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Review

Protein disulfide isomerases in neurodegeneration: From disease mechanisms to biomedical applications

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ABSTRACT

Protein disulfide isomerases (PDIs) are a family of foldases and chaperones primarily located at the endoplasmic reticulum that catalyze the formation and isomerization of disulfide bonds thereby facilitating protein folding. PDIs also perform important physiological functions in protein quality control, cell death, and cell signaling. Protein misfolding is involved in the etiology of the most common neurodegenerative diseases, including Alzheimer, Parkinson, amyotrophic lateral sclerosis, Prion-related disorders, among others. Accumulating evidence indicate altered expression of PDIs as a prominent and common feature of these neurodegenerative conditions. Here we overview most recent advances in our understanding of the possible functional contribution of PDIs to neurodegeneration, depicting a complex and poorly understood scenario. Possible therapeutic benefits of targeting PDIs in a disease context and their use as biomarkers are discussed.

1. Introduction

Protein disulfide isomerases (PDIs) are emerging as important factors in health and disease in recent years (reviewed in [1]). Special attention has been given to the contribution of PDIs to the pathogenesis of many neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), prion related disorders (PrDs) and amyotrophic lateral sclerosis (ALS). The accumulation of abnormal protein inclusions is a hallmark of all these pathologies [2], which are collectively classified as protein misfolding disorders (PMDs) [2]. These diseases are generally related with aging and a progressive decline and failure of cellular protein folding and quality control systems [3,4]. Specific mutations in an underlying gene (familial cases) or structural changes of a normal protein (sporadic cases) are the cause of the accumulation of misfolded proteins in PMDs, which are present in the form of small oligomers, aggregates or large inclusions or extracellular deposits [5]. Although the mechanisms involved in the pathogenicity of most PMDs are not fully understood, disturbance of basic neuronal processes are consistently reported as a potential cause of neurodegeneration, highlighting alterations in axonal transport, synapse formation and maintenance, redox balance, proteasome function, mitochondrial function and protein homeostasis [6].

2. ER stress in neurodegeneration

Chronic protein folding stress at the endoplasmic reticulum (ER) is linked to a number of neurodegenerative diseases [7]. In fact, many specific defects are observed in the secretory pathway in PMDs, and involve physical interactions of disease-related proteins with components of the ER-associated degradation (ERAD) machinery, ER/Golgi trafficking, ER folding network, ER calcium homeostasis, protein degradation pathways (proteasome and autophagy), among other disturbances [7–9]. These defects trigger the accumulation of abnormally folded proteins at the ER lumen, generating a cellular condition known as ER stress [10].

One third of the proteome of most cells is normally folded and matured in the secretory pathway. Numerous ER resident chaperones, foldases, processing enzymes, and co-factors assemble into protein folding networks, which also mediate quality control mechanisms. Protein folding is a complex process reflected in the failure to properly fold around 10–30% of newly synthesized proteins,

