Misfolding leads the way to unraveling signaling pathways in the pathophysiology of prion diseases

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ABSTRACT. A misfolded version of the prion protein represents an essential component in the pathophysiology of fatal neurodegenerative prion diseases, which affect humans and animals alike. They may be of sporadic origin, acquired through exogenous introduction of infectious misfolded prion protein, or caused by genetic alterations in the prion protein coding gene. We have recently described a novel pathway linking retention of mutant prion protein in the early secretory pathway to activation of p38-MAPK and a neurodegenerative phenotype in transgenic mice. Here we review the consequences that mutations in prion protein have on intracellular transport and stress responses focusing on protein quality control. We also discuss the neurotoxic signaling elicited by the accumulation of mutant prion protein in the endoplasmic reticulum and the Golgi apparatus. Improved knowledge about these processes will help us to better understand complex pathogenesis of prion diseases, a prerequisite for therapeutic strategies.

KEYWORDS. misfolding, neurodegeneration, p38-MAPK, prion disease, protein quality control

INTRODUCTION

The prion protein (PrP\(\text{C}\)) is a glycosylphosphatidylinositol (GPI)-anchored protein located at the plasma membrane in lipid-enriched microdomains commonly known as lipid rafts or detergent resistant membranes. A plethora of functions ranging from neurodevelopment to neuroprotection have been proposed for PrP\(\text{C}\) but the fact that it is enriched at the neuronal synapse suggests a potential role in neuromodulation, possibly by its involvement in neuronal signal transduction cascades. In its misfolded state, it plays a fundamental role in neurodegenerative prion diseases. In humans, prion diseases can be sporadic, acquired or genetic. In the latter, mutations in the \textit{PRNP} gene encoding the prion protein lead to a broad spectrum of prion diseases
with a wide variation in penetrance.\textsuperscript{7,8} Interestingly, \textit{PRNP} mutations present in the structured C-terminal domain of PrP\textsuperscript{C} may result in either a fully penetrant prion disease (E200K), a dementia clinically not resembling a prion disease (R208C) or a variant rarely associated with dementia (R208H).\textsuperscript{8} Some mutations leading to genetic human prion diseases are also present in the C-terminal signal sequence for the GPI-anchor attachment, implying that the GPI-anchor signal sequence itself, though not being present in the mature protein, can also play a role in neurodegeneration.\textsuperscript{9}

Plasma membrane attachment via a GPI-anchor is a unique and evolutionary highly conserved mean to bestow lipid raft localization. Thus, GPI-anchored proteins are able to carry out specific functions related to signal transduction and membrane trafficking.\textsuperscript{10} The GPI-anchor is composed of a highly conserved glycan core of three mannose residues, a glucosamine and a phosphatidylinositol (PI) group attached \textit{en block} through an amide bond to the ethanolamine phosphate of the nascent protein at the ER. This core GPI-anchor undergoes several remodeling steps through the passage to the ER and Golgi with the protein finally localizing in lipid rafts at the outer leaflet of the plasma membrane.

Protein homeostasis or proteostasis is fundamental for the viability of cells. It is estimated that about 10\% of total PrP\textsuperscript{C} is improperly folded when synthesized\textsuperscript{11} and, therefore, strict protein quality control (PQC) is of outstanding importance. In the case of secreted and membrane proteins, quality control takes place at the ER (ERQC) and Golgi apparatus.\textsuperscript{12} Misfolded proteins are retained at the ER and those that repeatedly fail to fold properly are directed to the cytosol where they are ubiquitinated to be degraded by the proteasome (the so-called ER-associated degradation (ERAD) pathway).\textsuperscript{13} Regarding degradation, GPI-anchored proteins can behave differently than other membrane proteins. In some instances, misfolded GPI-anchored proteins are not targeted to ERAD but seem to follow alternative PQC pathways instead, such as the rapid ER stress-induced export (RESET) pathway.\textsuperscript{14} Our own studies investigating a PrP version with a C-terminal deletion indicate that in stress conditions, either artificially induced in cell culture or caused by age-associated decay, this pathway cannot be fully executed, and leads to activation of p38-MAP kinase with subsequent neurodegeneration.\textsuperscript{15,16} Here we briefly summarize new findings related to PQC of GPI-anchored proteins with special attention to PrP\textsuperscript{C} and the implicated signaling pathways that could play a role in neurodegeneration.

