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Review

ER stress and neurodegenerative diseases

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Abstract

Endoplasmic reticulum (ER) stress is caused by disturbances in the structure and function of the ER with the accumulation of misfolded proteins and alterations in the calcium homeostasis. The ER response is characterized by changes in specific proteins, causing translational attenuation, induction of ER chaperones and degradation of misfolded proteins. In case of prolonged or aggravated ER stress, cellular signals leading to cell death are activated. ER stress has been suggested to be involved in some human neuronal diseases, such as Parkinson's disease, Alzheimer's and prion disease, as well as other disorders. The exact contributions to and casual effects of ER stress in the various disease processes, however, are not known. Here we will discuss the possible role of ER stress in neurodegenerative diseases, and highlight current knowledge in this field that may reveal novel insight into disease mechanisms and help to design better therapies for these disorders.

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Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β-peptide; ALS, amyotrophic lateral sclerosis; ASK1, apoptosis signal-regulating kinase-1; ER, endoplasmic reticulum; FAD, familial AD; FALS, familial ALS; GRP78, 78 Kda glucose-regulated protein; HD, Huntington's disease; IP3-R, inositol 1,4,5-triphosphate receptor; JNK, c-Jun NH2-terminal kinase; LSD, lysosomal storage diseases; NCL, neuronal ceroid lipofuscinoses; Pael-R, Pael receptor; PD, Parkinson's disease; PMD, Pelizaeus-Merzbacher disease; polyQ, polyglutamine; PS-1, presenilin-1; PS-2, presenilin-2; PrP, prion protein; RING, really interesting new gene; SCA, spinocerebellar ataxia; SOD1, Cu/Zn superoxide dismutase; TSE, transmissible spongiform encephalopathy; UCHL-1, ubiquitin carboxyl-terminal hydrolase-1; UPS, ubiquitin proteasome system; UPR, unfolded protein response; 6-OHDA, 6-hydroxydopamine

Introduction

Endoplasmic reticulum (ER) is an important organelle for the synthesis, correct folding, post-translation modification and transport of nascent proteins to different destinies.[1–3] The ER exerts a quality control of proteins ensuring correct handling of the final product that is crucial for normal cell function.[1–3] Disturbance in the function or loss of integrity of the ER leads to ER stress that can be caused, for example, by accumulation of unfolded proteins and by changes in calcium homeostasis within the ER. ER stress activates signaling pathways including the unfolded protein response (UPR) that counteracts the effects of the original stress having either an environmental or genetic cause.[1–3] The UPR and its signaling components are described in great detail in recent reviews. In general, the UPR changes the expressions of specific proteins, such as those for the ER chaperones, enhances degradation of misfolded (mutant or unfolded) protein, and inhibits protein synthesis to decrease the load within the ER.[1–3] However, if the function of the ER is severely impaired, genes and pathway leading to cell death and/or inhibition of survival are also activated.[1–3] The cellular signals involved in ER stress-mediated cell death and apoptosis are complex and yet not fully understood.[2,3] It is known that disturbed functions of the ubiquitin proteasome system (UPS), responsible for the degradation of cytosolic, ER and synaptic proteins, can contribute to ER stress.[4–6]

A common sign of many neurodegenerative diseases is the accumulation and deposits of misfolded proteins that affects various cell signaling systems, as well as neuronal connectivity and cell death.[7,8] It is generally thought that the activity of UPS is mitigated in these diseases either by the protein aggregates or by enhanced oxidative stress and other toxic products.[7,8] Dysfunctional UPS in turn cause more accumulation of proteins in the cell leading also to ER stress and aggravation of the disease.[4,5,9] To this come effects of environmental toxins, reactive oxygen species (ROS) and other signals that influence mitochondria and lead to activation of the caspase family of cysteine proteases causing cell death.[2,3,10] Level of intracellular calcium and calcium release from the ER are important in ER–mitochondrial interactions and in the control of cell death (Figure 1).[11] In the following, we will review studies on ER stress and its function in different neurodegenerative diseases by analyzing data gathered about molecular mechanisms and cellular pathways involving the ER and discuss how these cellular events may contribute to the disease process in human neurological disorders.

