TDP-43 Proteinopathy and ALS: Insights into Disease Mechanisms and Therapeutic Targets

Emma L. Scotter · Han-Jou Chen · Christopher E. Shaw

Published online: 5 February 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract Therapeutic options for patients with amyotrophic lateral sclerosis (ALS) are currently limited. However, recent studies show that almost all cases of ALS, as well as tau-negative frontotemporal dementia (FTD), share a common neuropathology characterized by the deposition of TAR-DNA binding protein (TDP)-43-positive protein inclusions, offering an attractive target for the design and testing of novel therapeutics. Here we demonstrate how diverse environmental stressors linked to stress granule formation, as well as mutations in genes encoding RNA processing proteins and protein degradation adaptors, initiate ALS pathogenesis via TDP-43. We review the progressive development of TDP-43 proteinopathy from cytoplasmic mislocalization and misfolding through to macroaggregation and the addition of phosphate and ubiquitin moieties. Drawing from cellular and animal studies, we explore the feasibility of therapeutics that act at each point in pathogenesis, from mitigating genetic risk using antisense oligonucleotides to modulating TDP-43 proteinopathy itself using small molecule activators of autophagy, the ubiquitin-proteasome system, or the chaperone network. We present the case that preventing the misfolding of TDP-43 and/or enhancing its clearance represents the most important target for effectively treating ALS and frontotemporal dementia.

Keywords ALS · FTD · TDP · TARDBP · Proteinopathy · C9ORF72

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease, and is characterized by the progressive loss of upper and lower motor neurons from the spinal cord, brain stem, and motor cortex, leading to muscle weakness and eventual respiratory failure. Approximately 5–10 % of ALS cases are familial with the remaining 90 % being sporadic, indicating that both genetic and environmental factors contribute to risk. Despite this diverse etiology of disease, 97 % of patients display a common phenotype in disease-affected tissues, namely the deposition of the TAR-DNA binding protein (TDP)-43 [1, 2]. Deposition of TDP-43 is also the major feature of tau-negative frontotemporal dementia (FTD), which shows clinical overlap with ALS [1, 3]. This convergence of genetic and environmental risk factors upon TDP-43 is hugely informative with regard to general disease mechanisms. Here we explore the pathways linking risk factors to the development of TDP-43 proteinopathy, and linking proteinopathy to the development of disease, with a view to identifying key points for therapeutic intervention.

TDP-43 and TDP-43 Proteinopathy

TDP-43 Protein Function

Encoded by TARDBP, TDP-43 is a ubiquitously expressed DNA/RNA-binding protein [4]. TDP-43 contains 2 RNA recognition motifs, a nuclear localization sequence (NLS), a
nuclear export signal [5], and a glycine-rich C-terminus that mediates protein–protein interactions [6, 7]. TDP-43 predominantly resides in the nucleus, but is capable of nucleocytoplasmic shuttling [5]. In the nucleus, TDP-43 plays a critical role in regulating RNA splicing, as well as modulating microRNA biogenesis [8, 9]. TDP-43 can regulate the stability of its own mRNA, providing a mechanism for the autoregulation of TDP-43 protein levels [10, 11]. In addition to TDP-43 RNA, TDP-43 regulates the splicing and stability of a large number of other transcripts [10, 12–15], and thus influences diverse cellular processes.

Although mostly nuclear, up to ~30% of TDP-43 protein can be found in the cytoplasm [16], with nuclear efflux regulated by both activity and stress [17]. TDP-43 is a key component of dendritic and somatodendritic RNA transport granules in neurons [18, 19], and plays an important role in neuronal plasticity by regulating local protein synthesis in dendrites [17]. TDP-43 is also involved in the cytoplasmic stress granule response [20]—the formation of protein complexes that sequester mRNAs redundant for survival [21]—meaning TDP-43 function is particularly important under conditions of cellular stress. Understanding the functions of endogenous TDP-43 is crucial to establishing whether loss of these functions might be key to disease pathogenesis, and to developing effective therapeutics.