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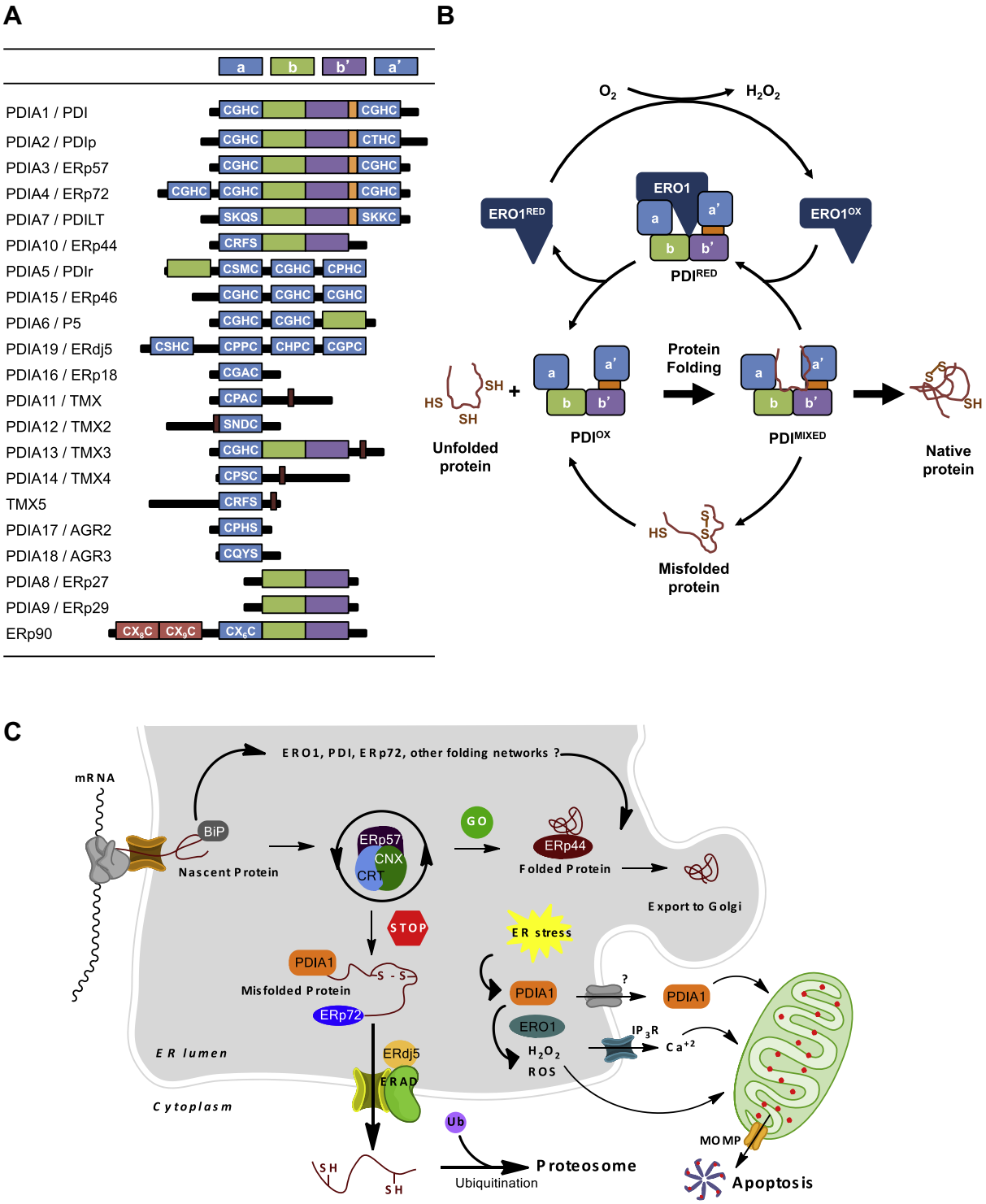

Fig. 1. The PDI family of proteins. (A) Domain structure of PDI family members is indicated with the following color representation: In blue, catalytic domains **a** and **a**′, in green and purple, non-catalytic **b** and **b**′ domains, respectively. The x-linker between **b**′ and **a**′ is shown in orange and transmembrane domains in dark red. (B) Schematic representation of the protein folding activity of PDI and the redox relay of PDI and ERO1, resulting in the generation of H_2O_2. PDI^{OX}: oxidized PDI; PDI^{RED}: reduced PDI; PDI^{MIXED}: PDI with the bound substrate; $ERO1^{OX}$: oxidized ERO1; $ERO1^{RED}$: reduced ERO1. PDI domains are colored as shown in (A). (C) Schematic representation of the function of PDIs at the ER. Briefly, newly synthesized unfolded polypeptides are translocated into the ER. Folding of glycoproteins requires CNX (green) or CRT (light blue) and ERp57 (purple), the components of the CNX/CRT cycle. Non-glycosylated polypeptides may acquire a folded state by additional protein folding networks, to promote their folding, including, for example, the participation of PDIA1 or ERp72. ERp44 (dark red) retains client proteins in the ER preventing premature transport to the Golgi until the folding process is completed (GO). Then, folded proteins are exported through the secretory pathway. Misfolded proteins (STOP) may bind to PDIA1 (orange) and ERp72 (blue) and retro-translocate to the cytosol via the ERAD pathway, where ERdj5 has an important function in disrupting disulfide bonds. Under ER stress conditions, PDIA1 can be released to the cytosol through activation of BAX and BAK at the ER, targeting the mitochondria. This event triggers apoptosome assembly resulting in cell death. By modulation of the redox state of calcium channels, such as the IP3 receptor and the calcium pump SERCA, PDIs also control ER calcium release. In addition, abnormal H_2O_2 production by ERO1 (dark blue) could result in ROS production. These two events may also contribute to trigger mitochondrial outer membrane permeabilization and the release of cytochrome c (red circles) and the activation of the apoptosome. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which are targeted to the cytosol for proteasome-mediated degradation through the ERAD pathway [11].

