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ER Stress and Unfolded Protein Response in Amyotrophic Lateral Sclerosis

Kohsuke Kanekura · Hiroaki Suzuki · Sadakazu Aiso · Masaaki Matsuoka

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Abstract Several theories on the pathomechanism of amyotrophic lateral sclerosis (ALS) have been proposed: misfolded protein aggregates, mitochondrial dysfunction, increased glutamate toxicity, increased oxidative stress, disturbance of intracellular trafficking, and so on. In parallel, a number of drugs that have been developed to alleviate the putative key pathomechanism of ALS have been under clinical trials. Unfortunately, however, almost all studies have finished unsuccessfully. This fact indicates that the key ALS pathomechanism still remains a tough enigma. Recent studies with autopsied ALS patients and studies using mutant SOD1 (mSOD1) transgenic mice have suggested that endoplasmic reticulum (ER) stress-related toxicity may be a relevant ALS pathomechanism. Levels of ER stress-related proteins were upregulated in motor neurons in the spinal cords of ALS patients. It was also shown that mSOD1, translocated to the ER, caused ER stress in neurons in the spinal cord of mSOD1 transgenic mice. We recently reported that the newly identified ALS-causative gene, vesicle-associated membrane protein-associated protein B (VAPB), plays a pivotal role in unfolded protein response (UPR), a physiological reaction against ER stress. The ALS-linked P56S mutation in VAPB nullifies the function of VAPB, resulting in motoneuronal vulnerability to ER stress. In this review, we summarize recent advances in research on the ALS pathomechanism especially addressing the putative involvement of ER stress and UPR dysfunction.

Keywords Amyotrophic lateral sclerosis · ER stress · Unfolded protein response

K. Kanekura · H. Suzuki · M. Matsuoka (✉)
Department of Cell Biology and Neuroscience,
School of Medicine, Keio University,
35 Shinanomachi, Shinjuku-ku,
Tokyo 160-8582, Japan
e-mail: sakimatu@sc.itc.keio.ac.jp

K. Kanekura · H. Suzuki · S. Aiso
Department of Anatomy, School of Medicine, Keio University,
Tokyo, Japan

H. Suzuki
Japan Society for the Promotion of Science Research Fellow,
Tokyo, Japan

Introduction

In 1869, a French neurologist Jean-Martin Charcot and his colleague Alexis Joffroy reported a strange phenomenon observed in two patients who died from progressive weakness and degeneration of both upper and lower motor neurons with gliosis. Despite over 135 years of vigorous investigations after the discovery of the disease, amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig disease, still remains a fatal merciless disorder that affects two to eight per 100,000 persons in the world [1].

ALS is characterized by progressive neurodegeneration specifically affecting both upper and motor neurons. Typical cases occur sporadically (~90%) in their middle ages and become fatal within 2–5 years from the disease onset mostly because of respiratory failure while cognitive function of patients remains completely intact even in the terminal phase of the disease. Several ALS-specific pathological hallmarks have been identified [2–5]: the degeneration of motoneurons in the spinal anterior horn; the Bunina body, also known as Lewy body-like hyaline inclusion body, seen in the survived motoneurons; spheroids consisting of accumulated neurofilaments in the

proximal axon; involution of the rough endoplasmic reticulum (ER), which is known as chromatolysis; and fragmented Golgi apparatus.

Sporadic and Familial ALS

Five percent to 10% ALS patients have familial background while the other cases occur sporadically [1]. The etiology of sporadic ALS (SALS) remains almost unknown although various hypotheses have been proposed including impairment of splicing of GluR2, an AMPA receptor subunit [6], ALS autoantibody [7], metal imbalance [8], and excitatory neurotoxin such as BMAA [9]. Generally speaking, race and area do not affect SALS incidence [1]. However, there are two endemic regions for SALS, Guam island and Kii peninsula in Japan [10]. It was also reported that veterans of the Gulf War have higher possibility to suffer from the disease [11]. These facts suggest that some environmental factors may contribute to the development of SALS.

To date, multiple familial ALS-causative genes have been identified: ALS1/SOD1 [12], ALS2/alsin [13, 14], ALS4/senataxin [15], and ALS8/vesicle-associated membrane protein-associated protein B (VAPB) [16]. In addition, mutations in p150 subunit of dynactin [17], angiogenin [18], and TDP-43 [19] have been recognized to be closely linked to the onset of familial ALS. Based on the fact that physiological functions of these familial ALS-related proteins do not appear to be overlapped, it is assumed that abnormalities in several independent cellular functions ultimately result in the development of ALS. Using familial ALS-related genes, neuroscientists have been trying to seek the key pathomechanism for ALS.

SOD1 (Superoxide Dismutase 1)

In 1993, an international research team identified the first familial ALS-linked gene, Cu/Zn superoxide dismutase (SOD1), from autosomal-dominant ALS patients [12]. SOD1, a ubiquitously expressed enzyme, consists of 153 amino acids, holds a catalytic cupper and zinc ions essential for its function, and protects cellular organelles from oxidative stress by dismutating superoxide produced by oxidative phosphorylation in mitochondria. Twenty percent of familial ALS cases are caused by mutations in the *SOD1* gene [1]. To date, more than a hundred different point mutations that result in amino acid substitutions or incorrect translation termination in the *SOD1* gene are known to cause autosomal-dominant ALS. Based on the observation that almost all missense mutations in SOD1 causes ALS and thereby generated mutant SOD1 proteins share similar characteristics of forming aggregate [20] and being poly-ubiquitinated [21], it is speculated that the conformation of wild-type SOD1 protein is strictly maintained by all parts of SOD1 proteins, tiny structural changes result in its misfolding, and misfolded mSOD1 is thought to cause ALS by a common mechanism.

