● PERSPECTIVE

ERp57 in neurodegeneration and regeneration

The protein disulfide isomerases (PDIs) family has a central function in the folding of proteins synthetized through the secretory pathway. ERp57, also known as Grp58 or PDIA3, is one of the main studied members of this family. ERp57 catalyzes the formation, disruption and isomerization of disulfide bonds of glycoproteins mediated by a cooperative interaction with the endoplasmic reticulum (ER) chaperones calnexin and calreticulin (Turano et al., 2002) (**Figure 1A**, **B**). In the past years, several studies have linked ERp57 and its closest homologue PDI (also known as PDIA1) to diseases affecting the central nervous system, including amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), Alzheimer's disease (AD), among others (Andreu et al., 2012). These neurodegenerative conditions are characterized by the presence of abnormal protein aggregates containing specific proteins, which are now classified as protein misfolding disorders (PMDs) (Hetz and Mollereau, 2014).

Alterations in the function of the ER trigger the accumulation of misfolded/unfolded proteins on its lumen, a cellular condition named "ER stress". Cells undergoing ER stress engage an adaptive reaction known as the unfolded protein response (UPR), which aims to re-establish cell's homeostasis. PDIs are major target genes induced by the UPR transcriptional program (Matus et al., 2013). In the last decade, accumulating studies have associated the occurrence of ER stress to PMDs, but the actual role of the UPR in the neurodegenerative process is just starting to be elucidated (Hetz and Mollereau, 2014). In this context, the contribution of PDIs to neurodegenerative diseases is still an open question and the possible physiological role of has family in the nervous system is even less explored.

Recent studies have identified distinct alterations to the function of ERp57 and PDIA1 in PMDs (reviewed in Andreu et al., 2012). For example, ERp57 levels are highly expressed in tissue from patients and mouse models affected with Prion disease or ALS (Andreu et al., 2012). ERp57 has also been proposed as a possible biomarker for sporadic ALS, whereas chemical inactivation of PDIA1 through nitrosylation of its active site was reported in patients with sporadic PD, AD and ALS. In general, there is a direct correlation between the accumulation of protein aggregates in PMDs and the upregulation of PDIs, which often co-localize with disease-related protein inclusions (**Figure 1C**). However, only recently the functional contribution of PDIs to diseases affecting the nervous system has been explored.

On an attempt to characterize the impact of ERp57 to neurodegeneration *in vivo*, we generated a transgenic mouse that overexpresses ERp57 in the nervous system (Castillo et al.,2015; Torres et al., 2015). Based on evidence indicating the inactivation of PDIs in PD (Andreu et al., 2012), we challenged ERp57 transgenic mice with the neurotoxin 6-OHDA to trigger dopaminergic neuron degeneration. In contrast to the initial prediction, no protection of dopaminergic neurons was observed upon ERp57 overexpression. Similarly, no changes in striatal innervation or motor performance were detected in these animals after exposure to 6-OHDA (Castillo et al., 2015). Interestingly, when ERp57 was targeted in the context of peripheral nerve injury, a marked effect was uncovered in axonal regeneration (Castillo et al., 2015). ERp57 transgenic mice showed significant locomotor recovery after injury, possibly due to a reduction in the amount of degenerated myelin sheets

and accelerated regeneration. These results depicted for the first time a functional role of the ER proteostasis machinery in axonal regeneration. We speculate that the lack of protection in the PD model could be due to the oxidative inactivation of ectopic ERp57 caused to the redox imbalance triggered by 6-OHDA.

Defining the contribution of ERp57 to neurodegeneration still demands more efforts. This is particularly true for ALS, where protein misfolding and ER stress is one of the major pathological events in the disease process as reported in different animal models of the disease and by the analysis of patient-derived samples (reviewed in Matus et al., 2013). ER stress is one of the earliest perturbations detected in motoneurons even before any denervation is detected and may underlay the differential neuronal vulnerability of specific subpopulation of motoneurons (Matus et al., 2013). Moreover, a genetic screening identified point mutations in the genes encoding ERp57 and PDIA1 as risk factors to develop ALS (Gonzalez-Perez et al., 2015). We recently characterized the possible impact of these PDI variants to motoneuron function (Woehlbier et al., 2016). Using complementary approaches, including zebra fish and three independent *in vitro* models of cultured motoneurons, we uncovered a novel role of PDIA1 and ERp57 in promoting neurite outgrowth and connectivity, an activity fully loss when the ALS-linked mutants were expressed (Woehlbier et al., 2016). Furthermore, we explored the possible physiological function of ERp57 in the nervous system by generating a conditional knockout mouse. Targeting *ERp57/PDIA3* in neurons resulted in altered motor performance and early death of the animals (Woehlbier et al., 2016). These effects were associated with dramatic alterations in the function and morphology of neuromuscular junctions, leading to changes in muscle physiology. All the phenotypes identified in ERp57 deficient mice resembled early stages of ALS.