TDP-43 Proteinopathy

TDP-43 protein was identified as a major component of the ubiquitinated neuronal cytoplasmic inclusions deposited in cortical neurons in FTD and in spinal motor neurons in ALS [1]. TDP-43-positive inclusions have subsequently been shown to be common to 97% of ALS cases [22, 23], whether sporadic or familial. The main exceptions are cases caused by mutations in SOD1 or FUS [24–28]. Neurodegenerative diseases linked to the deposition of TDP-43 are termed “TDP-43 proteinopathies”, and “TDP-43 proteinopathy” also describes the characteristic histopathological transformation of TDP-43 that occurs in disease [29]. This transformation is evidenced by the deposition of full-length and fragmented TDP-43 protein as detergent-resistant, ubiquitinated and hyperphosphorylated aggregates in the cytoplasm, with associated clearing of TDP-43 from the nucleus [1]. The regional spread of TDP-43 proteinopathy from spinal and cortical motor neurons and glia to other cortical regions can be used to stage ALS progression [30], which suggests that some or all of the features of transformed TDP-43 protein are linked to pathogenesis.

However, a key question in ALS research is which of these features of TDP-43 proteinopathy are required for the development of disease and thus represent therapeutic targets. Is ALS pathogenesis linked to the loss of wild-type TDP-43 function through protein misfolding and failure to interact with binding partners, or is it linked to a gain of toxic function of the aforementioned TDP-43 aggregates, which are the hallmark of TDP-43 proteinopathy? A number of studies have examined the roles of TDP-43 gain or loss of function in disease. Overexpression of wild-type TDP-43 recapitulates TDP-43 proteinopathy and disease phenotypes in a range of animal models [31–33], supporting a role for gain of toxic function in disease. Initial studies testing a loss-of-function hypothesis used knock-out of TDP-43 from mice, which resulted in embryonic lethality [34–36]. This demonstrated TDP-43 to play a vital role in early development without necessarily demonstrating that loss of function could be degenerative in adulthood. However, conditional and partial knockout models soon demonstrated that loss of TDP-43 function can, indeed, induce motor neuron defects, a progressive motor phenotype reminiscent of human disease, and even typical TDP-43 proteinopathy [37–39]. Interestingly, either overexpression or knockdown of TDP-43 selectively in glia or muscle also recapitulates ALS-like phenotypes [40, 41]. The emerging picture is that both gain and loss of TDP-43 function may be mechanistic in disease, and, as we will demonstrate, TDP-43 misfolding may link the two. We will demonstrate that therapies that remedy TDP-43 misfolding should be prioritized to best target the spectrum of disease with the fewest assumptions around mechanism.

Disease Mechanisms in the TDP-43 Proteinopathies

Disease Mechanisms Upstream of TDP-43 Proteinopathies

We have introduced the concept that TDP-43 is the convergence point for a range of upstream risk factors for ALS. Here we briefly review these genetic and environmental risk factors for the development of ALS, and for the development of TDP proteinopathy, and explore the potential for targeting therapeutics towards these diverse risk factors.

Genetic Factors in ALS

Currently, genetic causes are known for approximately 15% of all ALS cases; accounting for 11% of sporadic ALS and 68% of familial ALS [42]. Many of the ALS-linked genes group together functionally to implicate specific cellular processes in the pathogenesis of ALS (Table 1 and Fig. 1).

TARDBP mutations Mutations in TARDBP are a rare cause of ALS [43]. To date, 38 nonsynonymous TARDBP mutations have been identified in both familial and sporadic ALS, most clustering in the region encoding the C-terminus, and accounting for approximately 1–2% of total cases [43–58]. Like wild-type TDP-43 proteinopathy, mutant TDP-43 in TARDBP-linked ALS patient tissue is characterized by cytoplasmic
accumulation as aggregated and insoluble deposits [44, 55], nuclear clearing in a subset of motor neurons [44], and C-terminal fragmentation [55]. But what is the relationship of mutant TDP-43 proteinopathy to pathogenesis?

Cellular and transgenic animal models indicate that both overt proteinopathy, as well as preproteinopathic changes in TDP-43 may be at play in TARDBP-linked disease. Loss of RNA processing and axonal transport function of mutant TDP-43 has been suggested to precede other features of pathology [18, 19, 59], and the failure of mutant TDP-43 to rescue motor neuron defects caused by knockout of endogenous TDP-43 supports the notion that TARDBP mutations cause loss of function [18, 39]. However, they also promote C-terminal fragmentation [43, 45, 46, 60], cytoplasmic mislocalization [16, 61, 62], aggregation [18, 63–65], and altered proteostasis [66–69]. These structural abnormalities may therefore underpin both loss of function and proteinopathetic transformation of mutant TDP-43. The fact that TARDBP mutations cause ALS provides robust evidence that altered TDP-43 structure (i.e., misfolding) and the resultant loss and gain of function is not simply a cellular response to disease but is pathogenic.

