Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterised by the progressive loss of motor neurons, leading to paralysis and death within several years of onset. Although protein misfolding is a key feature of ALS, the upstream triggers of disease remain elusive. Recently, endoplasmic reticulum (ER) stress was identified as an early and central feature in ALS disease models as well as in human patient tissues, indicating that ER stress could be an important process in disease pathogenesis. One important chaperone induced by ER stress is protein disulphide isomerase (PDI), which is both upregulated and posttranslationally inhibited by S-nitrosylation in ALS. In this paper, we present evidence from studies of genetics, model organisms, and patient tissues which indicate an active role for PDI and ER stress in ALS disease processes.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal, rapidly progressing neurodegenerative disorder primarily affecting motor neurons of the spinal cord, brainstem, and cortex [1]. The disease is characterised by muscle weakness leading to paralysis and death usually within several years of diagnosis [2]. Similar to other neurodegenerative diseases, protein misfolding and abnormal intracellular protein inclusions are pathological hallmarks of ALS. However, the pathogenic mechanisms of disease are yet to be elucidated [3]. Approximately 10% of ALS cases are inherited, and up to 20% of these familial cases are caused by mutations in the gene encoding superoxide dismutase 1 (SOD1) [4, 5]. SOD1-linked disease remains the most studied and best characterised form of ALS, although successful therapeutic interventions from mutant SOD1 models have thus far failed to translate to patients [1]. Additional factors involved in disease have recently been identified, including the proteins TAR DNA binding protein 43 (TDP-43) and fused in sarcoma (FUS) as key players in disease pathogenesis [6], and understanding the molecular mechanisms of ALS will allow the design of effective therapeutics in the future. Recent evidence has identified endoplasmic reticulum (ER) stress and in particular the chaperone protein disulphide isomerase (PDI), as important in ALS disease processes [7, 8]. The role of PDI as a protective factor in ALS, with a particular focus on disulphide bond-mediated SOD1 misfolding, forms the central topic of this paper.

2. Endoplasmic Reticulum Stress

The ER is an important organelle for the folding and post-translational modification of many proteins, and it also acts as a significant intracellular calcium store [9]. The ER forms close associations with other organelles, including mitochondria, the Golgi apparatus and the nucleus, and is therefore a central player in cell physiology [10, 11]. Since there is a high rate of protein production in the ER, prevention of protein misfolding is an important function of this organelle. The protein folding capacity of the ER is adjusted according to requirements in order to maintain homeostasis [12].

Review Article
Mechanisms of Neuroprotection by Protein Disulphide Isomerase in Amyotrophic Lateral Sclerosis

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Proteins misfold as part of normal physiology, and when the rate of protein synthesis is increased, the burden of unfolded proteins in the ER also increases. This results in ER stress, which triggers signalling pathways collectively known as the unfolded protein response (UPR) [13]. Cellular insults including dysregulation of calcium stores, changes in the ER redox state, nutrient deprivation as well as disturbances in the Golgi, endosomal and vesicular transport systems, and induction of mitochondrial-associated apoptosis, can all elicit ER stress [14–16].

The UPR is a homeostatic mechanism which is initially protective by upregulation of chaperone proteins such as PDI, inhibition of general protein translation, and expansion of ER volume. The UPR is mediated by activation of three upstream sensors of ER stress, namely, PKR-like endoplasmic reticulum kinase (PERK), inositol-requiring kinase 1 (IRE1), and activating transcription factor 6 (ATF6) [17–21]. In the basal state, immunoglobulin binding protein (BiP) is bound to the upstream sensors, which maintains them in an inactive state. Upon ER stress induction, BiP dissociates from PERK, IRE1 and ATF6 to preferentially bind hydrophobic regions of the accumulating misfolded proteins, thereby activating the upstream sensors [13]. PERK activation leads to phosphorylation of eukaryotic translation initiation factor 2 subunit α (eIF2α), which attenuates general translation initiation, as well as upregulation of ER stress-specific proteins including activating transcription factor 4 (ATF4) [12]. IRE1 activation allows splicing of the mRNA of X box-binding protein 1 (XBP1), which is another ER stress-specific transcription factor, and activates the c-Jun N-terminal kinase (JNK) pathway, which leads to ER volume expansion [22, 23]. ATF6 is transported to the Golgi apparatus following release from BiP, where it is specifically cleaved to produce an additional ER stress-specific transcription factor [9, 22].