ER Stress and Cell Death Pathways in Neurons

Death of neurons, like that of other cells, is regulated by in principle two pathways.[12,13] The extrinsic pathway is caused by the activity of cell membrane death receptors through

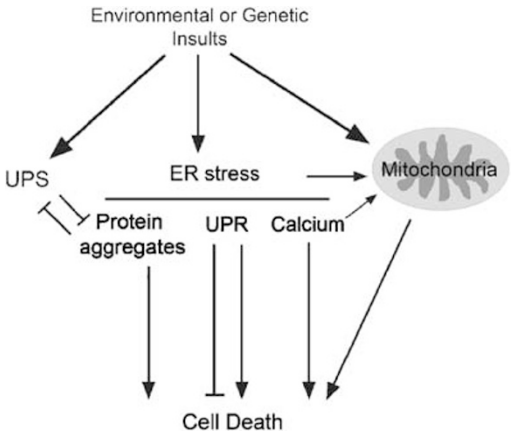

Figure 1 ER stress and neurodegenerative disease. Schematic view of changes and interactions during ER stress and the link to the ubiquitin proteasome system (UPS). The ER stress response is triggered by insults underlying also various neurodegenerative disorders. These include accumulation of abnormal or misfolded proteins in the ER and cytosol, oxidative injury, impaired calcium handling and a dysfunctional UPS. The unfolded protein response (UPR) serves to protect the ER and restores function by inducing chaperons, blocking translation and increasing protein folding in the ER. Prolonged ER stress causes further accumulation of aberrant proteins in the ER that constitutes a *circulus vitiosus* with inhibition of the proteasome activity, protein deposits and an enhanced calcium release that ultimately leads to activation of cell death pathways (see text for details). The ER communicates with mitochondria and sustained ER stress augments signals for executions of cell death. ER-mediated signals to the nucleus are also important but not depicted here. The cellular pathways elicited by the ER responses vary between the different disorders

activation of caspase-8 that cleaves downstream substrates, including other caspases.[10,14] Various stressors and insults that converge on the mitochondria trigger the intrinsic pathway. These evoke changes in membrane permeability transition with release of cytochrome-*c* and other proapoptotic molecules from the inner membrane space.[14] Cytochrome-*c* binds to the protein, Apaf-1, and activates caspase-9 and subsequently caspase-3. The two pathways converge on caspase-3 and they can augment each other, as exemplified by truncated Bid that after caspase-8 cleavage acts on the mitochondria.[10] In the nervous system, the extrinsic cell death pathway plays a less prevailing role compared with for example the immune system. However, in brain inflammatory responses activation of death receptors do occur with enhanced caspase-8 cleavage. Inflammation is also a part of many neurodegenerative disorders and contributes to the overall disease process.[15] Apart from the classical caspase-dependent cell death, other types of death mechanisms have also been described in various cell types including neurons that may or may not involve the mitochondria.[13]

To combat cell death neurons express a variety of antiapoptotic proteins that counteract cell degeneration caused by both environmental and genetic insults.[12,13] The multidomain Bcl-2 family consists of both antiapoptotic, such as Bcl-2 and Bcl-xL and proapoptotic proteins, as exemplified by Bax, Bak and Bik.[14] Bcl-2 controls the integrity of the mitochondrial membrane under normal conditions.[14] BH-3 only proteins are proapoptotic and induce cell death either by inhibiting Bcl-2 or inducing oligomerization of Bax.[14] It has been shown that some BH-3 protein, such as Bik and the recently cloned Spike are largely localized to the ER.[16,17] In addition, the BH-3 protein, Bim translocates to the ER membrane and is important for ER stress-mediated cell death.[18] However, little is so far known about the roles of BH-3 only proteins in ER stress-induced neuronal death or in human neurodegenerative disorders.

Recently, it has been recognized that there exists an important crosstalk between the ER and mitochondria in the execution of cell death.[2,3] It has been suggested that the Bcl-2 family proteins play a crucial role in ER–mitochondria interactions, and Bcl-2 and Bcl-xL associate with mitochondria and with the ER membrane.[2,3] Bcl-2 in conjunction with Bax and Bak, and with ER calcium channels, such as the inositol 1,4,5-triphosphate receptors (IP3-R), can regulate ER calcium levels and release into the cytosol.[19] Intracellular levels of calcium are of ultimate importance for many neuronal functions including cell death.[11] The sensitivity of the mitochondria to induce cell death varies with the amounts of calcium, ROS and other metabolites present in the cell.[11,14] Apart from Bcl-2 proteins, other molecules that take part in the ER–mitochondria communications have been identified.[2,3] Bap31 is a Bcl-2-binding transmembrane protein, important for protein trafficking.[2] Bap31 can be cleaved by caspases, release calcium from the ER, and subsequently cytochrome-*c* from the mitochondria.[2] Pacs-2 is a novel gene that influences various ER functions and cell death with participation of the mitochondria.[20] The significance of these proteins in ER stress in neurological diseases are, however, so far unknown. Studies have shown that the ER harbors caspase-12 that can be specifically cleaved/activated during ER stress.[21] Caspase-12 could in turn activate downstream caspases inducing cell death.[22,23] The association of caspase-12 with human diseases is not clear, as the gene shows large deletions in the human genome.[10,24] It is possible that other caspases take over the function of caspase-12 in humans, as suggested for the human caspase-4.[25] However, the role of caspases-12 and -4 as initiators of ER stress-induced cell death is a matter of current debate.[24] Procaspase-2 and a novel procaspase-8 isoform are also associated with the ER in some cell types.[2]