SOD1 detoxifies superoxide anions that are harmful to cells [22]. Based on the fact that some ALS-linked mSOD1's lose their enzymatic activities [23, 24], loss of the enzymatic function of SOD1 had been deemed to be linked to the onset of the disease. However, the level of decreased enzymatic activity of SOD1 did not correlate with the severity of the disease and some ALS-linked mSOD1 have almost normal enzymatic activity to remove superoxide [25]. Furthermore, SOD1 knockout mice did not show motor neuron degeneration [26]. All these experimental results declined the "loss of function" theory on mSOD1-induced ALS. Instead, the establishment of G93A-SOD1 transgenic mice as a representative ALS mouse model to mimic human ALS-like phenotypes by Gurney et al. in 1994 [27] strongly supported the notion that mutant SOD1 causes ALS by gain of some unknown neurotoxicities.

How does mSOD1 show neurotoxicity? A number of studies have demonstrated that various mutations increase the unfolded states of SOD1. As a result, SOD1 mutants are prone to form aggregates and to be ubiquitinated. In fact, ubiquitin- and SOD1-immunopositive cytosolic inclusions are often seen in the motor neurons in the spinal cord of ALS patients [28] and rodent ALS models [29]. While cytosolic aggregates are pathological hallmarks in neurodegenerative diseases such as Huntington's disease [30], Parkinson's disease [31], and ALS, it remains largely unknown how these misfolded aggregates show neurotoxicity. Some reports speculated that coaggregation of components of cell machineries essential for neuronal survival such as the proteasomal systems, chaperones, mitochondria, and axonal transport machinery into cell inclusions results in neurotoxicity. Urushitani et al. demonstrated that mSOD1 was highly ubiquitinated and transported to the proteasome to be degraded where they clogged the proteasomal systems if overloaded [32]. They speculated that malfunction of the proteasomal systems inhibited degradation of many cellular proteins and ultimately caused motor neuron toxicity. However, there is no direct evidence differentiating whether malfunction of the proteasome is a consequence of overloading of misfolded proteins or the primary cause of protein misfolding. In addition to malfunction of the proteasomal systems, mSOD1 contributes to the generation of oxidative stress [33]. Mutant SOD1 may also do harm to mitochondria by forming aggregates on the cytoplasmic surface and/or in the intermembrane space of mitochondria [34, 35]. In contrast to these studies supporting the idea that aggregation is

toxic, some reports have suggested that aggregation is not toxic but may be rather protective [36, 37].

Zhang et al. [38] reported that mSOD1 interacted with dynein, a huge cargo protein playing a pivotal role in the retrograde protein transport along axons and might inhibit axonal transport. A very recent study with NADPH oxidase knockout mice has indicated that SOD1 can directly regulate NADPH oxidase-dependent O_2^- production by binding Rac1 and inhibiting its GTPase activity, independently of its original SOD1 enzyme activity, and the mutations in SOD1 transform SOD1 to be constitutively active in NADPH oxidase-dependent production of O_2^- and may ultimately cause toxicity to motor neurons [39].

Besides these motor neuron-autonomous death mechanisms, it has also been hypothesized that inflammatory reaction contributed to the progression of motor neuron death [40]. Recent evidence has indicated that glial cells surrounding motor neurons exert an aggravating effect on motor neuron death [41]. Sasabe et al. indicated that D-serine whose levels were upregulated in ALS spinal cords contributed to the progression of ALS by increasing NMDA toxicity [42].

Based on these various hypotheses, a number of clinical trials including celecoxib (a cyclooxygenase II inhibitor) [43], minocycline (a putative apoptosis inhibitor and an inhibitor for activation of microglia) [44], TCH346 (an antiapoptotic compound) [45], and creatine [46] were performed. Unfortunately, however, they all finished unsuccessfully.

In addition to these hypotheses, the ER stress theory has recently emerged. A study with autopsied tissue samples of ALS patients and *in vivo* experiments indicated the involvement of ER stress in ALS [47, 48]. In neurons in the spinal cords of ALS patients and ALS model mice, levels of ER stress-related proteins were upregulated [47, 48]. These results suggest that ER stress-related cell death pathways are activated. It has been generally speculated that ER stress, triggered by accumulation of misfolded proteins in ER, causes toxicity especially in cells actively secreting proteins including plasma cells [49] and pancreatic β cells [50] and in long-turnover cells such as neurons [51]. Especially, in motor neurons, a large amount of proteins continuously produced to maintain the homoeostasis of the larger cell bodies are thought to be prone to trigger severe ER stress. Accordingly, it is an attractive hypothesis that ER stress causes selective death of motor neurons in ALS.

ER Stress and Unfolded Protein Response

ER is a reticulated organelle in which proteins are synthesized and modified for proper folding. In normal cells, approximately 30% of newly synthesized proteins are misfolded [52] and some portions of these proteins are refolded to have correct structures, assisted by ER chaperones such as BiP/GRP78 (Bip), whereas the other portions of the proteins remain misfolded, accumulate in ER, and cause ER stress [53]. Under such ER stress condition, an adaptive self-defense response named unfolded protein response (UPR) is triggered to decrease the load of misfolded proteins. Several cellular conditions such as high demand for protein secretion [49, 50], viral infection [54], deprivation of nutrient/oxygen [55, 56], and missense mutations [57, 58] enhance protein misfolding and lead to severer ER stress and overwhelming accumulation of unfolded or misfolded proteins that causes fatal ER stress.