ER stress triggers an adaptive program termed the unfolded protein response (UPR), an integrated signaling cascade that controls the expression of a large spectrum of target genes involved in almost every aspect of ER physiology [10]. Initially, the UPR increases the folding capacity of the ER through a massive up-regulation of ER chaperones and foldases, including well known components such as BiP/Grp78, Grp94, calreticulin (CRT), calnexin (CNX) and several PDI family members [12]. In addition, the removal of misfolded proteins from the ER via ERAD is stimulated by the UPR by controlling key genes of the pathway, including ER degradation-enhancing alpha-mannosidase-like 1 (EDEM1) and Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain protein (HERP) [11,13].

A triade of ER transmembrane proteins operate as stress sensors initiating the UPR. Inositol requiring kinase 1 (IRE1), activating transcription factor 6 (ATF6), and PKR-like ER kinase (PERK) monitor fluctuations in the protein folding capacity of the ER and transduce stress signals to the cytosol and nucleus, controlling the expression of specific transcription factors and signaling events [13,14]. When UPR adaptive programs are not sufficient to recover cellular homeostasis, its signaling outputs are shifted towards the induction of apoptosis [15,16].

PDIs are important targets of the UPR as revealed in numerous gene expression profile studies of cells undergoing ER stress (see examples in [17–19]). In fact, most proteins trafficking through the secretory pathway depend on disulfide bond formation and/or isomerization [20]. Hence, PDI family members fulfill key roles as ER foldases by catalyzing disulfide reduction (breakage), formation (oxidation) and isomerization (rearrangement) thereby promoting native protein folding (Fig. 1B) [21]. Although some PDI family members have been related to neurodegeneration, their specific contribution to particular pathological conditions is only beginning to be understood. In this review, we overview recent findings indicating important and divergent roles of PDI family members in PMDs, suggesting their participation not only in protein folding and quality control, but also in other processes such as apoptosis, production of reactive oxygen species (ROS), protein aggregation, cell signaling, and ERAD.

3. The protein disulfide isomerase family

In this section we discuss the biological and mechanistic aspects involving PDIs with neurodegenerative diseases. Disulfide bonds in proteins are generated by oxidation of two free thiols originating from two cysteine residues. The presence of disulfide bonds within (intramolecular) and between (intermolecular) proteins has important consequences for the structural stability of proteins or the formation of protein complexes. In addition, disulfide bonds may act as regulatory switches providing a mechanism to control the function of proteins[21].

PDIs are a large protein family comprised of 21 known members of the thioredoxin superfamily, classified based on sequence and structural homology (reviewed in [22]) (Fig. 1A). In addition to their function as foldases, PDIs can act as molecular chaperones by inhibiting the aggregation of unfolded/misfolded proteins at the ER [20,23]. PDI family members are characterized by a modular arrangement of thioredoxin-like catalytic **a** domains and non-catalytic **b** domains resulting in structural and functional versatility within the family. The catalytic **a** domains contain CXXC active-site motifs that react with thiols of newly synthesized proteins and determine the redox potential of PDIs and their role as a reductase, oxidase or isomerase [22]. In an oxidized state, PDIs can transfer disulfides to the substrate catalyzing its oxidation leading to a reduced active site [22]. Conversely, in a reduced state the disulfide of the substrate can be reduced and the PDI active site becomes oxidized again. The non-catalytic **b** domains act as stabilizers of the catalytic **a** domains and are responsible for substrate recruitment, and provide interaction sites for cofactors [22].

PDI (here termed PDIA1 to differentiate from the general referral to members of the PDI family) was the first PDI family member identified and contains four domains, **a**, **b**, **b**′ and **a**′, arranged in a U-shaped structure [24]. Studies in yeast indicated an essential function of PDIs for cell survival [25,26]. Depletion of PDIA1 in mammalian cells results in a delay in disulfide bond formation of secretory proteins highlighting its vital role in oxidative protein folding [27]. Despite the extensive progress in our understanding of the structure and enzymatic properties of PDIA1, little is known about specific functions of PDIA1 in vivo. PDIA1 was found to have the broadest substrate specificity of the PDI family members investigated so far [28], a property possibly due to the presence of a large substrate binding site in the **b**′ domain recognizing unfolded proteins. The **b** domain on the other hand regulates the substrate binding capacity of the **b**′ domain which also can be regulated by an 19 amino acid x-linker region between the **b**′ and **a**′ domains (reviewed in [28]).

