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REVIEW

Targeting the endoplasmic reticulum in prion disease treatment: breakthroughs and challenges

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Abstract: Prion diseases are infectious, predominantly fatal neurodegenerative diseases characterized by abnormal prion protein (PrP) accumulation and neuronal loss. Studies on experimental animal models and clinical features of human prion diseases have shown unfolded PrP accumulation results in endoplasmic reticulum (ER) stress. While ER stress-mediated apoptosis is responsible for neuronal loss in prion diseases, ER stress also activates the unfolded protein response (UPR) in an effort to restore ER homeostasis. Of the UPR signaling pathways, the PERK-eIF2α pathway is implicated in the pathogenesis of prion diseases. The proteasome protein degradation system is also activated during the UPR. Increasing evidence indicates that proteasome and autophagy activities are affected in prion diseases. These findings suggest that ER stress/UPR contributes to the onset of prion diseases. Hence, strategies that target the ER are useful approaches in treating prion diseases. Additionally, immunotherapeutic approaches for prion diseases have been developed in recent decades. Single-chain fragment variable antibodies targeting the accumulation of PrP are also beneficial in the prevention of abnormal PrP propagation. This review discusses pathogenic mechanisms related to the ER and potential strategies for treating prion diseases.

Keywords: prion, endoplasmic reticulum, unfolded protein response, therapy

Introduction: pathogenesis and clinical manifestations of transmissible spongiform encephalopathies

Transmissible spongiform encephalopathies, also well-recognized as prion diseases, include Creutzfeldt-Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia (FFI), and Kuru in humans, scrapie in sheep and goats, transmissible mink encephalopathy in minks, chronic wasting disease in deer and elk, and bovine spongiform encephalopathy in cattle.[1] These diseases are both fatal and infectious, and are characterized by similar features of pathogenesis. Common features of prion disease pathology are the deposition of abnormal forms of cellular prion proteins (PrP), spongiform vacuolation, severe neuronal loss, and astrocytosis and microgliosis. PrP itself is widely considered the infectious source.

Normally, newly synthesized PrP undergoes several post-translational modifications within the endoplasmic reticulum (ER) and Golgi apparatus to give rise to mature PrP, which is localized at the cell surface through a glycosylphosphatidylinositol (GPI) anchor.[2] PrP also contains a conserved hydrophobic sequence that can be oriented in either direction at the lipid bilayer, resulting in another two different transmembrane forms designated ^{Ctm}PrP and ^{Ntm}PrP. In this case, a centrally positioned, highly

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conserved hydrophobic region in ^{Ctm}PrP and ^{Ntm}PrP acts as a transmembrane anchor, and directs the C-terminal and N-terminal regions, respectively, into the lumen of the ER.[3] PrP has at least two structural forms, the normal cellular form (PrP^{C}) and the misfolded form (PrP^{Sc}). The two isoforms have identical amino acid sequences, but differ in their monomeric conformation and state of aggregation. PrP^{C} is a soluble, α-helix-rich protein, while PrP^{Sc} is an insoluble protein, high in β-sheet structure, and partially resistant to proteolytic digestion.[1,4–7] PrP^{Sc} functions as a template promoting the conversion of PrP^{C} to abnormal PrP^{Sc} structural forms.[8–10] Many studies have shown PrP^{Sc} production in vitro in the absence of genetic material. In such situations, PrP^{Sc} is able to self-replicate by facilitating the conversion of PrP^{C} to PrP^{Sc}.[11–14] PrP^{Sc} replication starts from the formation of an ordered nucleus as the seeds and then proceeds to polymerization to form aggregates.[15] To date, this concept is widely accepted. Interestingly, PrP^{C} knockout mice infected with an infectious PrP species did not display neurodegeneration, suggesting the loss of PrP was not involved in disease pathogenesis.[16,17] Some studies indicated that PrP^{C} functions as a high-affinity copper-binding protein.[18–20] Moreover, PrP^{C} regulates *N*-methyl-D-aspartate receptors, and might be involved in pathogenesis of Alzheimer's disease (AD).[21,22] However, the true function of PrPs is debatable.