In yeast cells, an inositol-requiring enzyme 1 (Ire1)/Hac1 signal is the only pathway of the UPR [59]. Ire1, as its name implies, is originally identified from an inositol-auxotrophic mutant and the yeast clone without Ire1 needs inositol to grow normally [60]. Ire1 encodes a transmembrane protein Ire1p that works as both a serine/threonine kinase and an endoribonuclease [61]. When Ire1p is activated, Ire1p forms an oligomer, induces autophosphorylation and gains RNase activity. Activated Ire1p then triggers splicing of HAC1 mRNA. Spliced HAC1 mRNA encodes a transcription factor with a basic leucine zipper domain Hac1p that induces the expression of UPR-related genes such as KAR2 (a homologue of mammalian ER chaperone Bip) to grow under ER stress conditions [62]. The deletion of the *Ire1* or *Hac1* gene causes not only vulnerability to ER stress but also inositol auxotrophy, and this fact indicates that there is probably a close relationship between UPR and inositol metabolism.

Recent extensive studies on the mammalian UPR system identified three types of ER-resident stress sensors, ATF6, IRE1, and PERK, in mammals [63]. All of IRE1, PERK (an ER-resident type I transmembrane protein kinase), and ATF6 (an ER-localized leucine zipper transcription factor) are kept to be inactivated by their association with an ER chaperone, BiP, via their luminal domain under the normal condition. Once ER stress occurs, Bip dissociates from them to be engaged in refolding the accumulated misfolded proteins. As a consequence, free IRE1 and PERK oligomerize and autophosphorylate, which results in their activation [64]. After dissociating from the luminal domain of Bip, ATF6 translocates to the Golgi apparatus and is cleaved by proteases S1P and S2P, which results in its activation as a transcriptional factor [65].

Mammalian UPR consists of four well-organized elements: (1) suppression of protein translation, (2) induction of ER chaperones, (3) enhancement of ER-associated degradation (ERAD), and (4) induction of apoptosis.

1. Suppression of protein translation

Activated PERK phosphorylates and inactivates eukaryotic initiation factor 2α to attenuate general protein

translation. As a consequence, production of misfolded proteins is suppressed [66].

2. Induction of ER chaperones

Activated IRE1 generates spliced XBP1. Both activated ATF6 and spliced XBP1 work as transcription factors that induce expression of ER chaperons such as Bip and GRP94. Upregulated levels of ER chaperones cause refolding of accumulated misfolded proteins in ER [67].

3. Degradation of misfolded proteins via the ubiquitin–proteasome pathways

To decrease the accumulated misfolded proteins in ER, the misfolded proteins are transported to the cytosol via the translocon and then degraded by the ubiquitin–proteasome systems [68].

4. Induction of apoptosis

Under a severe ER stress condition, the activated IRE1 interacts with TRAF2 and activates an apoptosis-stimulating kinase 1 (ASK1)/c-*jun* N-terminal kinase (JNK) cascade that triggers apoptosis [69]. Expression of apoptosis-related transcription factor CHOP is also induced via the PERK pathway [70].

Through these well-organized self-defense systems, cells try to escape from ER stress. Malfunction of the UPR system and/or overwhelming ER stress may result in fatal outcome to cells, especially to secreting cells actively producing proteins such as pancreatic β cells [50] and slow/no turnover cells with large cell bodies such as neurons [51].

Mutant SOD1 and ER Stress

G93A-SOD1 transgenic mice show progressive and selective loss of motor neurons and mimic clinical manifestations of human ALS [27, 71] and are, therefore, regarded to be the most established ALS animal model. Pathologically, swollen mitochondria [72], fragmented Golgi apparatus [73], and ubiquitin-immunopositive aggregates [74] are seen in G93A-SOD1 transgenic mice as well as in spinal cords of human sporadic ALS.

Mutations in the *SOD1* gene cause major conformational change of SOD1. Mutant SOD1 shows characteristics, biochemically quite different from wt-SOD1, such as hydrophobicity, aggregation [75], and aberrantly increased affinity for various proteins [76]. Tobisawa et al. [77] reported that overexpression of mSOD1 induced the upregulation of Bip expression in COS7 cells and Bip expression was also upregulated in the spinal cord of L84V-SOD1 transgenic mice, suggesting that mSOD1 caused ER stress (Fig. 1). Wootz et al. [78] demonstrated that caspase-12, an ER-stress-related caspase, was activated in the G93A-SOD1 transgenic mice while neither Bip nor CHOP was upregulated in the mice. Kikuchi et al. [48] found that mSOD1, accumulated in ER, triggers ER stress in the spinal cord, but not in other tissues of G93A-SOD1 mice at the end stage of the disease. In addition, it was shown that expression of protein disulfide isomerase (PDI), an ER-localized chaperone molecule that defensively promotes protein folding, was upregulated in the spinal cord of G93A-SOD1 mice [79]. Taking these results altogether, it is reasonably hypothesized that misfolded mSOD1, accumulated in the ER of spinal cord motor neurons, may trigger ER stress and causes neurotoxicity.