We previously demonstrated that a mutant TDP-43 allele (M337V) can be selectively silenced using small interfering RNA [70], and, indeed, mutation-specific therapy may become feasible with the recent demonstration of safety of silencing agents in humans [71]. However, generic TDP-43-based refolding or reduction strategies to be discussed ahead in this review may be more broadly useful, given the number of unique TARDBP mutations linked to disease.

Protein degradation gene mutations ALS-linked mutations in SQSTM1 [72], VCP [73], UBQLN2 [74], and OPTN [75] are rare but together implicate impaired protein turnover in TDP-43 proteinopathy and in ALS pathogenesis. They encode p62, valosin-containing protein (VCP), ubiquilin 2, and optineurin, respectively; effectors of the autophagy and/or ubiquitin-proteasome system (UPS) protein degradation pathways [76–80]. TDP-43 proteostasis is normally maintained by the coordinated action of the UPS and autophagy, which is particularly important for clearing TDP-43 oligomers and aggregates [81–87].

Notably, VCP and p62 are required for the formation of “aggresomes” [84, 88, 89], which are large perinuclear inclusions decorated with ubiquitin, ubiquilin, and p62. The TDP-43-positive aggregates that are the hallmark ALS pathology are likely aggresomes [76, 90]. Aggresomes act as a staging center for “aggrephagy”—the removal of misfolded proteins by autophagy [91, 92]—and augmentation of aggrephagy is thus emerging as a potential therapeutic strategy in ALS [90].

But is the removal of TDP-43 a logical approach in ALS linked to global impairment of protein degradation, when TDP-43 is just one of many substrates of these systems? At the high concentrations caused by global impairment in protein degradation, TDP-43, like other neurodegenerative

| Gene      | ALS/FTD | Percentage of cases | TDP-43 deposits | Characteristic features | Refs* |
|-----------|---------|---------------------|-----------------|-------------------------|-------|
|           | ALS     | Sporadic | Familial | ALS | FTD | Sporadic | Familial |                |                |
| RNA processing | C9ORF72  | Both    | 4–7   | 39  | 6   | 25    | +       | DPRs, RNA foci | [177]          |
|           | TARDBP  | Both    | 1     | 4   | <1  | +     | –       | –              |                |
| Protein degradation | MATR3    | ALS     | 1     | 1   | –   | +     | Matrin 3 elevated/ inclusions | [121]          |
|           | hnRNP A1| ALS, MSP | <1    | 2   | –   | +†    | hnRNP A1 inclusions† | [122]          |
|           | FUS     | Both    | 1     | 4   | <1  | –     | +     | –              |                |
| Protein degradation | UBQLN2  | Both    | <1    | <1  | <1  | +     | UBQLN2 inclusions | [42, 159]       |
|           | VCP     | Both, MSP| 1     | 1   | <1  | –     | Nuclear TDP-43 inclusions | [42, 159]       |
|           | SQSTM1  | ALS, PDB | <1    | 1   | –   | +     | Increased p62 inclusions | [42]           |
| Protein degradation | OPTN   | ALS, POAG | <1    | <1  | –   | +     | OPTN inclusions | [42]           |
| Other     | SOD1    | ALS     | 1–2   | 12  | –   | –     | SOD-1 inclusions | [42]           |

*TDP-43=TAR-DNA binding protein-43; MSP=multisystem proteinopathy (previously “inclusion body myopathy with frontotemporal dementia Paget’s disease of bone and amyotrophic lateral sclerosis”); PDB=Paget’s disease of bone; POAG=primary open angle glaucoma; DPRs=dipeptide repeats; FUS=fused in sarcoma; UBQLN2=ubiquilin 2; OPTN=optineurin; SOD-1=superoxide dismutase-1
*Reference for percentage of cases attributable to the gene
†In muscle tissue in MSP. No gene-positive tissue from ALS patients tested

Table 1 Genetic factors in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) implicating RNA processing and protein degradation pathways

Scotter et al.
disease proteins [93–95], and RNA-binding proteins (RBPs) [96], is highly prone to aggregation [64]. Thus, TDP-43 may be particularly adversely affected by genetic mutations affecting protein homeostasis. Supporting this idea, the predominant pathology in ALS linked to VCP mutation is the nuclear accumulation of TDP-43 rather than VCP protein itself or other degradation substrates [97]. Therefore, it is feasible that the misfolding and accumulation of TDP-43 and not other proteins is the “smoking gun”, and clearance of misfolded TDP-43 might represent a common druggable target.