Prolonged ER stress, as occurs in ALS, causes cell death via apoptotic signalling. ER stress-activated apoptosis is mediated in part by the release of Ca2+ from the ER and upregulation of proapoptotic factors, including CCAAT/enhancer binding protein-(C/EBP-) homologous protein (CHOP), as well as activation of ER stress-specific caspases [24, 25].

3. ER Stress in ALS

ER stress is induced in many diseases, such as diabetes, tumour development, hypercholesterolemia and autoimmune disorders, and by viral infection [26]. Many neuronal disorders also have ER stress as a component, including Huntington’s disease [27, 28], HIV-associated dementia [29], Alzheimer’s disease [30], Parkinson’s disease [31, 32], Creutzfeldt-Jacob disease [33] and Pick’s disease [34]. In addition, aging inhibits the early protective responses of ER stress [35].

In ALS, PDI is one of the most upregulated proteins in the spinal cords of presymptomatic transgenic SOD1(G93A) rats and mice, which are the most commonly used animal models of disease [7, 36]. Additionally, activation of the full UPR occurs prior to symptom onset in these animals, indicating an active role for ER stress in pathogenesis [7]. Indeed, ER stress occurs specifically in the vulnerable fast-fatiguable motor neurons prior to activation of other disease-associated mechanisms [37]. ER stress also occurs in sporadic ALS patient spinal cord tissues, indicating that these findings are not confined to mutant SOD1 models of disease [38, 39].

Recent genetic studies have confirmed the importance of ER stress in ALS. Genetic ablation of Ask1, a target of IRE1, or Puma, an ER-stress-related BH3-only protein, both decrease UPR activation and slow disease in SOD1(G93A) mice [40, 41]. Ablation of Xbp1, a key modulator of the UPR, stimulated macroautophagy in motor neurons, delaying disease onset and increasing survival of SOD1(G93A) mice [42]. Furthermore, administration of salubrinal, a small molecule inhibitor of ER stress, to SOD1(G93A) mice significantly delayed disease progression [37]. These studies indicate that modulation of ER stress could be potentially useful in therapy for ALS patients, although further investigation of the mechanisms of protection and design of targeted molecules is required.

4. Protein Disulphide Isomerase

Protein disulphide isomerase (PDI) is an important chaperone induced by ER stress which is involved in the formation, reduction and isomerisation of disulphide bonds in protein substrates. PDI is therefore an important cellular defence against protein misfolding [43]. Recently, PDI upregulation was detected in both mutant SOD1 transgenic rodent and human ALS patient spinal cord tissues, suggesting that PDI could act as a protective molecule in disease [7, 38]. PDI expression is also induced in other neurodegenerative diseases such as Parkinson’s and Alzheimer’s diseases [30, 31], suggesting that PDI has a broad role in neurological disorders related to protein aggregation.

5. The Structure and Expression of PDI

There are at least 19 members of the human PDI protein family, all of which possess at least one thioredoxin-like domain and an ER targeting sequence [44]. Full-length PDI has two thioredoxin-like domains which both contain a CGHC dicysteine active site motif (termed the a and a’ domains), two intervening homologous domains (b and b’), with an x-linker region, and a C-terminal c domain containing a KDEL ER-retention motif which is also potentially involved in Ca2+ binding [45]. Mutagenesis studies identified the b’ domain of PDI as the principal substrate-binding site, however other domains also contribute to the binding of proteins [46]. The CGHC motifs of the a and a’ domain active sites cooperate in forming disulphide bonds, although each motif possesses independent enzymatic activity in the full-length protein [47].