ERp57 (also known as Grp58 and PDIA3) displays similar domain organization as PDIA1. However, ERp57 is well known as a key component of the CNX–CRT cycle. As part of a protein complex with CNX and CRT it is responsible for the folding of highly glycosylated and disulfide bond-containing proteins [28]. ERp57 is required as a scaffolding protein for the assembly of the peptide loading complex of MHC class I molecules, a function independent of its enzymatic activity [29]. This alternative function is associated with a physical interaction with tapasin [30], which requires covalent bonding between them [31,32]. Additional functions of ERp57 as a modulator of ER calcium homeostasis and in STAT3 signaling have been suggested (reviewed in [33]).

Specific functions of other PDI family members are poorly understood. PDIp/PDIA2 displays a similar domain organization as PDIA1, nevertheless its physiological role is unknown. Initially, PDIp was found to be expressed in pancreatic acinar cells, however it is also highly expressed in dopaminergic neurons suggesting that it supports the increased secretory demand of these cells [34]. ERp72/PDIA4 on the other hand, has been suggested to compensate for ERp57 deficiency in knockout cells [35]. ERp72 contains three catalytic **a** domains and two non-catalytic **b** domains [24]. How ERp72 binds substrates is unclear. Nevertheless, ERp72 was found to be part of a large complex of chaperones in the ER including other PDI family members [36]. For detailed analysis of PDI structure and function see [28,37]. Overall, despite the fact that the PDI family of proteins is formed by a large group of highly conserved components, only a few PDIs have been studied. Very little information is available about the substrate specificity of individual PDI family members and their relation to other components of the ER protein-folding network or their roles in other cellular locations, such as the cytosol, mitochondria, or nucleus.

4. Multiple functions of PDIs in neurodegenerative diseases

In the next sections we overview different studies identifying distinct contributions of PDI family members in diverse neurodegenerative conditions. Available data suggests important roles of PDIs in attenuating ER stress and the specific aggregation of PMDs-related proteins, in addition to modulating cell death (both pro- and anti-apoptotic activities) [38], and the local ER redox status, among other important functions (summarized in Fig. 2A).

A

Disease	Effect	Reference
ALS	• PDIA1 and ERp57 are up-regulated in ALS mouse models and patients. • PDIA1 knockdown or overexpression increases or decreases mutant SOD1 aggregation, respectively. • PDIA1 inactivation by S-nitrosylated is detected in tissue from ALS mouse models and patients. • PDIA1 co-localizes with FUS and TDP-43 inclusions in ALS-derived tissue.	[42, 51, 52, 54, 55, 57]
PD	• S-nitrosylated PDIA1 is found in the brain of PD patients. • PDIp is upregulated in the brain of PD patients, it is found in Lewy bodies, and is induced in αSynuclein transgenic mice. • PDI upregulation is observed in many toxicological models of PD.	[34,38, 60]
AD	• ERp57 interacts with β-amyloid and prevents its aggregation. • PDIA1 is present in neurofibrillary tangles. • S-nitrosylated PDIA1 is detected in the brain of AD patients. • Inhibition of PDIA1 and ERp57 protects against amyloid β toxicity.	[38, 61, 62, 70, 100]
PrDs	• ERp57 is upregulated in the brain of CJD patients and in animal models of infectious prion disease. • ERp57 and PDIA1 protect cells against misfolded PrP. • PDIA1 is nitrosylated in PrD models.	[39, 44, 45-49]
HD	• Inhibition of PDIA1 or ERp57 suppresses neuronal degeneration induced by mutant Huntingtin.	[100]

B

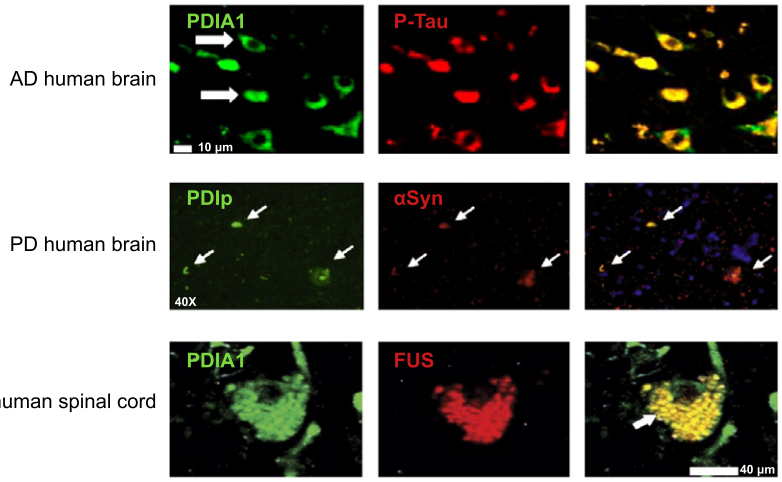

Fig. 2. Evidence implicating PDI family of proteins in neurodegenerative diseases. (A) Summary of the most relevant findings relating PDI family members with multiple neurodegenerative conditions. (B) Examples of in vivo co-localization between protein inclusions related to the etiology of AD, PD, and ALS with distinct PDI family members. Data was obtained in human post mortem samples from patients affected with PMDs. Images were originally published in [34,61,65]. Copyright authorization was obtained from each journal.

