Involvement of endoplasmic reticulum stress in TDP-43-linked neurodegenerative disease

Adam K. Walker, Ph.D.
Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA
Email: adwa@upenn.edu

Abstract
Phosphorylated and ubiquitinated TAR DNA binding protein 43 (TDP-43) pathology is the neurodegenerative hallmark of the vast majority of amyotrophic lateral sclerosis (ALS) and approximately half of all frontotemporal lobar degeneration (FTLD) cases. An RNA/DNA-binding protein with diverse roles in the nucleus, TDP-43 accumulates in the cytoplasm of affected neurons and glia in disease, indicating that both a loss of normal nuclear function and additional gain of toxicity due to increased cytoplasmic presence likely occur in these TDP-43 proteinopathies. Abundance and splicing of hundreds of transcripts are affected by dysfunction of TDP-43, making identification of the key pathways involved in disease pathogenesis a difficult task. However, endoplasmic reticulum (ER) stress and activation of the unfolded protein response have been described in sporadic ALS patient tissues as well as in cell and animals models of widely-studied superoxide dismutase 1 (SOD1) mutation-linked ALS. These responses to misfolded proteins have more recently been demonstrated in models of TDP-43-linked disease, and modulation of ER stress, which is intimately associated with oxidative stress and formation of stress granules in cells, could therefore represent one avenue for potential amelioration of ALS and FTLD. This review summarizes recent findings describing the involvement of ER stress in TDP-43 proteinopathies.

Keywords: amyotrophic lateral sclerosis, endoplasmic reticulum, frontotemporal lobar degeneration, neurodegeneration, protein aggregation

Introduction

TAR DNA binding protein of 43 kDa (TDP-43) was identified in 2006 as the pathological protein in cases of the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) [1]. Although ALS primarily affects motor neurons of the spinal cord and FTLD primarily affects neurons of the temporal and frontal lobes of the brain, both diseases show overlap of histopathology and clinical presentation. Therefore, ALS and FTLD are now considered ends of a spectrum of disorders [2-4]. In diseased tissue, TDP-43 redistributes from its primary location in the nucleus to the cytoplasm of affected neurons and glial cells, where large intracellular inclusions containing ubiquitin and phosphorylated TDP-43 are formed [1, 5, 6].

TDP-43 is ubiquitously expressed, and several studies in mice have shown that TDP-43 is an essential protein for development [7-9]. TDP-43 is an RNA/DNA-binding protein which affects transcription and splicing of hundreds of mRNAs and is involved in a remarkably diverse set of cellular processes [10]. Indeed, TDP-43 even binds to the 3' untranslated region its own mRNA and thus regulates its own protein levels [11, 12]. Recognition of the wide and varied roles of TDP-43 has made identification of key upstream processes in TDP-43-related disease a difficult process. However, cellular stress responses are involved in regulation of TDP-43 subcellular distribution and likely play a role in disease-related protein aggregation [13-16].

One such cellular stressor, endoplasmic reticulum (ER) stress, is a widely recognized process involved in both sporadic and less

common mutant SOD1-linked familial ALS [reviewed in 17]. More recently, TDP-43 mis-localization and aggregation has also been shown to be affected by ER stress in TDP-43 disease models, as discussed below. Further insight into the role of ER stress in TDP-43-linked disease is required in order to define whether targeting of these important cellular pathways could be used as a future therapeutic strategy. Figure 1 shows key processes involved in the cellular development of TDP-43 proteinopathy, with a focus on ER stress.