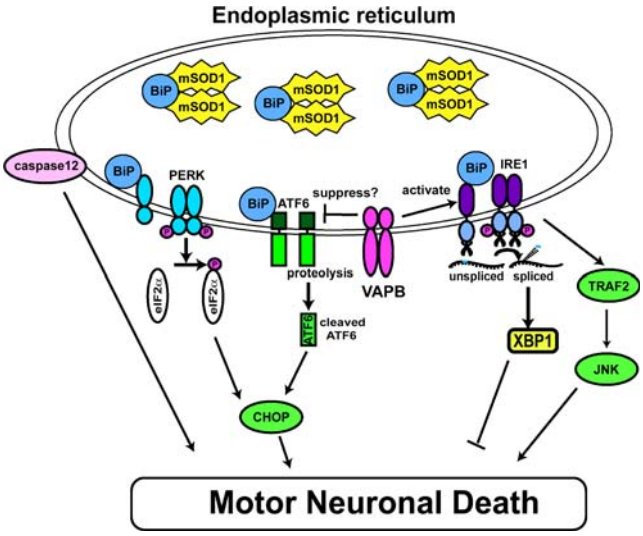

Fig. 1 ER stress- and ALS-related motoneuronal cell death. In the endoplasmic reticulum, Bip binds to mSOD1 that tends to be misfolded, which causes increase in free PERK, ATF6, and IRE1. Free PERK, ATF6, and IRE1 then triggers downstream signal transduction pathways, ultimately leading to motoneuronal cell death. XBP1, downstream of IRE1, is thought to exert an inhibitory effect on motoneuronal cell death. VAPB induces the activation of the IRE1/XBP pathway [86, 93] while it might suppress the ATF6 pathway [94]. The familial ALS-linked VAPB mutant, P56S-VAPB, is a loss-of-function mutant and simultaneously inhibits the function of coexpressed wild-type VAPB [86, 87, 93]

Several investigators provide hypotheses to explain how mutant SOD1 causes ER stress. Atkin et al. [79] reported that mSOD1 inhibits PDI, which may lead to the ultimate induction of apoptosis. Recently, Nishitoh et al. [80] demonstrated that by causing dysfunction of an ER-resident protein Derlin-1 that is one of the essential component of ERAD, mSOD1 induced ER stress-mediated cell death via apoptosis-stimulating kinase-1 (ASK1). Elimination of the *ASK1* gene ameliorates motor neuron loss of G93A-SOD1 transgenic mice. A BH3-only proapoptotic Bcl-2 family protein PUMA is also known to play a pivotal role in neuronal cell death caused by ER stress [81]. To examine the contribution of PUMA in the SOD1-related ALS,

Kieran et al. showed that disruption of the *PUMA* gene delayed disease progression [82]. Because its beneficial effect was limited to a minor extent, however, it was assumed that other proapoptotic Bcl-2 family proteins such as Bim and Bid may also be involved in the mutant SOD1-related ER stress.

VAPB/ALS8

Further evidence linking ER stress to ALS was provided by the discovery of a novel ALS-causative gene numbered ALS8. In 2004, a Brazilian group identified vesicle-associated membrane protein-associated protein B (VAPB) as an autosomal-dominant ALS-causative gene from a large Brazilian family [16]. Only one point mutation (Pro56Ser) is known to cause autosomal-dominant ALS and adult-onset spinal muscular atrophy for a total of 200 patients who share a common ancient ancestor [83].

The VAP family proteins consisting of VAPA (synonym, VAP33), VAPB, and VAPC (a splicing variant of VAPB) in human were originally identified as homologues of vesicle-associated membrane protein (VAMP)-associated protein (VAP) whose size is 33 kDa in *Aplysia californica* (aVAP33) that is involved in the release of neurotransmitters [84, 85]. VAPB is a type II transmembrane protein localizing in the ER membrane and known to dimerize with VAPA, VAPB itself, VAMP1, and VAMP2 via the C-terminal transmembrane domain [84].

In our previous study [86], to understand the ALS pathomechanism by ALS8, we biochemically and biologically characterized ALS-linked P56S-VAPB, compared with wt-VAPB, and found clear differences between them. First of all, the P56S-VAPB mutant is resistant to solubilization in buffers containing nonionic detergents such as Triton X-100. Some fraction of Triton X-100-insoluble P56S-VAPB migrated slower as supershifted ladders by SDS-PAGE analysis, indicating that it was misfolded or posttranslationally modified. Combined with the observation that P56S-VAPB tends to be polyubiquitinated, we concluded that the P56S mutation caused misfolding of VAPB. In addition, immunocytochemical analysis indicated that P56S-VAPB formed fine aggregates while wt-VAPB showed a reticulated subcellular distribution, colocalized with ER. These data together suggest that P56S-VAPB is a "loss of function" mutant. In addition to such "loss of function" phenotypes, we found that P56S-VAPB could work as a putatively dominant-negative mutant, based on the observation that P56S-VAPB selectively recruits coexpressed wt-VAPB into its insoluble aggregates. Teuling et al. [87] reported a similar phenotype of P56S-VAPB.