Apart from Bap31, other ER proteins can be cleaved by caspases during cell death. These include the IP3 receptors, and the presenilin-1 (PS1) and -2 (PS2) that are ER transmembrane proteins important in Alzheimer's disease (AD).[26] Presenilins are antiapoptotic but loose this ability after caspase cleavage. Figure 2 shows schematically some known ER proteins and how they are coupled to the function of the ER in cell death control. Apart from these proteins, ER stress and caspase-12 activation in neurons is influenced by the presence of the neuronal calcium-binding protein, hippocalcin and by upstream signals elicited by brain-derived neurotrophic factor.[27,28] There are probably other proteins and interactions yet to be discovered that take part in the ER–mitochondria crosstalk and in the control of cell death by the ER.

ER Stress and Parkinson's Disease

Parkinson's disease is a progressive degenerative disease with loss of dopaminergic neurons in the substantia nigra pars

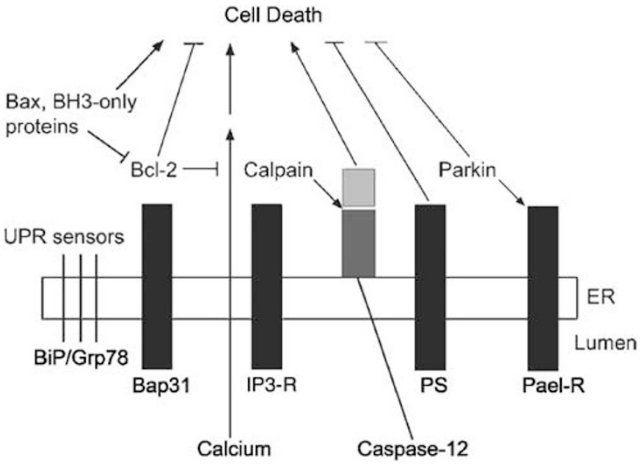

Figure 2 ER proteins in control of neuronal death. Bcl-2 in conjunction with IP3 receptors (IP3-R) control ER calcium stores and release into the cytosol. Higher calcium levels sensitize mitochondria to other insults inducing cell death. Bax and the BH3-only proteins inhibit Bcl-2. Bcl-2 binds Bap31 that normally exerts an antiapoptotic function in the ER. The various ER chaperones, such as Bip/Grp78 and Grp84 are protective and control protein folding and components of the UPR. ER specific caspases, such as the caspase-12, is thought to directly induce cell death. The calcium-dependent enzyme, calpain actives caspase-12, as shown in some cells. Presenilins (PS) are transmembrane proteins that in mutant forms contribute to AD. Pael receptor (Pael-R) is a putative G-protein-coupled membrane protein that tends to unfold and cause ER stress. Pael-R is a substrate for the ubiquitin E3 ligase, Parkin that is mutated in early onset PD with impaired proteasomal function. The exact roles of the different ER proteins in cell death control in various neurodegenerative disorders remain to be studied further. See text for details

compacta, with the presence of intraneuronal cytoplasmic inclusion bodies, known as Lewy bodies in the neurons.[29] The mechanisms behind the selective neuronal death in PD are not fully understood.[29,30] There is evidence that excitotoxicity together with disturbed energy metabolism; decreased activity of complex one in mitochondria, in addition to dysfunction of the UPS can contribute to dopaminergic neuronal death.[29,30] Environmental factors, such as pesticides and other conditions have been suggested to cause sporadic PD, which constitutes more than 90% of the patients.[29,31] Recent studies discussed below indicate that also ER stress in conjunction with abnormal protein degradation can contribute to the pathophysiology of PD.