These observations provided the first clues about the possible biological function of ERp57 in the brain, supporting a role in neuronal connectivity. Biochemical analysis revealed that mutations in PDIA1 modified its enzymatic activity, whereas mutations in ERp57 altered the physical association with calnexin and calreticulin (Woehlbier et al., 2016). In agreement with the effects of ERp57 deficiency in neuromuscular junctions, analysis in the levels of selected synaptic proteins indicated that the expression of synaptic vesicle 2 (SV2) protein was reduced in ERp57 deficient animals (Woehlbier et al., 2016). We proposed that ERp57 mediated in part the folding of synaptic proteins, like SV2, as a component of the calnexin and calreticulin cycle. In addition, we recently reported that ERp57 assists the folding of other disease-related proteins including the Prion protein (Torres et al., 2015). Thus, altered folding of specific neuronal clients may underlay the phenotypes of altering the function of PDIs in diseases effecting the nervous system. Since ERp57 has been also linked to the modulation of ER calcium release and cytoskeleton dynamics (LeBlanc and Nemere, 2014), we speculated that ERp57 may also contribute to neurite outgrowth by modulating the function of the cytoskeleton.

ERp57 is upregulated when the UPR is engaged in most experimental systems, suggesting that this foldase may reduce the load of abnormal proteins by enhancing the folding capacity of the ER. Although initial studies linked the induction ERp57/ Grp58 with neuroprotection against protein folding stress (Hetz et al., 2005), recent efforts have shown that its expression does not significantly impact cell fate under ER stress. We explored this question using gain- and loss-of-function strategies in various cellular models and also transgenic mice overexpressing ERp57. Overall, modulating ERp57 levels did not have any differential effect on the susceptibility of cells to ER stress (Torres
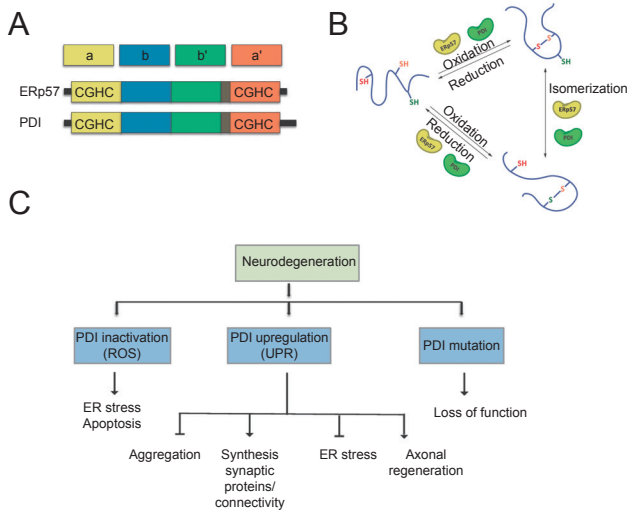
Figure 1 ERp57 and protein disulfide isomerase (PDI) in neurodegeneration.
(A) Primary structure of ERp57 and PDIA1 (indicated as PDI) indicating the catalytic domains (a and a' in yellow and orange, respectively) and non catalytic domains (b and b' in blue and green, respectively). (B) Function of ERp57 and PDIA1 in disulfide bond formation. Both proteins participate in the formation (reduction), oxidation and isomerization (rearrangement) of disulfide bonds of folded proteins at the endoplasmic reticulum (ER). (C) Possible involvement of PDIs in neurodegeneration. Alterations and upregulation of ERp57 and PDIA1 is detected in PMDs. PDIs can be inactivated in oxidative conditions through S-nitrosylation in PDIA1, which may trigger chronic ER stress. Upregulation of PDIs may decrease the accumulation of toxic misfolded proteins, it could promote the synthesis of synaptic proteins (like SV2), decrease ER stress levels and improve axonal regeneration.

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et al., 2015; Woehlbier et al., 2016). Similarly, ERp57 deficient animals in the nervous system do not develop signs of ER stress. These unexpected results may be explained by the fact that ERp57 folds a small subset of ER clients, and not all glycosylated proteins containing disulfide bonds. Based on this new evidence, we believe the major role of ERp57 in the nervous system and PMDs involves the control of neuronal proteostasis at the level of selected synaptic proteins.

Together, the available evidence is depicting a novel scenario, placing PDIs as interesting targets for disease intervention. Due to the fact synaptic dysfunction are salient features of most neurodegenerative conditions, the possible consequences of enforcing ERp57 expression in the brain (*i.e.*, using gene therapy) should be explored as a strategy to improve neuronal survival, synaptic function, and enhance tissue regeneration where ERp57 could act over protein substrates involved in synaptic functions. It remains to be elucidated if other functions beyond the ER can be attributed to the beneficial effects of ERp57 expression. Of note, ERp57 has been described in subcelular localizations beyond the ER, including the nucleus, the plasma membrane and cytoplasm (Turano et al., 2002). The new mouse models generated to manipulate ERp57 levels in the nervous system represent relevant tools to assess the function of this foldase to diverse diseases. Since the PDI family of proteins involves more than 20 members, we predict that this field will accelerate and develop toward the identification of novel functions of PDIs and other components of the ER proteostasis network on a variety of pathologies involving altered function of the secretory pathway.

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