What is the physiological function of VAPB related to ALS development? Although several reports implied the involvement of mammalian VAPB in lipid metabolism [88], protein transport [89], and maintenance of ER structure [90], the physiological function of VAPB related to motoneuronal death remained unknown. In comparison to the physiological function of mammalian VAPB, that of the yeast VAP homologue, called suppressor of choline sensitivity 2 (Scs2), has been characterized in detail. Scs2 is reportedly involved in inositol metabolism and the ablation of the *Scs2* gene results in inositol auxotrophy, indicating that the yeast strain cannot grow in inositol-free medium [91]. On the other hand, overexpression of Scs2 recovers the inositol-auxotrophic phenotype, caused by the defect of other inositol metabolism-related genes such as *ire1* and *hac1* [92]. Conversely, the deletion of the *ire1* or *hac1* gene is known to show increased vulnerability to severe ER stress in yeast cells, as reported previously [62]. We also preliminarily confirmed that the deletion of the *Scs2* gene caused increase in vulnerability to ER stress [93]. These findings together support the idea that intracellular signals of inositol metabolism and UPR are largely overlapped in yeast cells. Based on this notion, we predicted that VAPB is also involved in the UPR signal, especially in the IRE1/XBP1 (a mammalian counterpart of yeast Hac1) pathway in mammal, and demonstrated that wt-VAPB enhanced splicing of XBP1 by IRE1 (Fig. 1). Furthermore, the ALS-linked P56S-VAPB mutant completely loses the function to mediate UPR.

In contrast to our study showing that wt-VAPB is a positive regulator of UPR, Gkogkas et al. recently identified a UPR-related transcription factor, ATF6, as a novel interacter of VAPB by a yeast two-hybrid screening and demonstrated that VAPB negatively modulated the activity of ATF6 [94]. VAPB interacts with ATF6 via its cytosolic MSP domain and colocalizes with ATF6 at the ER membrane. They demonstrated that P56S-VAPB is a more potent inhibitor of ATF6 and malfunction of VAPB probably causes misregulation of UPR, resulting in motor neuronal death. Although further investigations are required, malfunction of UPR by the P56S-VAPB is worthy of attention.

Sporadic ALS and ER Stress

Further evidence indicating the involvement of ER stress in the ALS pathogenesis was shown by immunohistochemical studies of ALS autopsy cases. There are three ER pathological hallmarks of ALS: (1) chromatolysis, a destructive and fragmentative change of rough ER (also known as Nissl substance) [95]; (2) irregular enlargement of rough ER [96]; and (3) reduction in the number of ribosome on the rough ER [97]. These ALS-linked morphological changes of ER suggest the possible involve-

ment of ER stress associated with impaired protein production in ALS. A most recent study [47] indicated that, in the motor neurons in the spinal cord of ALS patients, expression levels of PDI and Bip and phosphorylation levels of eukaryotic initiation factor 2a (eIF2a) were increased. This finding indicates that ER stress occurred in the spinal motor neurons. In agreement, Oyanagi et al. [97] characterized rough ER in the motor neurons of ALS patients in detail and found that ribosomal detachment caused rough ER distention and shrinking of motor neurons in the anterior horn of the spinal cords. Furthermore, Atkin et al. [98] reported that activation of all three UPR cascades occurred in motor neurons in the spinal cords of sporadic ALS patients and levels of PDI was upregulated in the cerebrospinal fluid of ALS patients. Combined with the observations with ALS animal models, these findings support the idea that ER stress-related death is a highly possible mechanism underlying motor neuron death in ALS.

Challenge to Control UPR by Chemical Compounds

Because malfunction of UPR and disorganized ER stress may contribute to the development of ALS in addition to a variety of diseases such as diabetes mellitus, a small molecule that blocks ER stress could be a potent therapeutic drug for diverse diseases. One of those drug candidates is valproate, which has been commonly used for bipolar disorder and epilepsy. Patients suffered from bipolar disorders often need long-term pharmacotherapy with mood-stabilizing drugs such as valproate and lithium chloride while the molecular mechanism that underlies its clinical efficacy remains elusive. This is because of the fact that a variety of intracellular signals are triggered by valproate. Wang et al. [99] found that upregulation of BiP is induced by valproate. Recent reports demonstrated that valproate also induced other ER chaperones such as GRP94 and calreticulin [100]. These facts suggest that valproate may be capable of suppressing ER stress. In fact, Kim et al. [101] reported that valproate protects cells from ER-induced lipid accumulation and apoptosis by inhibiting glycogen synthase kinase-3. Shao et al. [102] also found that chronic treatment of lithium, another traditional mood-stabilizing drug, induced Bip, GRP94, and calreticulin, whereas that of carbamazepine or lamotrigine did not. Interestingly, a single nucleotide polymorphism (−116C→G) in the promoter region of XBP1 was reported to serve a risk factor of bipolar disorder [103]. This mutation affects the putative XBP1 binding sequence and probably impairs a positive feedback loop of XBP1. All these findings indicate that UPR is involved in the bipolar disorder and mood-stabilizing drugs could be also potent drugs for ER stress-mediated diseases. Screening of small compounds that conquers ER stress has been performed by several groups and some attractive drug candidates such as salubrinal have been identified. Salubrinal, identified by Boyce et al. [104], is a specific inhibitor for dephosphorylation of eIF2α and this molecule protects cells against ER stress-induced apoptosis.