4.1. PDIs in neuroprotection

ER stress is a salient feature of most PMDs [7]. The upregulation of PDIs as part of the general UPR transcriptional response may contribute to the reduction of the general overload of misfolded proteins inside the ER. In fact, expression of PDIs has been shown to protect against ER stress in different experimental settings (examples in [38–42]). The upregulation of several PDI family members have been extensively reported in mouse models of PMDs and also in post-mortem human samples from patients affected with neurodegenerative diseases (Fig. 2B). The first study describing a functional role of PDIs in the brain was performed more than a decade ago in a brain ischemia-reperfusion model [43], where through a proteomic study, PDIA1 was identified as a major protein induced in a cell culture model of hypoxia. PDIA1 is highly expressed in the brain of ischemic rats and its overexpression in vivo resulted in improved neuronal survival upon ischemia reperfusion [43]. Creutzfeldt–Jakob (CJD) disease, a PrD, was the first human brain disease shown to be associated with an upregulation of PDIs. A proteomic study of sporadic CJD brain tissue showed that ERp57 is induced in the pathology, representing the only consistent hit of this analysis [44]. We were able to confirm this observation in sporadic CJD, but also in a case of new variant CJD [45]. Moreover, we and others, described an increased expression of PDIA1 and ERp57 in murine models of infectious scrapie-prions [39,45–49]. ERp57 levels directly correlated with the levels of prion misfolding and inversely correlated with the extent of neuronal damage [39,50]. Functional studies also indicate that PDIA1 or ERp57 expression protects cells against the toxicity of misfolded PrP in vitro [39,49].

A large amount of reports link PDIs with the pathogenesis of ALS. A pioneer proteomic study of symptomatic mutant SOD1

transgenic mice (an ALS model) lead to the identification of PDIA1 and ERp57 as the two main proteins upregulated in the spinal cord [51,52]. Furthermore, PDIA1 overexpression in cell culture protects against mutant SOD1 neurotoxicity [42]. Consistent with this observation, increased levels of PDIA1 and ERp57 were found in the spinal cord and CSF from human sporadic ALS patients [53–55]. A recent proteomic screening for ALS biomarkers in peripheral mononuclear cells from ALS patients identified PDIA1 and ERp57 as major proteins induced [56]. Remarkably, changes in ERp57 expression had the highest score as a biomarker to monitor disease progression [56], suggesting its potential use to monitor the effectiveness of clinical trials. In addition, it was suggested that a member of the reticulon family, Reticulon-4A (NOGO-A), has a protective role in an ALS mouse model [57]. In the same study, therapeutic effects of NOGO-A expression were associated with intracellular redistribution of PDIA1, indicating a regulatory role of NOGO-A in PDIA1 function [57].

In two gene expression profile studies of PD cell culture models, the upregulation of PDIA1 and ERp57 were also identified as significant changes of a global ER stress response [58,59]. In addition, PDIp is induced in a toxicological mouse model of PD, as well as in brain tissue derived from PD patients [34]. Increased expression of PDIA1 is also observed in αSynuclein transgenic mice [60]. However, no studies have addressed the functional contribution of PDIs to PD in vivo. Augmented levels of PDIA1 were also reported in the brain of AD patients, co-expressed in cells containing neurofibrillary tangles [61,62]. Together these data suggest that augmentation of the levels of PDIs may represent a protective response to neurodegenerative conditions associated with abnormal protein aggregation, contributing to the re-establishment of protein homeostasis.