Studies of rare familial forms of PD have revealed cellular pathways and mechanisms important for the disease as whole.[30] Proteins that have been linked to autosomal recessive or dominant familial PD include α-synuclein, Parkin, DJ-1, PINK1, ubiquitin carboxyl-terminal hydrolase-1 (UCHL-1) and the recently cloned LRRK2/dardarin.[29,30,32,33] The mechanisms by which mutations in these genes cause PD vary, but the signals evoked converge on cellular functions concerned with mitochondria, and protein handling in the UPS.[30] Recent identification of PINK1 and LRRK2/dardarin suggest that alterations in protein kinase activities may contribute to PD.[29,32] α-Synuclein is found in the Lewy bodies and the mutant protein aggregate and can inhibit the UPS.[30] Genetic data on families with extra copies of α-synuclein showed that wild-type protein could cause disease, indicating that α-synuclein can achieve a gain of function mechanism in PD.[30] UCHL-1 is part of the UPS and possesses both hydroxylase and ubiquitin ligase activities.[30] Parkin contains an aminoterminal ubiquitin-like domain and two really interesting new gene (RING) domains that function as an E3-ubiquitin ligase for protein degradation in the UPS.[33] Parkin is frequently mutated in early-onset PD with loss of ligase function.[33] The function of Parkin can be compromised by modifications induced by increased ROS, oxidative *S*-nitrosylation or by the protein becoming sequestered within the cells.[33] Recently, the Bcl-2-associated athanogen 5 protein was shown to bind Parkin thus inhibiting its activity.[34] Protein targets for Parkin ligase activity have been described and include the Pael receptor (Pael-R) that is a putative G-protein-coupled transmembrane protein.[5,33] Pael-R can misfold and form aggregates and the protein is also found in Lewy bodies, suggesting a direct function in PD.[33,35] Parkin suppresses Pael-R-induced toxicity by ubiquitination and degradation of the protein and can protect dopaminergic neurons against various insults.[33] Expression of Parkin can restore proteasome function and promote survival, while loss of function of Parkin causes ER stress with accumulation of cytotoxic fibrils and protein aggregates in cells.[33] Parkin is abundantly expressed within mesenchephalic dopaminergic neurons that may explain part of their selective vulnerability.[33] It is not clear whether the observed ER stress in PD is largely neuroprotective or whether it directly contributes to the disease process.

Another piece of evidence for the involvement of ER stress in PD, comes from studies of certain neurotoxins that are used as model compounds to mimic the disease process both in cell culture and *in vivo*.[36,37] Compounds, such as 6-hydroxydopamine (6-OHDA) and *N*-methyl-4-phenyl-1, 2,3,6-tetrahydroyridine or its active derivative, MPP+, induce oxidative stress and impair mitochondrial respiration and energy metabolism. Recent studies with cultured neuronal cells, including dopaminergic neurons, showed that these compounds trigger ER stress and induce a number of genes.[36,37] Gene profiling revealed that both ER chaperones and other components of the UPR such as the transcription factor, CHOP/Gadd153 were upregulated in exposed cells, in addition to the phosphorylation of the ER stress kinases, IRE and PERK.[36,37] These changes were specific for the two PD mimetic and rotenone, a mitochondrial inhibitor that causes degeneration of dopaminergic neurons but not with other agents.[37] There were some differences in the magnitude and pattern of gene responses induced by 6-OHDA and MPP+, but both compounds clearly elicited an UPR in the neurons.[36] Experiments carried out using neuronal cultures from Perk gene deleted mice revealed an increased sensitivity of the cells against treatment with 6-OHDA.[37] This suggests that neurons lacking Perk were unable to mount a proper UPR and are more vulnerable to 6-OHDA. It also supports the notion that an early UPR response may be neuroprotective for the dopaminergic neurons, while a sustained ER stress later leads to an upregulation of gene products that induce cell death. Study of the molecular basis for such a switch in the ER responses in dopaminergic neurons and its role in the pathophysiology of PD deserves close scrutiny.