Conclusion

A variety of neurodegenerative disease-related insults were reported to cause protein misfolding and severe ER stress *in vitro* and *in vivo*. For example, Alzheimer's disease-related presenilin mutant impairs UPR [105]. As a result, neurons harboring the mutant become vulnerable to ER stress. Parkinson's disease-causing genes products, synuclein and parkin, are involved in ER stress-mediated dopaminergic cell death *in vitro* and *in vivo* [106, 107]. Similar to these diseases, familial ALS-related gene may cause ER stress. Several studies show that ER stress occurs in motor neurons in the spinal cord of human ALS patients. Further investigation of ER stress-related neurotoxicity in ALS will open a new horizon to cure this fatal disease.

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References

1. Bruijn LI et al (2004) Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu Rev Neurosci 27:723–749
2. Kato S et al (1999) Recent advances in research on neuropathological aspects of familial amyotrophic lateral sclerosis with superoxide dismutase 1 gene mutations: neuronal Lewy body-like hyaline inclusions and astrocytic hyaline inclusions. Histol Histopathol 14:973–989
3. Toyoshima I et al (1989) Phosphorylation of neurofilament proteins and localization of axonal swellings in motor neuron disease. J Neurol Sci 89:269–277
4. Nakano I, Hirano A (1987) Atrophic cell processes of large motor neurons in the anterior horn in amyotrophic lateral sclerosis: observation with silver impregnation method. J Neuropathol Exp Neurol 46:40–49
5. Mourelatos Z et al (1994) Fragmentation of the Golgi apparatus of motor neurons in amyotrophic lateral sclerosis revealed by organelle-specific antibodies. Am J Pathol 144:1288–1300
6. Kawahara Y et al (2004) Glutamate receptors: RNA editing and death of motor neurons. Nature 427:801

7. Niebroj-Dobosz I et al (2006) Auto-antibodies against proteins of spinal cord cells in cerebrospinal fluid of patients with amyotrophic lateral sclerosis (ALS). Folia Neuropathol 44:191–196
8. Yasui M et al (1992) Magnesium and calcium contents in CNS tissues of amyotrophic lateral sclerosis patients from the Kii peninsula, Japan. Eur Neurol 32:95–98
9. Cox PA et al (2003) Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc Natl Acad Sci USA 100:13380–13383
10. Kurtzke JF et al (1982) Epidemiology of amyotrophic lateral sclerosis. Adv Neurol 36:281–302
11. Horner RD et al (2003) Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. Neurology 61:742–749
12. Rosen DR et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62, (Erratum in: Nature 364:362)
13. Yang Y et al (2001) The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat Genet 29:160–165
14. Hadano S et al (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat Genet 29:166–173
15. Chen YZ et al (2004) DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am J Hum Genet 74:1128–1135
16. Nishimura AL et al (2004) A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am J Hum Genet 75:822–831
17. Münch C et al (2005) Heterozygous R1101K mutation of the DCTN1 gene in a family with ALS and FTD. Ann Neurol 58:777–780
18. Greenway MJ et al (2006) ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. Nat Genet 38:411–413
19. Sreedharan J et al (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319:1668–1672
20. Johnston JA et al (2000) Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 97:12571–12576
21. Niwa J et al (2002) Dorfin ubiquitylates mutant SOD1 and prevents mutant SOD1-mediated neurotoxicity. J Biol Chem 277:36793–36798
22. Liochev SI, Fridovich I (2003) Mutant Cu,Zn superoxide dismutases and familial amyotrophic lateral sclerosis: evaluation of oxidative hypotheses. Free Radic Biol Med 34:1383–1389
23. Deng HX et al (1993) Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. Science 261:1047–1051
24. Bowling AC et al (1995) Superoxide dismutase concentration and activity in familial amyotrophic lateral sclerosis. J Neurochem 64:2366–2369
25. Borchelt DR et al (1994) Superoxide dismutase 1 with mutations linked to familial amyotrophic lateral sclerosis possesses significant activity. Proc Natl Acad Sci USA 91:8292–8296
26. Reaume AG et al (1996) Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. Nat Genet 13:43–47
27. Gurney ME et al (1994) Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 264:1772–1775, (Erratum in: Science 269:149)
28. Kato S et al (1996) Familial amyotrophic lateral sclerosis with a two base pair deletion in superoxide dismutase 1: gene multisystem degeneration with intracytoplasmic hyaline inclusions in astrocytes. J Neuropathol Exp Neurol 55:1089–1101
29. Bruijn LI et al (1997) ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron 18:327–338
30. Becher MW et al (1998) Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. Neurobiol Dis 4:387–397
31. Trojanowski JQ, Lee VM (1998) Aggregation of neurofilament and alpha-synuclein proteins in Lewy bodies: implications for the pathogenesis of Parkinson disease and Lewy body dementia. Arch Neurol 55:151–152
32. Urushitani M et al (2002) Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. J Neurochem 83:1030–1042
33. Liu D et al (1999) The roles of free radicals in amyotrophic lateral sclerosis: reactive oxygen species and elevated oxidation of protein, DNA, and membrane phospholipids. FASEB J 13:2318–2328
34. Pasinelli P et al (2004) Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. Neuron 43:19–30
35. Goldsteins G et al (2008) Deleterious role of superoxide dismutase in the mitochondrial intermembrane space. J Biol Chem 283:8446–8452
36. Witan H et al (2008) Heterodimer formation of wild-type and amyotrophic lateral sclerosis-causing mutant Cu/Zn-superoxide dismutase induces toxicity independent of protein aggregation. Hum Mol Genet 17:1373–1385
37. Prudencio M et al (2009) Modulation of mutant superoxide dismutase 1 aggregation by co-expression of wild-type. J Neurochem 103(4):1009–1018
38. Zhang F et al (2007) Interaction between familial amyotrophic lateral sclerosis (ALS)-linked SOD1 mutants and the dynein complex. J Biol Chem 282:16691–16699
39. Harraz MM et al (2008) SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model. J Clin Invest 118:659–670
40. Graves M et al (2004) Inflammation in amyotrophic lateral sclerosis spinal cord and brain is mediated by activated macrophages, mast cells and T cells. Amyotroph Lateral Scler Other Motor Neuron Disord 5:213–219
41. Boillée S et al (2006) ALS: a disease of motor neurons and their nonneuronal neighbors. Neuron 52:39–59
42. Sasabe J et al (2007) D-serine is a key determinant of glutamate toxicity in amyotrophic lateral sclerosis. EMBO J 26:4149–4159
43. Cudkowicz ME et al (2006) Trial of celecoxib in amyotrophic lateral sclerosis. Ann Neurol 60:22–31
44. Gordon PH et al (2007) Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. Lancet Neurol 6:1045–1053
45. Miller R et al (2007) Phase II/III randomized trial of TCH346 in patients with ALS. Neurology 69:776–784
46. Shefner JM et al (2004) A clinical trial of creatine in ALS. Neurology 63:1656–1661
47. Ilieva EV et al (2007) Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain 130:3111–3123
48. Kikuchi H et al (2006) Spinal cord endoplasmic reticulum stress associated with a microsomal accumulation of mutant superoxide dismutase-1 in an ALS model. Proc Natl Acad Sci USA 103:6025–6030
49. Gass JN et al (2002) Activation of an unfolded protein response during differentiation of antibody-secreting B cells. J Biol Chem 277:49047–49054
50. Lipson KL et al (2006) Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. Cell Metab 4:245–254

