resembling Bunina bodies was seen in Cases 1, 5, 8, 13,
and 14 (Fig. 10). It differed from Bunina bodies in that it did
not show any tubular or vesicular structures or accumulation
of neurofilaments. Honeycomb-like structures that were
continuous with the rER were observed in Cases 10 and 15
(Fig. 11). Each cell of the honeycomb-like structures was
usually translucent, and free ribosomes were found between
the cells. Colloid inclusions derived from Nissl substance
was seen in Cases 4, 8, and 9. Hirano bodies consisting of
crystalloid arrays of interlacing filaments displaying either a
lattice-like or herringbone configuration were observed in
Case 8. These bodies were directly connected with the rER,
particularly with the membranes of the ER lumen (Fig. 12).
Membrane-particle complexes consisting of stacks or con-
centric arrangements of membrane-bounded lumina were
found in Case 8 (Fig. 13). As in the control cases, central
chromatolytic neurons were observed to some extent in the

FIGURE 8. Membranous structures in an amyotrophic lateral
sclerosis patient motor neuron endoplasmic reticulum (ER). (A)
The surfaces of the membranous structures are not studded
with membrane-bound ribosomes and extend from the mem-
branes forming the ER lumen. (B) The membranous structures
form bundles. Scale bars = 0.5 μm.

FIGURE 9. Lamellar bodies in motor neurons of amyotrophic lateral sclerosis patients. (A) A lamellar body stack that is continuous
with ribosomes of the rough endoplasmic reticulum (rER). (B) There are 4 lamellar body stacks. (C) Multiple stacks of lamellar
bodies between the ER. Scale bars = 0.5 μm.

FIGURE 10. Electron-dense material in rough endoplasmic retic-
ulum (arrow) resembles a Bunina body (arrows) in an amyotro-
fic lateral sclerosis patient. Scale bars = 0.5 μm.
ALS cases. The specific rER alterations in each ALS case are summarized in Table 2.

DISCUSSION

A variety of cellular stress conditions such as expression of misfolded proteins and energy depletion can interfere with protein folding and cause ER stress (5). The ER stress signals such as the UPR transducer PERK, which launches the most immediate response to ER stress, and the downstream effector of PERK, eukaryotic initiation factor 2α, have been shown to be involved in the pathogenesis of Alzheimer disease (8, 19) and familial ALS (15, 20). Prolonged ER stress can contribute to cell death both by mitochondria-dependent and independent pathways (21). Recent studies suggest that increases in GRP-78 and its mRNA are early indicators of ER stress signal transduction, and that it serves as a master UPR regulator, which plays essential roles in activating the UPR transducers inositol-requiring enzyme 1, PERK, and activating transcription factor 6 in response to ER stress (22, 23). Moreover, GRP-78 facilitates the folding of newly synthesized proteins (5, 24). Thus, molecular chaperones are key players in protein homeostasis, regulating networks that control protein synthesis and degradation, helping proteins to fold in the ER lumen under normal conditions and preventing aggregation of misfolded proteins (25, 26). In addition to GRP-78 (27), the ER is rich in other Ca2+-dependent molecular chaperones such as GRP-94 (28), calreticulin (29), and calnexin (30), all of which stabilize protein-folding intermediates (31).

Glucose-regulated protein 78 and ER stress have been implicated in the pathogenesis of ALS (13). Glucose-regulated protein 78 is expressed in superoxide dismutase 1 (SOD-1)-positive LBHI in G93A mutant SOD1 transgenic mice. It may bind to, or be closely associated with, mutated SOD-1 within the LBHIs, suggesting dysfunction of GRP-78 and subsequent derangement of the mutant SOD-1 in these mice (13). In the present study, GRP-78-positive motor neurons were more frequently observed in patients with sporadic ALS compared with controls, suggesting accumulation of

**FIGURE 11.** A honeycomb-like structure with scattered ribosomes between translucent cells in the motor neuron of an amyotrophic lateral sclerosis patient. Scale bars = 0.5 μm.

**FIGURE 12.** A Hirano body consisting of crystalloid arrays of interlacing filaments that display a lattice-like configuration in an amyotrophic lateral sclerosis case. The Hirano body is directly connected to the rough endoplasmic reticulum (ER), particularly with the membranes of the ER lumina. Scale bars = 0.5 μm.