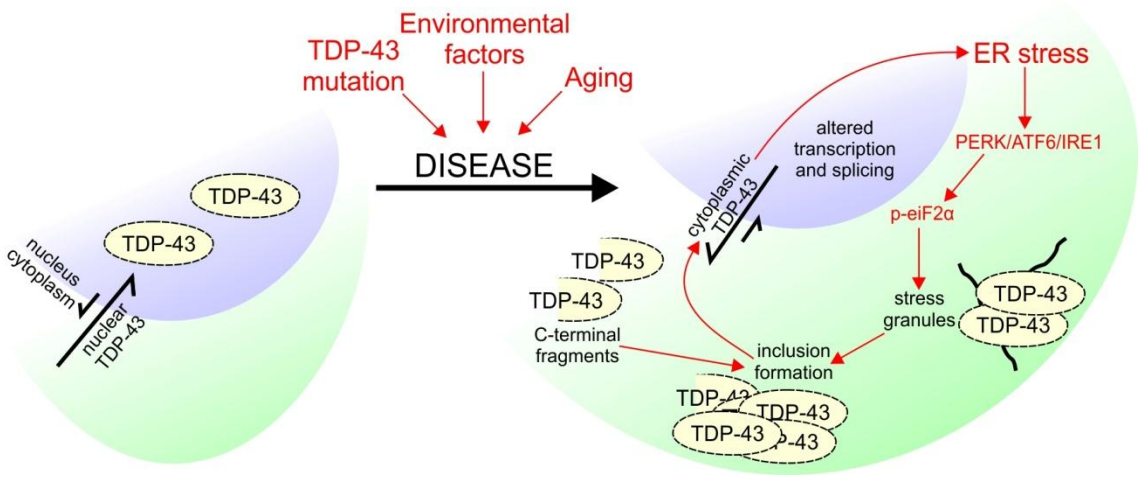

Figure 1. Involvement of ER stress in TDP-43 disease pathology. Under normal conditions, TDP-43 is located primarily in the nucleus. In disease states such as ALS and FTLD, which are influenced by multiple factors including genetic mutations, environmental factors and aging, TDP-43 is redistributed to the cytoplasm of affected cells. Among multiple cellular stress pathways, ER stress leads to phosphorylation of eiF2α which causes TDP-43-positive stress granule formation. Stress granules and C-terminal fragmented TDP-43 could contribute to TDP-43 inclusion formation, which further drives redistribution of TDP-43 to the cytoplasm. Clearance of nuclear TDP-43 also causes alterations in transcription and splicing due to loss of normal TDP-43 function.

ER stress in sporadic and familial ALS

Accumulation of misfolded proteins within the lumen of the ER causes ER stress, which activates a homeostatic mechanism known as the unfolded protein response (UPR) [reviewed in 18, 19]. Induction of ER stress leads to the activation of numerous pathways. ER stress is mediated by three upstream sensors, each of which is an ER-localized transmembrane protein: activating transcription factor 6 (ATF6), inositol requiring kinase 1 (IRE1) and protein kinase RNA-activated (PKR)-like ER kinase (PERK), which is also known as eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3) [reviewed in 20]. Under normal conditions, activation of these pathways allows for rapid clearance of misfolded proteins, via inhibition of global protein translation and specific up-regulation of chaperone proteins. ER stress also leads to an increase in ER-associated degradation, in which proteins are exported from the ER to the cytoplasm for proteasomal degradation. However, if the misfolded proteins are not cleared, cell death pathways can be activated, leading to apoptotic signaling [reviewed in 21] and subsequent cell loss in disease.

In relation to ALS, ER stress was first identified in cell and rodent models of mutant SOD1-linked disease [reviewed in 17], which was the most widely studied form of ALS following the first

description of ALS-causative SOD1 mutations in 1993 [22]. ER stress is induced in cell culture by expression of ALS-linked mutant, but not wildtype, SOD1 [23, 24]. Activation of all three ER stress sensor pathways also occurs in mutant SOD1 expressing rodents as well as in sporadic ALS patient tissues and in patients with the tau pathology-related form of FTLD known as Pick's disease [24-28]. Indeed, activation of ER stress is one of the earliest detectable pathological processes in vulnerable motor neurons in mutant SOD1 mice [29, 30]. More recently, ER stress has been demonstrated as a key pathway activated in motor neurons differentiated from induced pluirpotent stem (iPS) cells derived from human mutant SOD1 ALS patients [31]. These findings indicate that ER stress is involved in both mutant SOD1 models and in ALS and FTLD patients.