51. Oyadomari S, Mori M (2004) Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Diff 11:381–389
52. Schubert U et al (2000) Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 404:770–774
53. Ellgaard L et al (1999) Setting the standards: quality control in the secretory pathway. Science 286:1882–1888
54. Isler JA et al (2005) Human cytomegalovirus infection activates and regulates the unfolded protein response. J Virol 79:6890–6899
55. Lee AS (1992) Mammalian stress response: induction of the glucose-regulated protein family. Curr Opin Cell Biol 4:267–273
56. Feldman DE et al (2005) The unfolded protein response: a novel component of the hypoxic stress response in tumors. Mol Cancer Res 3:597–605
57. Bartoszewski R et al (2008) Activation of the unfolded protein response by F508 CFTR. Am J Respir Cell Mol Biol 39:448–457
58. Ito D, Suzuki N (2009) Seipinopathy: a novel endoplasmic reticulum stress-associated disease. Brain 132(1):8–15
59. Cox JS, Walter P (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell 87:391–404
60. Nikawa J, Yamashita S (1992) IRE1 encodes a putative protein kinase containing a membrane-spanning domain and is required for inositol phototrophy in *Saccharomyces cerevisiae*. Mol Microbiol 6:1441–1446
61. Sidrauski C, Walter P (1997) The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell 90:1031–1039
62. Nikawa J et al (1996) Saccharomyces cerevisiae IRE2/HAC1 is involved in IRE1-mediated KAR2 expression. Nucleic Acids Res 24:4222–4226
63. Kohno K (2007) How transmembrane proteins sense endoplasmic reticulum stress. Antioxid Redox Signal 9:2295–2303
64. Bertolotti A et al (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2:326–332
65. Ye J et al (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell 6:1355–1364
66. Harding HP et al (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397:271–274
67. Harding H et al (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 6:1099–1108
68. Werner ED et al (1996) Proteasome-dependent endoplasmic reticulum-associated protein degradation: an unconventional route to a familiar fate. Proc Natl Acad Sci USA 93:13797–13801
69. Urano F et al (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 287:664–666
70. Zinszner H et al (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 12:982–995
71. Guegan C et al (2001) Recruitment of the mitochondrial-dependent apoptotic pathway in amyotrophic lateral sclerosis. J Neurosci 21:6569–6657
72. Wong PC et al (1995) An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. Neuron 14:1105–1116
73. Mourelatos Z et al (1996) The Golgi apparatus of spinal cord motor neurons in transgenic mice expressing mutant Cu,Zn superoxide dismutase becomes fragmented in early, preclinical stages of the disease. Proc Natl Acad Sci USA 93:5472–5477
74. Stieber A et al (2000) Aggregation of ubiquitin and a mutant ALS-linked SOD1 protein correlate with disease progression and fragmentation of the Golgi apparatus. J Neurol Sci 173:53–62
75. Tiwari A et al (2005) Aberrantly increased hydrophobicity shared by mutants of Cu,Zn-superoxide dismutase in familial amyotrophic lateral sclerosis. J Biol Chem 280:29771–29779
76. Kanekura K et al (2004) Alsin, the product of ALS2 gene, suppresses SOD1 mutant neurotoxicity through RhoGEF domain by interacting with SOD1 mutants. J Biol Chem 279:19247–19256
77. Tobisawa S et al (2003) Mutant SOD1 linked to familial amyotrophic lateral sclerosis, but not wild-type SOD1, induces ER stress in COS7 cells and transgenic mice. Biochem Biophys Res Commun 303:496–503
78. Wootz H et al (2006) XIAP decreases caspase-12 cleavage and calpain activity in spinal cord of ALS transgenic mice. Exp Cell Res 312:1890–1898
79. Atkin JD et al (2006) Induction of the unfolded protein response in familial amyotrophic lateral sclerosis and association of protein-disulfide isomerase with superoxide dismutase 1. J Biol Chem 281:30152–30165
80. Nishitoh H et al (2008) ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. Genes Dev 22:1451–1464
81. Reimertz C et al (2003) Gene expression during ER stress-induced apoptosis in neurons: induction of the BH3-only protein Bbc3/PUMA and activation of the mitochondrial apoptosis pathway. J Cell Biol 162:587–597
82. Kieran D et al (2007) Deletion of the BH3-only protein puma protects motoneurons from ER stress-induced apoptosis and delays motoneuron loss in ALS mice. Proc Natl Acad Sci USA 104:20606–20611
83. Nishimura AL et al (2005) A common founder for amyotrophic lateral sclerosis type 8 (ALS8) in the Brazilian population. Hum Genet 118:499–500
84. Nishimura Y et al (1999) Molecular cloning and characterization of mammalian homologues of vesicle-associated membrane protein-associated (VAMP-associated) proteins. Biochem Biophys Res Commun 254:21–26
85. Skehel PA et al (1995) A VAMP-binding protein from Aplysia required for neurotransmitter release. Science 269:1580–1583
86. Kanekura K et al (2006) Characterization of amyotrophic lateral sclerosis-linked P56S mutation of vesicle-associated membrane protein-associated protein B (VAPB/ALS8). J Biol Chem 281:30223–30233
87. Teuling E et al (2007) Motor neuron disease-associated mutant vesicle-associated membrane protein-associated protein (VAP) B recruits wild-type VAPs into endoplasmic reticulum-derived tubular aggregates. J Neurosci 27:9801–9815
88. Kawano M et al (2006) Efficient trafficking of ceramide from the endoplasmic reticulum to the Golgi apparatus requires a VAMP-associated protein-interacting FFAT motif of CERT. J Biol Chem 281:30279–30288
89. Wyles JP et al (2002) Vesicle-associated membrane protein-associated protein-A (VAP-A) interacts with the oxysterol-binding protein to modify export from the endoplasmic reticulum. J Biol Chem 277:29908–29918
90. Amarilio R et al (2005) Differential regulation of endoplasmic reticulum structure through VAP-Nir protein interaction. J Biol Chem 280:5934–5944
91. Kagiwada S et al (1998) The *Saccharomyces cerevisiae* SCS2 gene product, a homolog of a synaptobrevin-associated protein, is an integral membrane protein of the endoplasmic reticulum