Point mutations or insertions in the PrP gene (known as the *PRNP* gene) are responsible for genetic prion diseases including familial CJD, GSS, and FFI.[23] Such mutations in the *PRNP* gene are likely to cause misfolding and aggregation of PrP. Sporadic prion diseases, which include the majority of CJD and sporadic fatal insomnia cases, are believed to arise from spontaneous misfolding of normal PrP^{C} or from rare somatic mutations in the *PRNP* gene.[24]

The clinical manifestations of human prion disease vary according to disease subtype. For example, GSS is characterized by chronic cerebellar ataxia with dementia, and the presence of multicentric amyloid plaques, while CJD is associated with subacute dementia and motor abnormalities, and with widespread spongiform degeneration in the cerebral cortex, striatum, and cerebellum. FFI shows a subacute condition with untreatable insomnia, dysautonomia, and severe selective atrophy of the anterior ventral and mediodorsal nuclei of the thalamus.[25]

Role of the ER and quality control systems in prion disease

Unfolded protein response

The ER is a specialized organelle in which newly-synthesized secreted or membrane proteins are folded and modified. ER stress is defined as the accumulation of unfolded and/or misfolded proteins in the ER in response to nutrient deprivation, perturbation of intracellular calcium homeostasis, oxidative stress, or the expression of mutated proteins, for example. ER stress activates a regulatory system termed the unfolded protein response (UPR) to manage defective proteins in the ER.[26] The UPR includes three signaling pathways: 1) translational attenuation to inhibit the further generation of unfolded proteins; 2) facilitating refolding of unfolded proteins by the induction of ER molecular chaperones; and 3) activating ER-associated degradation to degrade unfolded proteins accumulated in the ER via the ubiquitin proteasome system (UPS). If the aforementioned strategies fail, the cells enter into ER stress-induced apoptosis. The three major transducers of the UPR are PKR-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). PERK is an ER transmembrane protein kinase which phosphorylates eukaryotic initiation factor-2α (eIF2α) to induce translational attenuation.[27] The prolonged suppression of protein synthesis by inactive phosphorylated eIF2α (P-eIF2α) is not conducive to cell survival, hence the translational process is restored through GADD34-mediated dephosphorylation of P-eIF2α.[28] IRE1 is an ER transmembrane protein containing endoribonuclease and cytoplasmic protein kinase domains. Activated IRE1 catalyzes the unconventional splicing of X-box binding protein 1 (XBP-1) messenger RNA, which encodes a basic leucine zipper transcription factor. The spliced form of XBP-1 protein upregulates the synthesis of ER-resident molecular chaperones, including GRP78/BiP.[29,30] ATF6, a member of the CREB/ATF family, is a type II ER transmembrane protein which mediates transcriptional induction in response to ER stress. The membrane-anchored ATF6 is cleaved at the transmembrane region in response to ER stress, and the processed N-terminus of ATF6 is translocated to the nucleus where it promotes the transcription of molecular chaperones, such as GRP78/BiP and GRP94.[31–33]

Recently, ER stress has been implicated in the pathogenesis of prion diseases. Elevated levels of ER stress markers, including GRP78/BiP, GRP94, and GRP58, were observed in the cerebral cortex of prion disease patients, and in prion disease mouse models.[34–36] Purified PrP^{Sc} extracted from the brain of scrapie-infected mice invoked ER stress and induced the release of intracellular Ca^{2+} from ER stores.[37] It is believed that alteration of ER Ca^{2+} homeostasis and subsequent ER stress are involved in the progression of prion disease.[36,38] PrP^{Sc}-mediated release of Ca^{2+} from the ER to the cytosol induces loss in mitochondrial membrane potential, increases

reactive oxygen species levels, and results in apoptosis.[38–40] PrP^{Sc} is also responsible for protein accumulation in the ER, resulting in ER stress-induced apoptosis.[41] During PrP infection, ER stress occurs and translocation of nascent PrP reduces, then this leads to neurodegeneration.[42] In contrast, Nunziante et al showed that ER stress or proteasomal dysfunction enhances trafficking of PrP aggregates through the secretory pathway and raises accumulation of PrP^{Sc} significantly in persistently prion-infected cells.[43] Thus, trafficking of PrP during ER stress in PrP infection is open to dispute.