Certainly, small molecule activators of the UPS or autophagy have been shown to promote TDP-43 clearance and/or mitigate toxicity in models based on TDP-43 overexpression [66, 77, 98, 99]. Autophagy activators may offer selectivity in clearing misfolded TDP-43 [76, 79]; however, given the ability of TDP-43 to autoregulate, even nonselective clearance
strategies hold promise for safely restoring TDP-43 proteostasis.

**RNA processing pathway gene mutations** The identification of disease-linked mutations affecting other RNA-binding proteins suggests an important role for deficits in RNA processing, which is a key TDP-43 function, in disease pathogenesis. ALS-linked mutations in the RBP fused in sarcoma (FUS) lead to its mislocalization from the nucleus to the cytoplasm and aggregation [27], but they do not cause TDP-43 proteinopathy [26–28]. However, TDP-43 and FUS do share a subset of RNA targets, which may be part of a disease-relevant pathway [100, 101]. Indeed, RNA targets shared with TDP-43 may also be involved in ALS linked to other RNA processing genes.

The most common ALS-linked mutation is an intronic GGGGCC repeat expansion in C9ORF72 [102, 103], the pathology of which is characterized by classical TDP-43 inclusions in the motor cortex and spinal cord [102]. C9ORF72-linked disease can be distinguished by the additional presence of nuclear foci of repeat-containing RNA [102]. These RNA foci sequester RBPs [104, 105], leading to dysregulation of a large number of transcripts [106, 107]. C9ORF72 cases also harbor TDP-43-negative inclusions throughout the central nervous system, which are decorated by ubiquitin [102], p62 [14, 108], and/or ubiquilin 2 [109]. These inclusions contain dipeptide-repeat proteins (DPRs) translated in all 6 frames from repeat-containing RNA [110–112]. Overexpression of DPRs, particularly those that are arginine-rich (polyGR, polyPR) [113, 114], can cause toxicity independently of RNA foci formation [115, 116]. However, the distribution of dipeptide aggregates in ALS/FTD brain and spinal cord, at least for polyGA, shows poor correlation with neurodegeneration [117, 118]. Mapping of arginine-rich DPRs, including preaggeregated species, may reveal a role for DPRs in disease, but currently the marker most closely correlated with degeneration remains TDP-43 deposition [118]. Thus, while the reduction of repeat-containing transcript levels or foci formation holds promise in C9ORF72-mediated disease [106, 107, 119, 120], TDP-43 misfolding should also be pursued as a potential therapeutic target.

Mutations in the RNA-binding protein genes MATR3 and hnRNP A1 are also associated with ALS and TDP-43 proteinopathy [121, 122], potentially through direct binding of the affected RBP to TDP-43 [6, 121]. Taken together with FUS and C9ORF72, these ALS-linked genes indicate that RNA processing deficits can be a cause of ALS in the presence or absence of TDP-43 proteinopathy, and that rescue of RNA processing defects could be beneficial to patients. As is the case for C9ORF72-linked disease, the extent to which RNA processing defects are due to TDP-43 proteinopathy will determine whether TDP-43-based therapeutics are effective in patients.

**Environmental Stress**

For the majority of ALS cases, no genetic mutations have yet been identified to account for disease; therefore, the development of ALS and TDP-43 proteinopathy in susceptible individuals is thought to also involve environmental factors. This idea was first proposed following observations of unexpectedly high disease incidence rates in certain “hotspots”, such as the Kii peninsula of Japan and the island of Guam [36, 123, 124]. While patients in these regions show an increased frequency of ALS-linked genes or genetic modifiers such as MAPT [125–128], these only partly account for the observed rates of disease. Proposed environmental agents underlying the high incidence in these populations include dietary neurotoxins such as β-methylamino-L-alanine [129, 130], or mineral deficiencies [75].

There is an association between US military service and increased risk of ALS [131], which may implicate intense physical activity, or exposure to lead, pesticides, or other toxins. Exposure to electromagnetic fields [87, 114], agricultural chemicals [114, 115, 132], head injuries [113, 117], and smoking [116, 119] may also increase susceptibility. Increased incidence of ALS has been reported in professional football players [120, 133, 134]; however, this link has been disputed [135], and risk is not increased for other professional athletes [133]. While no single environmental factor has been unequivocally linked to increased ALS risk, the risk factors discussed can collectively be viewed as cellular stressors, and together implicate cellular stress in disease pathogenesis.