ER Stress and AD

AD is a devastating neurodegenerative disease characterized by the progressive decline of cognitive functions.[26] In AD, there is a loss of neurons in different brain regions among others within frontal cortex, the hippocampus and the basal forebrain. Pathological features of brain tissue from AD patients are extracellular senile plaques formed by aggregates of amyloid β-peptide (Aβ), and filamentous intracellular structures of the protein tau, called neurofibrillary tangles (NFT).[26] AD is divided into two classes, familial AD (FAD) with early onset disease and sporadic AD occurring later in life. Mutations in the Aβ precursor protein (APP), and the Presenilins, PS1 and PS2 are associated with FAD.[26,38] Mutations in these proteins cause alterations in the processing of Aβ from APP with the production of increased amounts or more toxic forms of Aβ in the plaques.[26,38] The reasons for the altered processing of APP in sporadic AD is not known but increased intracellular levels of calcium together with other insults probably play a role.[39] Fibrillar Aβ is toxic to neuronal cells and produces high amounts of ROS with the impairment of the mitochondrial redox activity.[39,40]

Recent studies have shown an involvement of ER stress and disturbed calcium homeostasis in AD.[11,39,41] Analyses of brain tissue from AD patients revealed alterations in calcium metabolism that are associated with the neurodegenerative process. Neurons containing NFT showed an increase in the levels of free and protein bound calcium compared with tangle free neuron.[11,12,39] Changes in calcium may be early events in AD as shown in studies on transgenic mice expressing mutant forms of APP or PS proteins.[11,12,39] Whether elevated cell calcium is the cause or a consequence of the increase in Aβ in AD remains to be investigated.

The PS1 and PS2 proteins are parts of the multiprotein γ-secretase complex that mediates the intramembranous cleavage of APP, as well as of Notch and some other proteins.[38] Mutations in these proteins cause changes in the pattern of APP processing in the cell and increases the amount of the more toxic Aβ-42 peptide.[38] The PS1 and PS2 proteins are abundantly expressed by brain neurons and are ER transmembrane proteins that provide a link between AD and ER stress.[38] Cells expressing PS1 mutants show altered calcium homeostasis, increased production Aβ, and an enhanced sensitivity to apoptosis induced by ER stress.[11,12,39] Likewise, PS1 mutant knock in mice displays abnormalities in ER calcium regulation and an increased vulnerability of neurons towards cell death and excitotoxic injury.[12] Mutant PS1 also binds and inhibit the ER kinase, IRE1 that sense the accumulation of misfolded proteins in the ER lumen.[1–3] IRE activates downstream signals and the transcription of the ER chaperone, 78 kDa glucose-regulated protein (GRP78), also called BiP.[41] Mutant PS1 suppresses the activation of UPR, indicating that the responsiveness of the ER is reduced in the presence of mutant PS1.

The PS and APP proteins are also targets for activated caspases during cell death. Thus, PS1 and PS2 are cleaved in the C-terminal region that leads to loss of the antiapoptotic activity of the wild-type proteins.[2] This can probably impair the function of the ER and accelerate the disease process. The role of caspase cleavage of wild-type and mutant APP is covered in great detail elsewhere.[42]

Kinetic studies on the aggregation of Aβ suggest that intermediate oligomers of Aβ are the primary neurotoxic agents in AD.[26] These can impair various signaling and synaptic functions of the neuron by influencing intracellular calcium levels and cellular membranes.[26,43] The role of ER in these processes remain to be studied in more detail.

Cells from caspase-12 gene deleted mice showed reduced susceptibility towards ER stress.[21] Particularly, cultured cortical neurons were resistant to death caused by the Aβ.[21] This indicates that ER stress and activation of caspase-12 may contribute to neuronal death in AD. However, as the presence of caspase-12 is less likely in human neurons, related molecules with similar functions may be present. Human caspase-4 shows similar characteristics to mouse caspase-12 and is localized to the ER and to the mitochondria. Caspase-4 is increased in AD brains, suggesting that this caspase may play a role in ER stress-induced cell death.[25]

Recent studies on the UPR revealed increased levels of Bip/Grp78 and the protein kinase, Perk in AD brain.[44] This shows the occurrence of ER stress in AD although the initial changes in these UPR mediators may reflect neuroprotection rather than nerve cell degeneration. The exact significance of the ER stress responses in AD and their relationship to ensuing neuronal death remain to be clarified.