Proteasome system

Ma et al proposed that UPS might play an important role in the pathogenesis of prion disease.[44] Proteasomes are large multicatalytic, cytoplasmic and nuclear protease complexes essential for cell survival. Additionally, the UPS is predominantly responsible for non-lysosomal protein degradation.[45–48] Inhibition of the UPS by proteasome inhibitors leads to accumulation of PrP^{Sc}-like species in cells. PrP^{Sc} generated from misfolded PrP^{C} was retro-transported to the cytosol and degraded by proteasomes.[44] The use of proteasome inhibitors induced conversion of accumulated PrP^{C} to PrP^{Sc}-like species, which persisted after removal of inhibitor, and led to apoptosis and infection of neighboring cells.[44] Some studies have suggested that functional impairment of the UPS is related to Prion disease.[49,50] A null mutation in the gene encoding Mahogunin, a putative E3 ubiquitin ligase, is responsible for spongiform-like neurodegeneration, which mimics prion disease histologically.[51,52] Moreover, E3 ubiquitin ligase Hectd2 was linked with prion disease incubation time in mice, and associated with sporadic variant CJD and Kuru.[53]

Additionally, three PrP mutations (V203I, E211Q, and Q212P) associated with familial prion disease caused PrP accumulation in the cytosol in response to proteasome inhibition, resulting in aggresome formation.[54] Following mild proteasome inhibition, PrP-infected neuronal cells succumbed to apoptosis, forming large PrP^{Sc} aggresomes, which complexed with HSC70, ubiquitin, proteasome subunits, and vimentin.[55] Thus, many studies have suggested that UPS is impaired in neurodegenerative diseases, however, the precise mechanism is still to be elucidated.

Autophagy

Recent evidence has emerged that autophagy plays a key role in prion disease. Autophagy is a fundamental bulk degradation system for cellular organelles and cytosolic proteins. Autophagy is also involved in UPR and is triggered by ER stress.[56–58] The importance of autophagy has been recognized in a number of neurodegenerative diseases including prion disease, AD, Parkinson's disease (PD), and Huntington's disease (HD).[59,60] Increased number of autophagosomes, or autophagic vacuoles (AVs), is a common feature of many neurodegenerative diseases. Large AVs were observed in neurons of CJD model mice and scrapie-infected hamsters, with similar ultrastructural features of autophagy observed for both experimental models. Moreover, multivesicular bodies and AVs were detected in prion-infected neurons and in various forms of human prion disease.[61–63] Although colocalization of PrP^{Sc} with autophagosomes was not observed in the brain of scrapie-infected hamster, PrP^{Sc} was colocalized with autophagosomes in prion-infected cells, which had been treated with bafilomycin A, an inhibitor of autolysosome formation.[64] Mutant PrP colocalized with autophagosomes in a Neuro-2a cell line expressing CJD-associated PrP mutants.[65] Chronic administration of rapamycin, an autophagy-inducing agent, to transgenic Tg(PrP-A116V) mice, which model genetic prion disease, prevented the deposition of PrP^{Sc} and significantly delayed disease onset.[66] Imatinib treatment of prion-infected mice delayed PrP^{Sc} invasion of neural cells and the onset of clinical disease, by activating lysosomal degradation of PrP^{Sc} at the initial stage of peripheral infection.[67] Additionally, both lithium and trehalose improved PrP^{Sc} clearance by induction of autophagy in prion-infected cells,[68,69] while PrP^{Sc} levels increased in cells treated with autophagy inhibitors.[60,68–70]

There is increasing evidence that proteasome inhibition induces autophagy, and this provides a complementary pathway for proteasome impairment. However, once the activated autophagic system reaches saturation, autophagy appears to be impaired. Autophagy has been shown to go awry in neurodegenerative diseases including AD, PD, HD, and amyotrophic lateral sclerosis.[71] Therefore, more detailed analyses are required to clarify whether a balance exists between the proteasome system and autophagy in vivo.

Elucidating ER-related cellular and biochemical mechanisms that lead to neuronal dysfunction

Many studies have demonstrated synaptic dysfunction and degeneration, in addition to neuronal loss in prion disease mouse models.[72–80] PrP^{C} misfolding and its subsequent accumulation in the ER is believed to be responsible for synaptic failure. Moreno et al discovered a link between PrP accumulation in the hippocampus of mice infected

with Rocky Mountain Laboratory (RML) prions, and the enhanced translational attenuation pathway of the UPR.[80] Sustained translational attenuation by phosphorylation of PERK and its target eIF2α, resulted in synaptic failure and neuronal loss, whereas enhancement of translational recovery in the hippocampi of RML-infected mice was neuroprotective. Overexpression of the eIF2α-specific phosphatase GADD34 rescued synaptic deficits and neuronal loss. Additionally, RNA interference mediated by lentivirally expressed short hairpin RNA for PrP reduced PrP levels and prevented synaptic degeneration. Conversely, salubrinal, an inhibitor of P-eIF2α dephosphorylation, increased P-eIF2α levels and aggravated neurodegeneration in prion-infected mice. Inhibition of PERK phosphorylation by GSK2606414, a selective inhibitor of PERK, also provided a neuroprotective effect in prion-infected mice.[81] Collectively, these results suggest the PERK-eIF2α pathway regulates pathogenesis of prion diseases.