4.2. Control of aggregation of disease-related proteins

A few reports have shown that the overexpression of PDIs may decrease the aggregation of proteins linked to PMDs. For example, knocking down PDIA1 increased the levels of mutant SOD1 aggregation whereas its overexpression had the opposite effect [42]. These findings are consistent with the known role of cysteine residues and intermolecular disulfide bonding in the aggregation of mutant SOD1 [63,64]. Moreover, a small molecule that mimics the active site of PDIs decreased mutant SOD1 aggregation in vitro [42]. PDIA1 co-localizes with abnormal protein inclusions associated with sporadic ALS [55]. Furthermore, mutant SOD1 is found in ER-enriched fractions and a physical interaction between PDIA1 and mutant SOD1 was described both in vitro and in vivo [51]. Similar interactions were recently described for another ALS-linked gene, fused in sarcoma (FUS) [65]. PDIA1 also co-localizes with TDP43 in human ALS tissue [55]. Finally, PDIA1-positive protein inclusions were also described in patients with multiple system atrophy [66], and Alzheimer's disease [61,62].

We and others showed that PDIs can physically interact with misfolded PrP [39,67], and general inhibition of PDI activity with bacitracin causes the generation of more aggregated PrP [67], consistent with the idea that inter-disulfide bond formation is also an important factor driving prion misfolding [68]. PDIA1 also was found to prevent αSynuclein aggregation in a cell-free system [69]. Finally, ERp57 is found in CSF of AD patients physically associated with amyloid-β [70], suggesting a role as a carrier protein that prevents aggregation of the β-amyloid peptide. These few reports propose an interesting scenario where PDIs may not only reduce general ER stress levels in PMDs as part of the UPR, but they could also modulate the aggregation of specific disease-related proteins possibly through a direct interaction. However, most of the data relating PDIs with PMDs so far are correlative with almost no mechanistic or molecular insights.

4.3. The ER redox balance and PDI activity

Sustained maintenance of specific redox conditions for the formation of correct disulfide bonds inside the ER requires equilibrium between the oxidized and reduced states of PDIs [71–75]. The ER as an oxidative environment compared to the cytosol favors protein folding and the formation of disulfide bonds between two cysteine residues by thiol oxidation [76]. Alteration of the ER redox state is detrimental for normal cell function, and thus well-controlled mechanisms maintain the redox balance inside this organelle [77]. Yeast and mammalian ERO1 catalyze PDI oxidation coupled to de novo disulfide formation and the reduction of molecular oxygen to hydrogen peroxide (H_2O_2) [78]. In mammals, ERO1α is broadly expressed, whereas ERO1β is greatly enriched in the endocrine pancreas and in immunoglobulin-secreting cells [79–81]. Under normal physiological conditions, ERO1 probably is the most important oxidase for disulfide formation. Yeasts lacking ERO1 are not viable [82,83], whereas ERO1α and β double knockout mice and cells are viable [81], suggesting that additional pathways for disulfide formation exist in mammalian cells. In fact, recently recognized ERO1-independent pathways for the efficient oxidative folding include enzymes working together with PDI proteins, such as peroxiredoxin (PRDX) 4, glutathione peroxidase (GPX7 and GPX8), quiescin sulfhydryl oxidase (QSOX) and vitamin K epoxide reductase (VKOR) (reviewed in [84]). Other studies also suggest that small molecules like H_2O_2, glutathione disulfide (GSSG) or dehydroascorbate directly oxidize cysteines in PDIA1 and substrate proteins to form disulfides bonds (reviewed in [84]). To prevent hyper-oxidizing conditions and overproduction of ROS different redox buffers operate inside the ER, where GSH is maintained at high levels [72]. The existence of additional pathways to generate disulfide bonds in proteins in the ER of mammalian cells suggests that PDIs may function more specifically to control folding of selected client proteins.

The accumulation of unfolded proteins in the ER lumen is sufficient to produce ROS [85,86], suggesting a vicious cycle of ER stress and local oxidative stress finally leading to cell death when unresolved [87]. In fact, the efficiency of ER protein folding and secretion can be enhanced by antioxidants [88]. Thus, uncontrolled ER stress or an imbalance between PDIs/ERO1 and other redox buffering systems may contribute to the generation of oxidative stress in neurodegenerative diseases, a hypothesis that remains to be further explored. On the other hand, an altered ER redox status could explain the occurrence of ER stress in some PMDs through direct inhibition of PDIs function (see next section).