ER Stress and Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is characterized by the degeneration of motorneurons in the spinal cord, cortex and brain stem, leading to muscle atrophy and paralysis.[45] The course of the disease is usually rapid and the underlying cause for cell demise is not fully understood. Disturbances in or a lack of appropriate neurotrophic factors, enhanced excitotoxicity, hypoxia, disturbed protein metabolism, cytoskeletal changes, elevated levels of ROS and calcium, as well as the accumulation of toxic products may all contribute to neurodegeneration.[45] Most ALS cases are sporadic, but about 5–10% of patients have an inherited variant of the disease, called familial ALS (FALS).[45] Some patients with FALS have mutations in the Cu/Zn superoxide dismutase (SOD1) enzyme that is involved in quenching ROS.[45] It is thought that the mutant SOD1 protein may acquire some adverse properties contributing to the disease process in FALS. Transgenic mice or rats expressing the human mutant SOD1 protein develop a motorneuron disease resembling ALS and are excellent models to study the disease process more closely.[45] Available data from studies on transgenic ALS mice carrying the mutant SOD1 gene, in addition to analyses of human ALS spinal cord specimens, indicate that motorneurons in ALS undergo cell degeneration with signs of apoptosis and activation of various caspases. In this respect, caspases-1, -3 and -9 have been particularly studied.[45,46] Dysfunctional mitochondria and changes in membrane integrity with release of proapoptotic molecules have been suggested to contribute to the disease progress in ALS.[45]

Recently, it was shown that mutant SOD1 aggregates in mitochondria from spinal cord in transgenic ALS and in humans.[47] Mutant SOD1 was found to interact with Bcl-2 providing a link to the regulation of motorneuron degeneration.[47] The functional role of the SOD1 aggregates in mitochondria is so far unknown. Apart from the mitochondria, less is known about the roles of other organelles and their dysfunction in ALS.

Intracellular cytoplasmic inclusions have been detected in motorneurons in transgenic ALS mice and in human samples.[45] In ALS mice these contain deposits of SOD1 but contain also other components, such as ubiquitin.[45] The aggregates can occur long prior to the debut of disease symptoms. The significance of the inclusion bodies in ALS is not clear at the moment, but they may be neurotoxic or inhibit other cellular functions.[45] Mutant SOD1 is a degraded by the E3 ubiquitin ligase, Dorfin through the UPS, and Dorfin localizes to the inclusion bodies in ALS.[48] In analogy with mutant α-synuclein in PD, aggregated SOD1 may in conjunction with other proteins impair the function of the UPS or adversely affect some other functions within motorneurons.[45] The motorneuron is not the only cell type that is affected by the disease process in ALS. Thus, it has been shown that the preferential expression of mutant SOD1 in motorneurons is not sufficient for development of ALS in mice.[45] The role of glial cells and glial–neuron interactions during development of the disease deserves close scrutiny.

Increased ROS production with oxidative damage to crucial proteins and other cell components may play a role in ALS. These changes may rise as a consequence of disturbed glutamate metabolism with prolonged stimulation of excitatory amino-acid receptors leading to increased intracellular calcium that can damage the mitochondria and the ER.[11,45]

Recently, it was reported that cleavage of caspase-12 occurred in the spinal cord of transgenic ALS mice indicative of ER stress.[49] The observed cleavage of caspase-12 in the ALS mice could be due to the activity of the calcium-dependent enzyme calpain that was also activated in the spinal cord of these mice.[49] Caspase-12 has been reported to be a substrate for calpain in some cells including neurons.[50] It remains to be studied whether the ER-mediated caspase activation play a role in the disease progression of ALS. It was recently shown that deletion of caspase-11, upstream of capsases-1 and -3, had no significant effect on the onset, progression or inflammatory responses in transgenic ALS mice.[51] This may suggest that caspase-11-independent pathways are activated in the ALS mice. Apart from caspase-12, other markers for ER stress, such as Bip/Grp78 were also altered in the ALS mice, although not the same extent.[49] Further evidence for ER stress in ALS comes from studies showing a large increase in Bip/Grp78 in spinal motorneurons of transgenic ALS mice prior to onset of motor symptoms.[52] It was also reported that mutant, but not wild-type SOD1 can aggregate and associate with the ER.[52] These findings lend credence to the view that ER stress is part of the mechanism by which mutant SOD1 contributes to FALS and motorneurondegeneration. The roles of ER stress and ER-mitochondria interactions for disease progression in ALS remain to be studied further.