PrP accumulation caused the ER stress-associated release of calcium from the ER, which leads to hyperactivation of calcium dependent phosphatase calcineurin (CaN).[82] Activated CaN mediated neurodegeneration in prion-infected mice. Interestingly, the administration of CaN inhibitor FK506 to prion-infected mice reduced the severity of clinical abnormalities and increased life-spans. Thus, PrP^{Sc}-mediated perturbations to ER calcium homeostasis have implications on the pathogenesis of prion diseases.

^{Ctm}PrP possesses an uncleaved signal peptide and GPI anchor, and its retention in the ER or Golgi apparatus is dependent on cell type.[83] Recently, Wang et al demonstrated recombinant mutant PrP associated with familial CJD induced ^{Ctm}PrP retention in the ER, upregulated the levels of GRP78, GRP58, and PERK, and led to ER stress-induced apoptosis in SH-SY5Y cells.[41]

These findings indicate that ER stress and the UPR are involved in neuronal dysfunction in prion diseases. Within murine models of prion disease, however, there are mice in which ER stress or the UPR are not triggered. One such example can be found in Tg(PG14) mice that express a mutant PrP with a nine-octapeptide repeat insertion associated with a genetic prion disease. Neither an increase in UPR-regulated gene expression, nor activation of the PERK-eIF2α pathway, was observed for Tg(PG14) mice.[84] Additionally, CaN activity was reduced, rather than promoted, in this mouse model.[85] These results suggest that pathogenesis of prion diseases is not explained by ER stress alone. Different pathogenic mechanisms might account for the observed heterogeneity in prion diseases. More extensive analyses are required to fully understand neuronal dysfunction and pathogenesis of prion diseases.

Discovery of ER-retained antibodies that disrupt PrP^C accumulation in the ER, and novel immunotherapeutic strategies

The development of antibodies directed against PrP is one approach in the treatment of prion diseases. In the last decade, single-chain fragment variable (scFv) antibodies have been developed for use in passive immunotherapy.[86] scFvs are produced by fusing the variable region in heavy (V_H) and light (V_L) chain fragments of an immunoglobulin G connected with an appropriate linker.[87] scFvs have several advantages over chimeric or humanized antibodies, for example, little immunogenicity, no complement fixation, and better tissue penetration due to their small size. Moreover, scFvs tend to have shorter half-lives. Additionally, because scFvs do not require glycosylation, they can be produced in a bacterial expression system.[88] Antibodies against PrP^C indirectly facilitate the clearance of PrP^{Sc}, inhibit the conversion of PrP^C to PrP^{Sc}, and promote degradation of PrP^C.[89] To date, at least 20 studies have used scFvs to target PrPs.[88] Many of these scFvs inhibited PrP aggregation and impaired PrP^{Sc}-associated cellular toxicity. Cardinale et al produced ER-retained anti-prion scFv antibodies, which confined PrP^C to the ER, prevented PrP^C translocation to the cell surface, and inhibited PrP^{Sc} accumulation in PC12 cells.[90]

The targeting of scFvs to specific sites within cells is achieved by in-frame fusion with intracellular trafficking sequences to scFvs. To generate ER-retained scFvs, they were designed with a signal sequence at the N-terminus and a retention peptide, KDEL (Lys-Asp-Glu-Leu), at the C-terminus.[91] In the same way, scFvs can be targeted to cytoplasm, nucleus, trans-Golgi, and plasma membrane with using specific trafficking sequences and retention signals for each organelle.[92] The delivery of scFvs would be attained by either adeno-associated viral vectors[93] or cell lines expressing scFvs.[89,94,95] Delayed onset of prion disease was observed in a mouse model treated with anti-PrP^C recombinant adeno-associated viral scFvs.[93] Also, secreted anti-PrP scFvs produced by cell lines prevented PrP^{Sc} formation[89,94] and significantly extended the life-span of scrapie-infected mice.[95]

Another strategy in the treatment of prion diseases would be the use of antibodies targeted against the laminin receptor (LRP/LR). The LRP/LR is thought to function as a receptor for PrP^C and PrP^{Sc},[96,97] and it has been shown to

play a critical role in the infection process of PrP.[98,99] The LRP/LR is necessary for internalization of bovine PrP^{Sc} in human cells,[100] and the administration of anti-LRP/LR scFvs to scrapie-infected mice reduced peripheral levels of PrP^{Sc}, even though survival period was not prolonged.[101,102]