4.4. Inactivation of PDIs by S-nitrosylation

Enhanced production of ROS has been proposed to be the cause of ER stress in pharmacological models of PD in vitro [89]. This phenomenon correlates with the oxidation of ERp57, leading to accumulation of high molecular weight ERp57-containing aggregates [90]. Accumulating evidence indicates that oxidative stress actually directly alters the activity of PDIs in many human diseases (reviewed in [91]). For example, S-nitrosylation of PDIA1 was initially described in brain tissue derived from PD and AD [38]. This chemical modification inactivates PDIA1 activity, possibly triggering ER stress in addition to eliminating its anti-apoptotic activity [38]. Similarly, PDIA1 S-nitrosylation is observed in spinal cord tissue derived from human ALS cases and mouse models of the disease [42]. Similarly, PDIA1 S-nitrosylation was recently reported in a PrD model [49]. One study also suggested that nitrosative stress could also trigger S-glutathionylation of PDIA1 [92]. Overall, many correlative reports have shown the simultaneous occurrence of ER stress and oxidative stress in PMDs in vivo (see examples in [93,94]). However, most of the available in vivo observations are

correlative and the actual contribution of PDIA1 S-nitrosylation to neurodegeneration remains to be established with more direct approaches.

4.5. Pro-apoptotic role of PDIs

Chronic or irreversible ER stress triggers apoptosis [16], where apoptotic signals converge into the activation of the main intrinsic death pathway at the mitochondria through activation of pro-apoptotic BCL-2 family members [13]. Activation of BAX and BAK is key to induce ER stress-mediated apoptosis facilitated by the release of cytochrome c and the assembly of the apoptosome [95]. BAX and BAK are also located at the ER membrane, where they control ER calcium homeostasis [95,96] and UPR signaling [97,98]. Remarkably, a recent report indicates that BCL-2 proteins also regulate the permeability of the ER membrane to luminal proteins during apoptosis, associated with the release of ER chaperones including PDIA1 and BiP [99]. This study suggested that PDIA1 might have a direct function in regulating apoptosis signaling.

A small molecule screening for compounds that decrease mutant huntingtin toxicity identified five molecules that all target and inhibit PDIA1 and ERp57 [100]. These unexpected findings were confirmed by direct manipulation of PDIA1 and ERp57 levels in models of HD and AD in the same study [100]. Remarkably, PDIA1 was shown to induce mitochondrial-outer membrane permeabilization in a BAK-dependent manner, triggering the canonical apoptosis pathway (Fig. 1C). This phenomenon was associated with the accumulation of PDIs in membrane contact sites between ER and mitochondria [100]. This novel pro-apoptotic function of PDIs may represent a new link between protein misfolding and cell death that contrasts with the known role of PDIs in alleviating ER stress and promoting cell survival (dual function).

4.6. Other possible biological functions of PDIs in neurodegeneration?

PDIs are located in many subcellular compartments, including the ER, cytosol, mitochondria, nucleus, plasma membrane as well as the extracellular space (reviewed in [101]). Their ubiquitous cellular distribution highlights their direct or indirect participation in many biological processes. Under various physiological conditions PDIs may modulate the structure and function of individual client proteins at distinct subcellular locations via their chaperone, foldase or isomerase activity. Owing to their ER localization, PDIs affect the folding of many proteins that transit through the secretory pathway which may depend on the cell type affected. ERp57 was described to participate in signaling events from the plasma membrane as well as in regulatory events in the nucleus where it was found to interact with several transcription factors and with DNA itself [33]. These observations suggest many additional functions of PDIs in the maintenance of cellular homeostasis. ERdj5 has a central role in ERAD, where it catalyzes the disruption of disulfide bonds to allow the retro-translocation of proteins to the cytosol for degradation [102]. This opens the possibility that the effects of PDIs on the aggregation of PMDs-related proteins may be related to enhanced degradation. PDIs also modulate the activity of ER calcium channels and pumps [103,104], which may impact diverse physiological processes dependent on calcium such as mitochondrial metabolism and apoptosis. It remains to be determined if all these alternative functions of PDIs may also contribute to neurodegeneration in PMDs.

4.7. Studies of PDIs function in vivo

The function of PDI family of proteins is poorly understood in vivo. A few knockout mouse models for PDIs have been described to date, including ERp57, ERdj5 and AGR2 [29,105–108]. Full ERp57 deficiency is lethal in mice at embryonic day 13.5, possibly due to STAT3 signaling modulation [109]. In this study, deletion of ERp57 had no effect on ER morphology, ER stress sensitivity, apoptosis or the expression of other ER chaperones in ERp57 deficient mouse embryonic fibroblast (MEF) cells [109]. B-cell specific ERp57 knockout mice show decreased MHC class I expression and suboptimal peptide loading [29]. Unexpectedly, B cells deficient for ERp57 produce normal levels of immunoglobulin, a highly glycosylated protein containing several disulfide bonds. Deletion of ERp57 in intestinal cells showed that enterocytes fail to display steroid hormone-stimulated calcium responses [105].