Many PDI family members have not been fully characterised in terms of structure and function, although it is becoming increasingly recognised that each member has distinct substrate specificities [48, 49]. For example, whereas PDI interacts with both glycosylated and nonglycosylated
proteins, ERp57 (also known as Pdia3 or Grp58) interacts specifically with glycosylated proteins [50]. Also, while ERp57−/− mice are nonviable [51], genetic ablation of either of the PD family members AGR2/Hag2 or ERdj5 produces viable mice, with specific defects in mucin production and salivary gland function, respectively, and increased ER stress in affected tissues [52, 53]. No models of genetic PDI ablation in higher eukaryotes have been reported, although PDI is essential in yeast [54]. Due to its widespread distribution and important cellular functions, genetic ablation of PDI in mammals would also likely be lethal. However, the potential for redundancy with the other PD family members remains undetermined, and delineation of the different roles and substrate specificities of the PD family members is an important area for future research [48].

6. The Functions of PDI

PDI is primarily a disulphide bond-modulating chaperone, however PDI also facilitates ER-associated degradation of misfolded proteins [55], and is involved in retrotranslocation of misfolded choler toxin from the ER to the cytoplasm by interaction with the ER transmembrane protein Derlin-1 [56, 57]. Other PD family members are involved in calcium homeostasis and antigen presentation [44], while PDI is important for the cellular export of some proteins, such as thyroglobulin [58].

PDI protects against aggregation of misfolded proteins in several neurodegenerative diseases. PDI decreases aggregation of the Parkinson’s disease-associated synphilin-1 protein in neuroblastoma cells, an activity which is dependent on the presence of the CGHC active site motifs [59]. PDI also prevents aggregation of α-synuclein in cell-free in vitro systems [60], and ERp57 prevents aggregation and subsequent neurotoxicity of prion protein in cell culture [33]. PDI colocalises with ubiquitin-positive inclusions of torsin-A in a transgenic mouse model of dystonia [61], and with neurofibrillary tangles in Alzheimer’s disease patient brain tissue [62]. Furthermore, PDI prevents neuronal and cardiomyocyte cell death caused by hypoxia-ischaemia in cell culture and in rodent models, at least partly by decreasing protein misfolding [63, 64]. In contrast to these findings, PDI does not decrease the number of inclusions formed by the variant of α1-antitrypsin linked with liver disease [65], indicating some disease and protein-specificity of PDI protection.

7. Subcellular Localisation of PDI

While PDI is conventionally considered as an ER lumenal protein, PDI has also been detected in the nucleus, extracellular matrix and on the cell surface, where it modulates several different functions [8, 66–72]. Cell surface PDI facilitates infection of HeLa cells by mouse polyoma virus [67], modulates thrombus formation on the surface of platelets [68] and facilitates dengue virus infection [73]. In addition, both PDI and ERp57 interact with misfolded prion protein on the cell surface, which could be important for prion accumulation and cell-to-cell transmission [74]. Also, ER stress can lead to the leakage of PDI to the cytoplasm [75]. These findings clearly indicate that PDI is found in other cellular locations under a variety of different conditions.

8. The Involvement of PDI in ALS

PDI is upregulated in the spinal cords of SOD1G93A transgenic mice and rats at the pre-symptomatic, symptomatic and end stages of disease [7, 38, 76, 77]. In addition, PDI levels are increased in spinal cords of sporadic ALS patients compared to nonneurological controls [38, 39]. Interestingly, PDI colocalises with inclusions in motor neurons of SOD1G93A mice [7], in human ALS patients [38], and also with inclusions formed by vesicle associated membrane protein-associated protein B (VAPB) in a Drosophila melanogaster model of a rare familial form of ALS caused by mutant VAPB [78]. These findings suggest that PDI is both upregulated in disease and recruited to areas of the cell containing aggregated protein. Furthermore, increased levels of PDI are detected in the cerebrospinal fluid (CSF) of ALS patients compared to nonneurological controls, suggesting that the level of PDI in CSF could be used as a potential biomarker of disease [38].