**Quality Control at the ER**

Once the protein folding capacity of the ER is overwhelmed, misfolded proteins accumulate in the ER lumen and induce ER stress. This activates the unfolded protein response (UPR), a signaling network that tries to re-establish homeostasis by increasing the folding capacity, transiently attenuating the translation of proteins while up-regulating ERAD components.\textsuperscript{17,18} If homeostasis cannot be restored, ER stress becomes chronic leading to cell death. Investigations of ER stress, UPR and ERAD in prion disease is an active field of research and has been extensively reviewed.\textsuperscript{18,19} It has been shown that PrP\textsuperscript{C} and some mutants of PrP (e.g. PrP with an unprocessed GPI-anchor or PrP expressing the D177N mutation) follow the ERAD pathway.\textsuperscript{11,20-22} Moreover, ERAD and the proteasome system influence prion diseases as evidenced by the fact that variations in the \textit{hectd2} gene (encoding E3 ligases that targets proteins to the proteasome) are linked to alterations in prion disease incubation time in mice and humans.\textsuperscript{23} UPR activation can likewise participate in the progression of prion disease in mice\textsuperscript{24-26} although this issue remains controversial since in other instances markers of the UPR could not be observed in human prion diseases and prion disease mouse models.\textsuperscript{27,28} More recently, it has been shown \textit{in vitro} that expression of PrP mutants disables retrotranslocation not only of PrP\textsuperscript{C} but also of other ERAD substrates but does not lead to activation of the UPR.\textsuperscript{29} As described elsewhere, induction of the UPR pathway in disease is complex and context-dependent.\textsuperscript{18,30}

Apart from the variability of the different models and the ways of inducing ER stress, it seems that the cell possesses several pathways
to ensure elimination of misfolded PrP (and probably other GPI-anchored proteins as well). Nevertheless, it has been repeatedly shown that certain PrP mutants can escape this ERQC and, more recently, Ashok et al. observed that when they expressed different PrP C mutants bearing C-terminal mutations found in human prion diseases, ERQC was bypassed. They demonstrated that GPI-anchored mutants could passage from ER to Golgi albeit being obviously misfolded. Once at the Golgi there was a rerouting to lysosomes where proteins were finally degraded. In a more detailed study, Satpute-Krishnan et al. described the novel RESET pathway, in which GPI-anchored PrP lacking its disulfide bond follows the secretory pathway, transiently reaching the plasma membrane to finally get degraded in the lysosome. Calnexin was identified as the retention factor to the ER and Tmp21 as the receptor that mediates the egress from ER to Golgi. Factors facilitating the transport to the plasma membrane and from there to the lysosome are currently unknown. The fact that a PrP mutant (H187R) with an unprocessed GPI-anchor is degraded via ERAD while the same protein with a functional GPI-anchor is degraded in the lysosome made the authors hypothesize that the GPI-anchor could sterically impair the ERAD pathway. This question has recently been addressed by Sikorska et al. When a mutated version of the GPI-anchored Gas1 (Gas1*) was expressed in a cell line, in which lipid remodeling of the GPI-anchor is blocked, Gas1* underwent ERAD degradation, whereas when the lipid remodeling and maturation of the GPI-anchor was complete, ER-to-Golgi transport was rescued. Thus, in the case of misfolded GPI-anchored proteins it is the presence of a lipid remodeled GPI-anchor that prevents the ERAD pathway and promotes an alternative quality control pathway (such as RESET).

Our own studies demonstrated that misfolded GPI-anchored PrP is directed to the secretory pathway in vivo and that, under stress conditions (in cells) or aging (in transgenic mice), it leads to the activation of neurotoxic signaling cascades. Aged transgenic mice expressing PrP partially lacking the C-terminal portion (Tg(PrPD214-229)) presented with a fatal neurodegenerative disease. Neuropathological analysis showed neuronal loss especially in the hippocampus and cerebellum and, strikingly, we could observe a considerable concomitant activation of p38-MAPK without obvious activation of UPR pathways. In a cell model expressing this C-terminally deleted PrP, we observed that, at steady state, PrPΔ214-229 was mainly retained at the ER and that, under stress conditions, it reached the Golgi where it remained, not being able to travel further. The latter retention at the Golgi led to p38-MAPK activation. As shown in Fig. 1, we hypothesize that PrPΔ214-229 follows the RESET pathway under steady-state conditions. Under ER stress or during aging, however, PrPΔ214-229 accumulates at the Golgi, which results in activation of p38-MAPK and cell death.