**FIGURE 13.** Membrane-particle complex in the cytoplasm of an amyotrophic lateral sclerosis case. (A) The complex is indicated by an arrow. Scale bars = 5 μm. (B) In a higher magnification of (A), the complex is shown to consist of membrane stacks that surround lumina. Scale bars = 0.5 μm.
of neurodegeneration of motor neurons in these diseases. Patients with sporadic ALS imply differences in mechanisms LBHI between G93A mutant SOD-1 transgenic mice and different patterns of GRP-78 and SOD-1 immunoreactivity of motor neurons in spinal cords in sporadic ALS (38). Based on proteasome immunoreactivity is increased both in glia and properties shown by mutated SOD-1 in vivo (37), whereas inhibition leads to the reproduction of the abnormal solubility motor neuron death in sporadic ALS (16, 35).

Endoplasmic reticulum stress signals are increased or activated in the motor neurons of mutant SOD-1 transgenic animals (14, 17, 43, 44) and in motor neuron-like culture cells expressing mutant SOD-1 (15). A full UPR, including induction of stress sensor kinases inositol-requiring enzyme 1, PERK, and activating transcription factor 6; chaperones; and apoptotic mediators, is present in spinal cords. In addition, the UPR chaperone protein disulfide isomerase is present in cerebrospinal fluid and is aggregated and widely distributed throughout the motor neurons of patients with sporadic ALS (45). The accumulation of mutant SOD-1 is more abundant in the ER lumen of motor neurons of the spinal cords than that of wild-type SOD-1, as revealed by immuno-electron microscopy (14). Mutant SOD-1 is also found in the immunoisolated trans-Golgi network and in microparticle preparations (46). These findings suggest that mutant SOD-1 can be secreted in the ER lumen (15, 46), although the presence of SOD-1 in the ER lumen remains unexplained because it is normally cytoplasmic and does not contain a signal peptide. In SOD-1-mediated ALS, mutant SOD-1 specifically interacts with Derlin-1, a protein that is essential for transporting misfolded proteins from the ER lumen to the cytoplasm for degradation (47). A recent study also demonstrated that ER stress is triggered early and is associated specifically with those cells that are affected preferentially in familial ALS-related mouse models (17).

The relationship between protein oxidative damage and proteasomal activity, ubiquitin-positive aggregates such as LBHIs and skeinlike inclusions may be related to oxidative stress and ER stress. Moderate oxidative modification of proteins increases their susceptibility for proteasome clearance, whereas higher rates of oxidative modification inhibit proteasomal activity (39). Thus, the decrease in proteasomal activity may explain the increased presence of ALS-characteristic inclusions and ubiquitination. The frequent presence of ubiquitin-positive inclusions of most patients with sporadic ALS in the present study may be compatible with proteasomal disturbance. The disruption of the ERAD is a potential consequence of such proteasomal impairment, finally contributing to ER stress (40). Moreover, immunohistochemical evidence, including our previous reports regarding sporadic ALS patients and mutant SOD-1 (G93A) transgenic mice, supports a role for increased oxidative damage in ALS (41, 42). Frequent ubiquitin-positive inclusions and the increase in GRP-78 immunoreactivity observed in the present study suggest the participation of oxidative damage as well as ER stress in motor neurons in sporadic ALS.

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Moreover, the accumulation of amorphous electron-dense material has been demonstrated within membrane-bound cisternae of tER in motor neurons of chronic N-methyl-D-aspartate–treated chick embryos, a model of glutamate receptor–mediated neurotoxicity (48). Thus, the accumulation of mutant SOD-1 and ER stress in motor neurons disturbs the restoration system for misfolded or unfolded proteins in the ER, probably leading to toxicity.

One previous report showed fragmented ER cisternae in degenerated motor neurons with central chromatolysis and irregularly distended ER cisternae with detachment of ribosomes in shrunken motor neurons (49), but there have been

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**TABLE 2.** Specific ER Abnormalities in ALS Cases