In ERdj5 knockout mice show increased levels of ER stress markers in the salivary gland [108]. ERdj5 expression alleviates ER stress triggered by α-amylase overexpression [108]. Anterior gradient homolog 2 (AGR2)/PDIA17 deficiency in mice drastically alters intestinal homeostasis [107]. AGR2 knockout mice are viable, but show decreased levels of mucin, an essential component of the protective mucus in the intestine. These mice also presented abnormal Paneth cell morphology with increased levels of ER stress. AGR2 deficient animals develop severe colitis [107]. Another study also showed that AGR2 deficient mice are more susceptible to colitis due to reduced production of intestinal mucin [106]. Related observations were also described on loss-of-function experiments in zebrafish [110]. AGR2 function has been also related to the metastatic capacity of pancreatic cancer in vivo [111]. Studies of ERp5/PDIA6 in zebrafish indicated an interesting developmental function in maintaining the asymmetry of several organs including the brain [112].

Deficiency in the ER disulfide oxidase ERO1α does not induce clear spontaneous phenotypes, whereas ERO1β deficiency alters pro-insulin folding, triggering glucose intolerance [81]. As mentioned, ERO1α and ERO1β double deficient animals are viable, having normal secretion of immunoglobulins [81], which is consistent with the existence of independent pathways for disulfide bond formation. Most attempts of generating knockout mouse models for ER chaperones failed, because deficiency lead to embryonic lethality, due to their vital roles during embryonic development [113]. Interestingly, knockout mice for CNX [114], BiP [115], and Sil1 [116], a nucleotide exchange factor for BiP, have revealed important roles of these proteins in the maintenance of protein homeostasis in the nervous system. However, to date the possible impact of PDI family members on brain function, as well as their contribution to motor or cognitive functions of the CNS, remains fully unexplored. It will be interesting to define if PDIs are involved in the synthesis and secretion of specific neurotransmitters, which could have a broad impact on neuronal function, an important aspect to consider for future clinical use of PDI inhibitors.

5. Concluding remarks

The role of the PDI family in the CNS remains highly elusive. In this review we discussed most evidence suggesting the involvement of these enzymes in neurodegenerative diseases. PDIs are a large family of foldases with complex yet poorly characterized functions with emerging roles in PMDs (Fig. 2). Of note, PDIs are linked to other important diseases such as cancer and infectious diseases, including HIV-AIDS, Hepatitis and Chlamydia infections. The broad involvement of PDIs in human pathologies underlines their importance in fundamental processes, and highlights the need for a better understanding of their biological function, which is crucial to evaluate their use as possible targets for disease intervention. Small molecules to inhibit PDI activity or gene therapy approaches for "gain of function" may have specific applications depending on the disease context. However, the field clearly needs to advance into detailed in vivo validation to specifically identify

the contribution of distinct PDI family members to particular pathological conditions using conditional knockout mouse models. In addition, a better definition of how ER protein-folding networks are arranged and what are their substrate specificities is necessary to move forward into therapeutic applications.

Monitoring the expression of distinct PDI family members or an overall PDI network signature in body fluids may also serve as an important tool for early disease diagnosis due to the fact that PDIA1 and ERp57 are present in CSF and blood of ALS patients [56] and have been identified as top hits in several proteomic screenings of human diseases. ERp57 and CRT are also upregulated on the cell surface of cancer cells, which has been exploited for immunotherapy [117]. Inhibitors of PDIs also represent efficient anticancer compounds [118,119]. Thus, we believe that cancer research may provide interesting compounds in the near future to manipulate PDIs in PMDs.

In summary, the information discussed here demonstrates the significance of PDIs as potential targets for new treatments and the potential use of PDIs as biomarkers for diagnosis and prognosis of neurodegenerative diseases. Since PDIs play key roles in diverse cellular contexts, the generation of more specific compounds to target distinct PDI family members will be of high relevance to prevent potential systemic effects of chronic drug administration. To solve this issue, gene therapy represents a reliable option to manipulate specific PDI-family members in a disease context, locally in the affected tissue. This concept has been already probed in animal models of brain and cardiomyocyte ischemia [43,120]. The development of new mouse models to study the biology of PDI family members in specific tissues will provide valuable information to define their function in physiology and disease. Finally, due to the fact that alterations in PDIs function is emerging as an important factor in many PMDs, the possible occurrence of genetic mutations in *PDI* genes as part of the etiology of PMDs represents an interesting open question to be explored through genetic screening in the human population.

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