Prospects for targeting the ER, and early diagnosis

As detailed above, evidence supporting involvement of ER stress in prion disease pathogenesis is increasing (Figure 1). Therefore, strategies that target the ER should be explored when considering therapeutic approaches to prion diseases. Prolonged phosphorylation of PERK and eIF2α resulted in synaptic failure and neuronal loss in RML prion-infected mice.[80] Conversely, the overexpression of GADD34, or the inhibition of PrP synthesis by RNA interference, rescued these defects. Moreover, the PERK inhibitor GSK2606414 inhibited clinical symptoms of disease in prion-infected mice.[81] Hence, strategies that suppress the PERK-eIF2α pathway, such as activation of GADD34, would be beneficial in prion disease therapy. Interestingly, salubrinal, which activates the PERK-eIF2α pathway of the UPR by inhibiting dephosphorylation of eIF2α, exacerbated disease presentation.[80] Salubrinal reduced α-synuclein accumulation in PD models,[103–105] prevented amyloid β-induced neuronal death,[106] reduced protein aggregation caused by N-terminal mutant *huntingtin*,[107] and reduced brain damage in a rat model of cerebral ischemia/reperfusion injury.[108] It is therefore of no surprise that salubrinal has been considered a potential therapeutic agent for ER stress-related neurodegenerative diseases including AD, PD, and HD. As discussed previously, however, the use of salubrinal in the prion disease model had quite the opposite effect.[80] These findings suggest that a certain amount of fine-tuning depending on aspects of ER stress conditions will be necessary in individual neurodegenerative diseases.[109] In either case, ER stress is observed for prion diseases, and as such, monitoring of ER stress may be a useful strategy for the early diagnosis of disease.

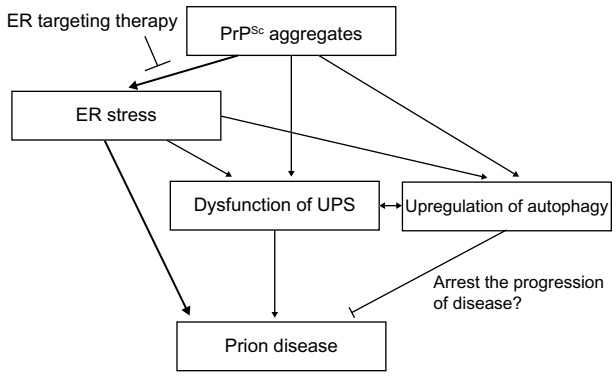

Figure 1 Overview of the relationship among prion disease, ER stress, UPS, and autophagy.
Abbreviations: ER, endoplasmic reticulum; PrP^{Sc}, the misfolded form of prion protein; UPS, ubiquitin proteasome system.

During the UPR, ER-associated degradation activation results in protein degradation via the UPS. Both proteasome system impairment and enhanced autophagy occur during prion infection (Figure 1). These findings suggest that strategies targeted to proteasomes and/or autophagy should be also considered in the treatment of prion diseases.

In recent years, antibody-based immunotherapy has focused on the development of antibodies that could neutralize the toxicity associated with PrP aggregation. Of these therapies, scFvs have proved the most promising. ER-targeted anti-PrP^{C} scFvs trapped PrPs in the ER, and blocked the accumulation of PrP^{Sc}.[90] Anti-LRP/LR scFvs were effective in reducing peripheral PrP^{Sc} propagation, but failed to prolong survival of scrapie-infected mice.[102]

Finally, recent experimental studies have provided evidence to indicate dissociation between PrP infectivity and neurotoxicity.[110,111] To date, it was widely accepted that PrP^{Sc} was responsible for both infectivity and neurotoxicity, but the idea that noninfectious PrP is the true pathogen is gaining momentum. Moreover, it has been reported that PrP oligomers show neurotoxicity rather than PrP^{Sc} aggregates in vitro and in vivo.[112,113] Hence, an approach eliminating only PrP^{Sc} aggregates as a treatment for prion disease may not abrogate PrP neurotoxicity. Inclusive of recent concepts, the application of a therapeutic agent in treating prion diseases requires further analysis, and it should include measures for the safe and precise delivery of the agent to the target site.

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Disclosure

The author declares no conflict of interest in this work.

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