and is required for inositol metabolism. J Bacteriol 180:1700–1708
92. Nikawa J et al (1995) Cloning and sequence of the SCS2 gene, which can suppress the defect of INO1 expression in an inositol auxotrophic mutant of *Saccharomyces cerevisiae*. J Biochem 118:39–44
93. Suzuki H et al (2009) ALS-linked P56S-VAPB, an aggregated loss-of-function mutant of VAPB, predisposes motor neurons to ER stress-induced death by inducing aggregation of co-expressed wild-type VAPB. J Neurochem 108(4):973–985
94. Gkogkas C et al (2008) VAPB interacts with and modulates the activity of ATF6. Hum Mol Genet 17:1517–1526
95. Cruz-Sánchez FF et al (1993) Amyotrophic lateral sclerosis brain banking: a proposal to standardize protocols and neuropathological diagnostic criteria. J Neural Transm Suppl 39:215–222
96. Wakayama I (1992) Morphometry of spinal motor neurons in amyotrophic lateral sclerosis with special reference to chromatolysis and intracytoplasmic inclusion bodies. Brain Res 586:12–18
97. Oyanagi K (2008) Spinal anterior horn cells in sporadic amyotrophic lateral sclerosis show ribosomal detachment from, and cisternal distention of the rough endoplasmic reticulum. Neuropathol Appl Neurobiol 34(6):650–658
98. Atkin JD et al (2008) Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. Neurobiol Dis 30:400–407
99. Wang JF et al (1999) Differential display PCR reveals novel targets for the mood-stabilizing drug valproate including the molecular chaperone GRP78. Mol Pharmacol 55:521–527
100. Bown CD et al (2000) Increased expression of endoplasmic reticulum stress proteins following chronic valproate treatment of rat C6 glioma cells. Neuropharmacology 39:2162–2169
101. Kim AJ et al (2005) Valproate protects cells from ER stress-induced lipid accumulation and apoptosis by inhibiting glycogen synthase kinase-3. J Cell Sci 118:89–99
102. Shao L et al (2006) Mood stabilizing drug lithium increases expression of endoplasmic reticulum stress proteins in primary cultured rat cerebral cortical cells. Life Sci 78:1317–1323
103. Kakiuchi C et al (2003) Impaired feedback regulation of XBP1 as a genetic risk factor for bipolar disorder. Nat Genet 35:171–175
104. Boyce M et al (2005) A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. Science 307:935–939
105. Katayama T et al (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. Nat Cell Biol 1:479–485
106. Smith WW et al (2005) Endoplasmic reticulum stress and mitochondrial cell death pathways mediate A53T mutant alpha-synuclein-induced toxicity. Hum Mol Genet 14:3801–3811
107. Imai Y et al (2001) An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. Cell 105:891–902