Recent experiments have further demonstrated the role of ER stress regulators in mouse models of mutant SOD1-linked ALS by direct genetic manipulation of key proteins. For example, haploinsufficiency for the upstream ER stress sensor PERK in $SOD1^{G85R}$ transgenic mice caused an acceleration in disease onset accompanied by decreased disease duration and shorter lifespan, possibly due to a general decrease in cellular ability to clear misfolded proteins, suggesting a protective role for PERK in this disease model [32]. In contrast, genetic ablation of the ER stress-responsive activating transcription factor 4 (ATF4) caused a delay in disease onset and extension of lifespan in $SOD1^{G86R}$ transgenic mice [33]. Furthermore, decreased levels of active GADD34, one of the phosphatases responsible for dephosphorylation of the PERK-target transcription factor eiF2α, slowed disease progression and significantly extended survival in $SOD1^{G85R}$ transgenic mice [34]. In these mice, decreased levels of GADD34 also lowered levels of aggregated mutant SOD1 in lumbar spinal cord at disease end stage, suggesting that the protective effect of lowered GADD34 levels could be attributed in part to enhanced protein clearance. Together these studies indicate an important modulating effect of PERK signaling in SOD1-linked disease, and cell-survival signaling pathways mediating enhanced protein clearance may be important in the disease process.

ER stress and stress granule formation in TDP-43-linked disease

Although well established in SOD1 models of ALS, only more recently has ER stress been linked with TDP-43 dysfunction. Cellular stress including oxidative stress, osmotic alteration and heat shock causes increased phosphorylation of eiF2α, which is also activated through ER stress via PERK, leading to a general decrease in global protein translation via the formation of cytoplasmic stress granules [35]. Stress granules incorporate a wide variety of mRNA and select RNA binding proteins, and act as a cellular holding compartment to prevent translation in the face of increased protein folding burden [36]. In several cell culture studies, a wide variety stressors, including ER, oxidative, and osmotic stresses and heat shock, have been shown to drive TDP-43 redistribution from the nucleus to the cytoplasm leading to rapid incorporation of TDP-43 into stress granules [14-16, 37-39].

Notably, in cell culture pharmacological induction of ER stress causes TDP-43-positive stress granule formation, and over-expression of ALS-linked mutant, and to a lesser extent wildtype, TDP-43 causes induction of ER stress [38, 39]. This suggests that in disease the presence of TDP-43 mutations or altered TDP-43 dynamics leads to ER stress, which in turn drives further dysfunction of TDP-43 [38]. Formation of TDP-43-positive stress granules has been proposed as one pathway to the formation of large cytoplasmic disease-defining TDP-43 pathological inclusions [40, 41].

The involvement of ER stress has also been evaluated in *in vivo* models of TDP-43-linked disease. In transgenic rats over-expressing ALS-linked mutant $TDP\text{-}43^{M337V}$, early in disease progression neurons showed a failure of normal unfolded protein response induction, despite progressive activation of ER stress in neighboring microglial cells [42]. In *C. elegans*, decrease in

the TDP-43 ortholog leads to increased sensitivity to oxidative stress [43]. Furthermore, pharmacological inhibition of eiF2α phosphatases using the small molecule salubrinal led to a rescue in motor phenotype in *C. elegans* expressing ALS-linked mutant TDP-43, likely at least partially due to an effect on chaperone protein expression [44].