Other mouse models of genetic human prion diseases showing retention of mutant PrP at the ER or Golgi have been described that likewise did not present with activation of the UPR. For example, Tg(PG14) mice expressing PrP with a N-terminal octapeptide insertion or other transgenic mouse models for fatal familial insomnia (FFI; Tg(FFI)) showed retention at the secretory pathway and decreased plasma membrane expression without signs of UPR activation. Remarkably, in these transgenic mice, mutant PrP accumulates in different organelles of the secretory pathway supporting the idea that phenotypic variation seen in genetic prion diseases could be caused by organelle-specific retention of mutant PrP.

How could ER and Golgi retention lead to cell death in these mice? In Tg(PG14) mice mentioned above, cell death has been linked to a defect in Ca^{2+} homeostasis caused by inefficient membrane delivery of voltage-gated calcium channels promoted by retention of the mutant PrP. In our study, where PrPΔ214-229 is retained at the ER, we did not investigate Ca^{2+} dynamics or delivery of voltage-gated calcium channels to membranes. Thus, it is conceivable that alterations in these aspects and transport deficit of other proteins at the secretory pathway may contribute to neurodegeneration in our mice. In addition, it would be interesting to investigate if Tg(PG14) or Tg(FFI) mice likewise present with activation of p38-MAPK seen in our PrPΔ214-229 mouse model.
Golgi Quality Control

Why is PrPΔ214-229 retained at the Golgi? How can retention of PrPΔ214-229 at the Golgi lead to cell death? The Golgi apparatus plays a central role as a sorting station and in post-translational modification of cargos in the secretory pathway. But it has recently become obvious that the Golgi apparatus has additional functions, such as quality control and in integrating signaling events.43

Golgi quality control is clearly less understood than ERQC. The fact that mutations in the C-terminal part of PrP cause lysosomal degradation lead to the concept that the Golgi apparatus behaves as a quality control organelle for GPI-anchored proteins (as shown previously for some yeast proteins or for the incomplete T-cell receptor complex in eukaryotes).33,44 Golgi quality control can route misfolded proteins either back to the ER or forward to the endosomal system for degradation.45 Hence it has been demonstrated that non-remodeled GPI-anchors that reach the Golgi are redirected to the ER by the p24 cargo receptor complex46 and misfolded proteins with a strong ER exit signal or lacking determinants recognizable by the ERQC (in the case of transmembrane proteins) can bypass ERAD and become a substrate for Golgi quality control leading to lysosomal degradation.47 It is noteworthy that one principle of quality control in sorting proteins from the Golgi to the lysosome (among others proposed “signals”45) is based on the state of protein oligomerization/aggregation rather than the exact folding state of a monomeric protein. Thus, for some Golgi-resident proteins, oligomerization leads to lysosomal targeting and subsequent degradation.48 This
may have implications for PrP<sup>C</sup> as, in cell culture, the drug Suramin leads to oligomerization of PrP<sup>C</sup> at the Golgi and is then re-routed to lysosomes.<sup>49,50</sup> Suramin also showed protective effects in prion disease, although the mechanism is unclear. Whether this sorting mechanism also applies for the re-routing of PrP<sup>Δ214-229</sup> to lysosomes needs further investigation. In our studies, we observed that the presence of PrP<sup>Δ214-229</sup> at the ER and Golgi after induction of cellular stress leads to p38-MAPK activation. This kinase is also activated in aged Tg (PrP<sup>Δ214-229</sup>) mice. At this point, we can only speculate about the mechanism of p38-MAPK, activation in our mice, but it seems likely that different factors are involved. It is well known that ER stress can activate p38-MAPK, playing an important role in UPR.<sup>51</sup> We did not observe activation of UPR in the transgenic mice and in cells we observed that induction of ER stress with Thapsigargin was leading to accumulation of PrP<sup>Δ214-229</sup> at the Golgi co-incidental with activation of p38-MAPK. What leads to ER-independent with activation of p38-MAPK? As pointed out above, the Golgi apparatus has several functions as an integrator but also as an initiator of signaling cascades. On the one hand, it has been shown that under severe ER-stress conditions, the KDEL-receptor, a cis-Golgi resident and key regulator of signaling in ER-to-Golgi trafficking,<sup>51-54</sup> is able to activate both p38- and JNK-MAPK. Although in the latter studies, p38-MAPK activation was linked to a protective role, this may likely be cell- and context-dependent and, in fact, p38-MAPK activation can also be associated with pro-inflammatory and cell death responses.<sup>55,56</sup> On the other hand, it has also been shown that when ER-to-Golgi trafficking is arrested by treatment with Brefeldin A, this leads to activation of p38- and JNK-MAPK and subsequent cell death.<sup>57</sup> Whether accumulation of PrP<sup>Δ214-229</sup> leads to impaired ER-to-Golgi communication thus leading to p38-MAPK activation, deserves further studies.