ER Stress and Transmissible Spongiform Encephalopathy

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are transmissible neurodegenerative disorders that include Creutzfeldt–Jakob disease, bovine spongiform encephalopathy (BSE) and scrapie.[53] The pathological hallmarks of TSEs are the cerebral accumulation of a misfolded and protease resistant form of the prion protein (PrP).[53,54] During the course of the disease spongiform degeneration of brain tissue occurs together with increased astrogliosis and extensive neuronal apoptosis. The pathological form of PrP (PrP^{Sc}) shows no changes in amino-acid sequence or post-translational modifications compared with normal PrP.[53,54] However, there is a conformational change in PrP^{Sc} with an increased amount of β-sheet present.[53,54]

Despite considerable efforts to study the mechanism for cell demise, it is not clear how accumulation of PrP^{Sc} leads to apoptosis. N2A neuroblastoma cells treated with PrP^{Sc} produce a fast and sustained increase in intracellular calcium levels.[55] Pretreatment with thapsigargin that depletes ER calcium stores reduced the increase in calcium observed with PrP^{Sc}.[55] This indicates that the ER is involved in the mechanisms by which PrP^{Sc} acts in nerve cells. ER calcium levels and signaling are tightly regulated by activity of various channel proteins and by the Bcl-2 family proteins (Figure 2).[11,19] The mechanism for the enhanced ER calcium release elicited by PrP^{Sc} remains to be studied further.

Apart from calcium, caspase-12 was upregulated in infected cells after PrP^{Sc}.[55] In keeping with this, brain tissue from PrP^{Sc}-infected mice and patients with Creutzfeldt–Jakob disease showed higher levels of caspase-12.[55] This data indicates that the activation of ER stress and caspase-12 may play a role in neuronal apoptosis associated with the accumulation of mutant prion protein in neurons. It was reported that in the murine scrapie model, the accumulation of PrP^{Sc} was closely followed by the induction of the ER chaperone Grp58.[56] *In vitro* studies using overexpression of Grp58 or its dowregulation by small interfering RNA revealed that this protein exerts a protective function against neurotoxicity caused by PrP^{Sc} protein.[56] This data shows the dual functions of ER stress in neurodegenerative diseases with an early largely protective response involving among other chaperone proteins, and the later induction of other gene products causing cell death. The results with Grp58 as a modifier of prion protein toxicity point to possible novel targets to consider for therapies of these grave disorders.

ER Stress and Polyglutamine Diseases

Expanded polyglutamine (polyQ) repeats found in different proteins can cause human inherited neurodegenerative diseases, such as Huntington's disease (HD), spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy and six spinocerebellar ataxias (SCA 1, 2, 3, 6, 7 and 17). These disorders are characterized by selective neuronal death and accumulation of intracellular protein aggregates observed in cultured cells, transgenic animals and in human post-mortem brain tissue.[57] The significance of the polyQ expansions and

protein deposits for disease pathophysiology remains unclear. It has been proposed that alterations in gene expression, changes in protein–protein interactions, dysfunctional mitochondrial and an impairment of the UPS contributed to the disease progression.[57,58] It is a matter of debate whether the polyQ protein aggregates are deleterious for neurons or whether they preserve vital functions in the cell.[58]

Mutant huntingtin can affect calcium metabolism in the cell and sensitize the IP3 receptors at the ER.[59] One piece of evidence for the role of ER stress in polyQ diseases comes from studies showing colocalization of polyQ fragments with various molecular chaperones, such as Hsp70 and Hsp40 that are induced in ER stress.[58] In addition, overexpression of Hsp70 suppresses polyQ toxicity in *Drosophila*.[58] This was also observed in some, but not all mouse models of polyQ diseases.[57,58]

ER stress is also triggered by the pathogenic SCA3 polyQ fragments, as shown by the activation of IRE1 and PERK and the induction of CHOP/Gadd153 and BiP/Grp78. This occurred after an impairment of proteasomes showing the interplay between the ER and the UPS.[9] Studies with deficient mouse embryonic fibroblasts showed that activation of the apoptosis signal-regulating kinase 1 (ASK1) is essential for polyQ induced ER-mediated cell death.[9] It is known that ASK1 can form a complex with IRE and TRAF2 proteins at the ER and subsequently activate downstream signals, such as the c-Jun NH2-terminal kinase (JNK).[2,3] However, ASK1 is not involved in cell death caused by the expanded androgen receptor (AR112Q), suggesting the presence of alternative pathways for cell death.[60] Expression of polyQ72 fragments was reported to activate caspase-12 in C2C5 cells that occurred independently of caspases-8 and -3.[61] It remains to be studied whether caspase-12 or other related ER caspases are activated in other mouse models of polyQ disorders and in brain samples of patients afflicted by these diseases.