Most recently, a yeast genetic screen for modifiers of TDP-43 toxicity identified multiple stress granule and ER stress components, including the yeast homolog of eiF2α [45]. This finding was further evaluated in *D. melanogaster* models of disease expressing human TDP-43, which develop progressive neurodegeneration [45, 46]. In these flies, knockdown of the *D. melanogaster* homologs of PERK and GADD34, an eiF2α phosphatase, modified motor phenotype – with PERK knockdown suppressing the phenotype and GADD34 knockdown enhancing the deficits [45]. GADD34 knockdown also lead to an increased accumulation of cytoplasmic TDP-43, indicating that modulation of eiF2α phosphorylation has downstream effects on TDP-43 sub-cellular distribution. Interestingly, treatment of flies with a recently-developed pharmacological inhibitor of PERK, GSK2606414, caused a decrease in eiF2α phosphorylation and ameliorated motor phenotype without directly affecting TDP-43 protein levels [45]. These findings were recapitulated in rat primary neurons, whereby treatment with GSK2606414 decreased the toxicity associated with over-expression of TDP-43 [45].

These studies raise the question of how TDP-43 is causing ER stress. Although TDP-43 was detected at low levels in the ER-containing microsome fraction in one study [47], it is unlikely that a substantial amount of TDP-43 is localized to the ER lumen. ALS-linked mutant SOD1 has been shown to induce ER stress by interaction with the cytoplasmic face of the ER membrane protein Derlin-1, by inhibition of ER-associated degradation-mediated protein clearance [48]. In addition, ER-Golgi transport is inhibited by mutant SOD1 in cell culture, and this represents an additional mechanism for potential induction of ER stress without requiring the presence of mutant SOD1 within the ER lumen [49]. Whether these mechanisms are similarly involved in triggering ER stress in TDP-43-linked disease remains to be determined. The additional involvement of TDP-43 in stress granule formation, which is directly activated by ER stress, also suggests that TDP-43 is able to affect additional pathways not involved in SOD1-linked disease models. Indeed, although decreased levels of GADD34 were protective in $SOD1^{G85R}$ transgenic mice [34], GADD34 knockdown in TDP-43-expressing *D. melanogaster* was detrimental [45], indicating likely differences in pathological processes in SOD1 versus TDP-43-linked disease related to both ER stress and stress granules.

A further question surrounds the selective vulnerability of subsets of neurons in TDP-43-linked disease. It has been demonstrated that the fast fatiguable motor neurons, which are the most vulnerable to degeneration in mutant SOD1 mice, are selectively prone to ER stress very early in the disease course [30]. In addition, knockdown of matrix metalloproteinase-9 (MMP-9), which is highly expressed exclusively by vulnerable motor neurons in the spinal cord, both ameliorates ER stress activation and delays motor neuron dysfunction in $SOD1^{G93A}$ mice [50]. This suggests that MMP-9 is one factor involved in selective vulnerability in disease. Furthermore, iPS cell-derived motor neurons from human ALS patients display aberrant hyperexcitability, which could be an upstream trigger of ER stress in vulnerable neurons [51]. The precise mechanisms regulating neuronal vulnerability in disease, and the factors that make subsets of neurons selectively susceptible to ER stress remain to be fully defined. Whether or not the same mechanisms are involved in selective vulnerability to TDP-43 pathology as in mutant SOD1-linked disease is also unclear.

Reliable mammalian models of TDP-43-linked disease which faithfully recapitulate key

pathological processes seen in human disease, including cytoplasmic accumulation and phospho-TDP-43 inclusion formation, remain to be developed [4]. However, once available, it will be important to further define the involvement of both ER stress and stress granule formation, given the positive results obtained from the currently a vailable, albeit arguably non-optimal, models. A particular focus on the phosphorylation of eiF2α may be warranted, since this appears to be a key modulator of ER stress and other disease-related pathways. Several small molecules have been developed to modulate ER stress, including those targeting the PERK pathyway, namely salubrinal [52] and GSK2606414 [53]. Further development of these and other lead compounds, and additional investigation of whether modulation of ER stress can ameliorate cellular dysfunction and provide meaningful functional benefits in *in vivo* TDP-43 models is required. These studies may mark the next step towards development of much-needed therapeutics for TDP-43-related diseases.

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