<table>
<thead>
<tr>
<th>ALS Case No.</th>
<th>ER Abnormalities</th>
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<tbody>
<tr>
<td>1</td>
<td>Lamellar body, electron-dense material</td>
</tr>
<tr>
<td>2</td>
<td>Lamellar body</td>
</tr>
<tr>
<td>3</td>
<td>Colloid inclusion</td>
</tr>
<tr>
<td>4</td>
<td>Electron-dense material</td>
</tr>
<tr>
<td>5</td>
<td>Colloid inclusion</td>
</tr>
<tr>
<td>6</td>
<td>Honeycomb-like structure</td>
</tr>
<tr>
<td>7</td>
<td>Accumulation of amorphous materials in the ER lumen, membranous structure, lamellar body, electron-dense material, colloid inclusion, Hirano body, membrane-particle complex</td>
</tr>
<tr>
<td>8</td>
<td>Colloid inclusion</td>
</tr>
<tr>
<td>9</td>
<td>Honeycomb-like structure</td>
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<td>Accumulation of amorphous materials in the ER lumen</td>
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ALS, amyotrophic lateral sclerosis; ER, endoplasmic reticulum.
no reports providing fine structural evidence that ER stress can lead to neurodegeneration of motor neurons in patients with sporadic ALS, or SOD-1-related or -nonrelated familial ALS. The present study is the first demonstration of the preferential accumulation of amorphous or granular material suggestive of misfolded or unfolded secreted or membrane proteins in dilated or distended ER lumen in normal-appearing motor neurons in sporadic ALS. Earlier studies have revealed that the initial signal for activating the UPR is the accumulation of unfolded proteins in the ER (50). Thus, the accumulation of these materials in the ER lumen of normal-appearing motor neurons appears to be an early pathological change in sporadic ALS. It also suggests that there may be a disrupted transport of newly synthesized proteins destined for appropriate sites, accumulation of misfolded proteins, and disturbance of transport of nascent proteins from the ER lumen to the cytoplasm. The accumulation of damaged proteins may eventually lead to the impairment of the ubiquitin-proteasome degradation system beyond the capacity of ERAD function, possibly even yielding ALS-characteristic inclusions such as skeinlike inclusions and LBHIs.

Extended waving membranous structures resulting from the ER membranes without membrane-bound ribosomes are another peculiar finding in the present study. The ER plays a central role in protein biosynthesis, and the ER membrane is the site of production of all transmembrane proteins for most of the organelles. Membrane-bound ribosomes attached to the cytosolic side of the ER membrane are engaged in the synthesis of proteins that are concurrently translocated into the ER. Thus, changes in the ER membranes without membrane-bound ribosomes suggest that there is no biosynthesis of new secretory proteins in those areas. On the other hand, the lumen of the ER is a unique environment, containing the highest concentrations of Ca^{2+} within the cell because of active transport of calcium ions by Ca^{2+} ATPase. The lumen is an oxidative environment, critical to the formation of disulfide bonds and proper folding of proteins. Several studies indicate that sensitivity to apoptosis correlates with the total ER Ca^{2+} load rather than with the free ER Ca^{2+} concentrations and depends on the ability of cells to transfer Ca^{2+} from the ER to the mitochondria. The decrease in the Ca^{2+} concentrations in the ER protects cells from apoptosis, and conversely, the increase of the ER Ca^{2+} load sensitizes cells to apoptotic stress (51). Thus, changes in the ER membranes as well as the accumulation of amorphous or granular materials in the ER lumen may lead to Ca^{2+} load imbalance in the ER lumen.

Other rER-associated alterations are lamellar bodies, electron-dense materials resembling Bunina bodies, honeycomb-like structures, membrane particle complexes, colloid inclusions, and Hirano bodies. Lamellar bodies are virtually restricted to Purkinje cells (52). The presence of electron-dense materials of the rER resembling Bunina bodies suggests that the rER may be at least partly involved in the process of formation of these bodies. Honeycomb-like structures have been observed continuous with the smooth ER in normal cerebella but are more common in a variety of pathological conditions (53–55). Membrane particle complexes have been found in the dorsal root ganglia of both normal (56) and abnormal experimental animals. Colloid inclusions have been observed exclusively in hypoglossal nuclei and anterior horn neurons of normal adults (57). Hirano bodies are observed under various pathological and physiological conditions.

In summary, more frequent GRP-78–positive normal-appearing motor neurons indicating UPR activation and morphological changes of the rER, including accumulation of amorphous or granular material in dilated ER lumina in normal-appearing motor neurons, support the concept of ER stress as part of the pathogenesis of neurodegeneration of motor neurons at early stages in patients with sporadic ALS. The precise relationships among the various morphological alterations of the rER and the neurodegenerative processes of motor neurons remain unclear at present. Therapies based on the modulation of ER stress may be warranted or applicable to patients with sporadic ALS.

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