ER Stress in Neuronal Storage Diseases

Lysosomal storage diseases (LSD) are inherited metabolic defects that result in accumulation of various materials within the cells.[62] There are at least 45 different conditions classified as LSD, of which G_{M1}-gangliosidosis is one of the most common. This disorder is characterized by accumulation of G_{M1}-ganglioside, a major sialoglycolipid of neuronal membranes, leading to neurodegeneration, manifested by motor and mental retardation in the patients.[62]

Using a mouse model of G_{M1}-gangliosidosis, ER stress and the UPR response were shown to accompany the disease.[62] There was an upregulation of BiP/Grp78, CHOP/Gadd153 and the activation of caspase-12 and JNK2 pathways causing cell death. Depletion of ER calcium stores was one of the first events in the disease, prior to the UPR.[62] Recently, it was shown that in G_{M2}-gangliosidosis, Sandhoff's disease the accumulation is due to a reduced uptake of calcium into the ER, which is driven by the sarcoplasmic/ER calcium ATPase.[63] So far the role of ER stress in the pathogenesis of Sandhoff's disease has not bees studied.

Pelizaeus-Merzbacher disease (PMD) is a X-linked recessive pediatric disorder characterized by diffuse hypomyelination of the central nervous system leading to a variety of symptoms.[64] Mutations, duplications or deletions in the phopholipid protein (PLP) gene cause PMD with the accumulation of PLP and/or DM20 proteins in the ER.[64] This is followed by the induction of the ER-mediated transcription factors, CHOP/Gadd153 and ATF3, as shown in cultured cells in different PMD mouse models and in patients.[64] The direct involvement of CHOP/Gadd153 in the oligodendrocytes was shown in studies using gene-deleted mice for this protein crossed with the disease causing PMD mice.[64] The exact gene targets for CHOP/Gadd153 in PMD are so far unknown.

Neuronal ceroid lipofuscinoses (NCLs) are rare neurodegenerative diseases in childhood.[65] CLN8 mutations give rise to northern epilepsy, called progressive epilepsy with mental retardation and mutant CLN8 protein localizes to ER.[65] The possible role of ER stress in CLN8 and other NCL disorders remain open.

ER Stress and Acute Neurodegeneration

Apart from the more chronic neurodegenerative diseases, ER stress is also shown to be present in acute brain disorders, such as ischemia. In focal cerebral ischemia in mice resulted in an activation of the UPR sensors, eIF2α and PERK due to the detachment of the chaperone, BiP/Grp78 (Figure 2).[66] Global ischemia in mice also induced ER stress induced and activation of the ER transcription factors, CHOP/Gadd153 and ATF-4.[66] It has been reported that hippocampal neurons from CHOP/Gadd153-deficient mice are more resistant to cell death induced by hypoxia-reoxygenation compared with controls.[67] Fewer neurons degenerated in the CHOP$-/-$ mice after ischemia, suggesting an important role for ER stress in ischemia/stroke.

In brain trauma, caspase-12 is activated as a consequence of ER stress.[68] With respect to function, the ER chaperones, Oxygen-regulated protein 150 kDa, and the 94 kDa glucose-regulated protein, GRP94 have been shown to afford protection against ischemia-induced cell death in brain.[69,70] This shows a direct functional role of the ER response in brain damage. It remains to be studied whether targeting of proteins linked to ER stress might be useful in the therapy of acute brain disorders.

Conclusions

As discussed above, the ER orchestrates the different cellular processes by which proteins are synthesized, correctly folded, modified and transported to their final destination. In neurodegenerative diseases many of the cellular processes involving ER might go wrong, leading to different degrees of ER stress that ultimately contribute to nerve cell demise. The questions remain, however, how much of cell degeneration is due to ER stress, and how much is caused by other pathways. As shown Figure 1, the different organelles and pathways communicate with each other. We know too little about the importance of such interactions in the control of cell death and about proteins taken part in ER–mitochondria communication. With regard to the functional significance of the ER stress it has both a protective component exemplified by the early

UPR, and a more deleterious prolonged response with disturbed calcium homeostasis, increased protein accumulation, loss of ER function and the activation of cell death cascades. Similarly, it has been suggested that the accumulation of mutant protein into cellular aggregates may be neuroprotective. This probably depends on the nature and the function of the protein in question. In general, it is also possible that intracellular protein deposits become deleterious only after reaching a certain level within the cells.

In view of this and considering future possibilities for treatment of neurodegenerative disorders it is important to know more about which part of the ER stress response to target and at what particular stage of the disease. Previous work on ER stress has mainly been performed using cultured non-neuronal cells expressing high amounts of exogenous proteins. Future work focusing on different neuronal systems and mouse models together with the analyses of human samples will ultimately increase our knowledge about the role of ER stress in the different neurodegenerative diseases.

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