**Consequences of PrP Accumulation in the Golgi Apparatus**

In prion infected mice expressing a PrP-green fluorescent protein fusion protein, accumulation of this protein can be seen in the Golgi apparatus long before onset of prion disease, thus raising the question if this may causally be involved in disease pathogenesis.<sup>38</sup> In another mouse model, expression of a transmembrane version of the prion protein leads to accumulation of this protein in the Golgi apparatus and subsequent neurodegeneration.<sup>59</sup> This is reminiscent of our Tg (PrP<sup>Δ214-229</sup>) mice and, as we do, Stewart et al. discuss the possibility that Golgi stress may directly or indirectly be involved in neurotoxicity. Again, it would be interesting to see if p38-MAPK activation may be a common pathway. The issue that prion infection itself may impair post-Golgi trafficking was recently highlighted by Uchiyama et al. when they investigated cell surface expression of PrP<sup>C</sup> and other neuronal membrane-bound proteins.<sup>60</sup> In prion-infected mice, they observed reduced expression of PrP<sup>C</sup> and other GPI-anchored proteins at the outer leaflet of neuronal membranes while these proteins accumulated in the Golgi well before onset of clinical symptoms. This reinforces the concept that misfolded GPI-anchored proteins use diverse transport routes and raises the question if a Golgi stress response may involve translational shutdown of distinct proteins. The molecular mechanisms underlying defective post-Golgi transport in prion diseases are only now being appreciated. It is suggested that the Rab GDP dissociation inhibitor α (GDI), which regulates the function of those Rabs involved in intracellular vesicular trafficking, might regulate this.<sup>61</sup> In fact, cells accumulating mutant PrP at the Golgi upregulate GDI and silencing of GDI rescues post-Golgi transport of reporter proteins.

**Implications in Human Prion Diseases and Future Directions**

Little is known about the signaling cascades underlying neurodegeneration in human prion diseases.<sup>62-64</sup> In sporadic CJD brains, intracellular PrP<sup>Sc</sup> deposits similar to those seen in Tg (PrP<sup>Δ214-229</sup>) mice can be observed (Fig. 2) raising the question if p38-MAPK activation may also be relevant in human prion diseases. Our knowledge on the molecular basis of prion disease has rapidly grown over the last
years and it has become obvious that misfolding of PrP\textsuperscript{C} and the generation of infectious “prions” represent only one out of several facets in the complex pathogenesis of prion diseases. Here we have presented our point of view regarding transport pathways and degradation of misfolded PrP. It seems that in our model misfolded PrP does not lead to UPR induction but rather follows the so-called RESET pathway. However, instead of being degraded in the lysosome, PrP\textDelta214-229 does not fully complete the RESET pathway but accumulates at the Golgi and leads to activation p38-MAPK and subsequent neurodegeneration. Future work will have to concentrate on establishing if these 2 features are causally linked and if p38-MAPK activation may represent a further mechanistic aspect of Golgi stress.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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