These studies indicate an important function for PDI in protection against mutant protein aggregation in ALS. This is supported by the increase in inclusion formation observed when mutant SOD1 expressing motor neuron-like NSC-34 cells were treated with the broad disulphide isomerase inhibitor bacitracin [7]. More recently, siRNA-mediated knockdown of PDI was also shown to increase mutant SOD1 inclusion formation in neuroblastoma cells, confirming the importance of PDI in modulating mutant SOD1 aggregation [8]. Furthermore, overexpression of PDI in neuroblastoma cells decreased the levels of insoluble mutant SOD1, inhibited inclusion formation and decreased apoptotic cell death [8]. Interestingly, a small molecular mimic of the PDI active site also decreased mutant SOD1 aggregation and inclusion formation, indicating that similar molecules may be beneficial for treatment of disease [8]. Further investigation of the effects of PDI mimics in animal models of ALS is therefore warranted.

The findings of upregulation of PDI in disease and the converse finding that overexpression is protective against mutant SOD1 in cell culture raised the question of why the increased levels of PDI in ALS were not beneficial. Recently, posttranslational modification of PDI by S-nitrosylation of critical active site cysteine residues, leading to inhibition of PDI enzymatic activity, was identified in Parkinson’s and Alzheimer’s disease brain tissues [59]. This same process of S-nitrosylation of PDI has now been confirmed to occur in spinal cord tissues of sporadic ALS patients as well as in transgenic SOD1G93A mice, and could explain a loss of protection by PDI in disease [8]. S-nitrosylation involves the covalent addition of nitrogen monoxide (NO) to thiol side chains of cysteine residues of proteins, which can influence many cellular processes by altering both protein function and protein-protein interactions [79]. S-nitrosylation can occur
in a specific substrate-dependent manner, when one or a few potential cysteine residues become modified. Nitrosative stress caused by the accumulation of reactive nitrogen species (RNS) such as peroxynitrite can cause aberrant S-nitrosylation [80]. Nitrosative stress is linked with excessive glutamate receptor activation, excitotoxicity and oxidative stress [80, 81], processes which are key events in neurodegenerative diseases including ALS [82]. Recently, the toxicity of mutant SOD1 in neuroblastoma cells has been linked with increased levels of nitric oxide [83].

In addition to the findings of S-nitrosylation of protein cysteine residues in ALS, a dramatic increase in tyrosine-nitrated proteins in the insoluble fractions of spinal cords from both SOD1G93A mice and ALS patients was identified in a recent proteomic screen [84]. PDI was one of the nitrated proteins increased in the insoluble protein fraction from SOD1G93A mice, suggesting that nitrosative stress could also lead to tyrosine nitration of PDI in ALS [84]. Previously, the PDI family member ERP57 was found to be nitrated on tyrosine residues in SOD1G93A mice [85].

Importantly, a specific mechanism for subcellular redistribution of PDI has recently been identified in ALS [86, 87]. Several different members of the reticulon family of integral ER membrane proteins were shown to modulate PDI distribution and reticulon overexpression caused a change in localisation of PDI from a normal ER distribution to a less homogenous punctate pattern [86]. In SOD1G93A mice, deletion of the gene encoding the reticulon-4A,B proteins accelerated disease processes, possibly by preventing the reticulon-mediated PDI redistribution [86]. The exact mechanism of the selective redistribution of PDI by reticulons remains to be determined, and how this mechanism is protective in disease remains unknown, although it is possible that redistribution allows selective interaction with a subset of PDI target proteins [87]. Although the levels of PDI are increased in the cerebrospinal fluid of transgenic SOD1G93A rats and ALS patients compared to controls [38], a further unanswered question is whether the levels of PDI in other locations, such as on the cell surface or in the cytoplasm, are also affected in disease. Overall, these findings indicate that several different processes, including posttranslational modifications and subcellular redistribution, are involved in modifying PDI function in ALS, with potential implications for disease pathogenesis.

9. Conclusion

The recent identification of ER stress as a central process involved in ALS, and the particular involvement of PDI as a protective factor in disease, highlights new areas of research which could have potential therapeutic application. Both ER stress and PDI S-nitrosylation occur not only in mutant SOD1-linked disease, but also in the more common sporadic forms, indicating that targeting these pathways could be useful in all forms of ALS. Additionally, pharmacological prevention of protein misfolding, stimulation of autophagy or inhibition of oxidative stress could also decrease ER stress, and may be beneficial in disease. Possible applications of this research include development of small molecule PDI mimics or ER stress inhibitors, which could prove beneficial in treating this devastating disease.

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