Indeed, cellular studies have drawn links between a diverse range of stressors and the properties of TDP-43 protein. Osmotic stress [136], oxidative stress [20, 137, 138], endoplasmic reticulum stress [66], and heat stress [138] can induce TDP-43 to redistribute from the nucleus to the cytoplasm and incorporate into stress granules. While the regulated aggregation of RBPs and RNA into stress granules is wholly reversible under normal conditions [99, 139], it has been proposed that in conditions of prolonged or repeated neuronal stress, stress granules might act as “seed” the irreversible pathological transformation of TDP-43 [96, 140, 141]. Certainly, TDP-43 within stress granules is detergent resistant and may become post-translationally modified [137, 138, 142], which are defining features of TDP-43 proteinopathy. Also, stress granule markers have been found by several groups to co-localize with TDP-43 aggregates in ALS patient spinal cord [137, 143], although not by others [20, 144]. Together, these findings suggest that myriad and diverse environmental stressors, which normally induce the reversible coalescence of TDP-43 into stress granules, might instead promote irreversible TDP-43 changes and that these are linked to a common pattern of degeneration. A number of antioxidants have
been trialed in patients with ALS, unfortunately without success [134], highlighting the need for better understanding of factors which precipitate proteinopathy and disease.

Disease Mechanisms via TDP-43 Proteinopathy: Toxic Features and Points of Intervention

The vast majority of ALS and FTD cases are of unknown etiology but are linked by TDP-43 proteinopathy, which is defined by cytoplasmic mislocalization, fragmentation, aggregation, and post-translational modification. Here we examine how each of these features of pathological TDP-43 is linked to ALS pathogenesis, and whether preventing these common features might be a valid therapeutic strategy.

Cytoplasmic Mislocalization of TDP-43

Mislocalization of TDP-43 in ALS and FTD is evidenced by the deposition of granular, skein-like, and macroaggregated TDP-43 in the cytoplasm, as well as clearing of TDP-43 from the nucleus [1]. Enhanced levels of TDP-43 in the cytoplasm can occur downstream of ALS-linked mutations [16, 39, 60, 145, 146], cellular stress [20, 136–138], or impaired degradation [147]. TDP-43 mislocalization can be induced in model systems by targeted mutation of the NLS; importantly, this sets in motion many pathological features of disease—NLS mutant TDP-43 is cytoplasmic, aggregated, and capable of recruiting wild-type TDP-43 [5]. Interestingly, several studies have shown that increased levels of cytoplasmic TDP-43 are toxic to cells, but that toxicity is independent of inclusion formation at the level of light microscopy [16, 148]. An important question therefore is whether pathogenic TDP-43 in the cytoplasm is natively folded or misfolded.

As a very early pathogenic event cytoplasmic mislocalization is a desirable intervention point, and selectively reducing mislocalized TDP-43 is linked to reduced toxicity in vivo. However, studies that demonstrated that link had targeted autophagy or interactions with stress granule components rather than TDP-43 nucleocytoplasmic shuttling per se [99, 141]. Without identifying the unique characteristics of the subset of cytoplasmic TDP-43 that exerts toxicity, developing therapeutics that do not affect TDP-43 undergoing normal nucleocytoplasmic shuttling may be challenging.

Fragmentation of TDP-43

Phosphorylated C-terminal fragments (CTFs) of TDP-43 are a major constituent of neuronal protein inclusions in ALS and FTD brains, but less so in spinal cord [1, 149]. Cleavage of TDP-43 is enhanced by C-terminal TDP-43 mutations [45, 43], by cellular stress [150–152], and proteasomal inhibition [124]. Cleavage generates CTFs, which mislocalize to the cytoplasm owing to removal of the NLS, and are aggregation-prone owing to the presence of a prion-like domain [153]. CTFs that correspond in size to those in ALS patient tissue may “seed” the formation of inclusions that are detergent-resistant and ubiquitinated [80], and able to sequester full-length TDP-43 [63, 154–156].

But is fragmentation of TDP-43 a target for therapeutic intervention? There is no current consensus as to whether cleavage enhances or mitigates TDP-43 toxicity [80, 150, 155]. It has been proposed that TDP-43 toxicity requires intact RNA binding capacity [157]; therefore, CTFs may not directly exert toxicity. Cleavage is also not a prerequisite for TDP-43 aggregation [151]; indeed, CTFs expressed at physiological levels require a second “hit” to precipitate misfolding and aggregation [158]. Importantly, cleavage may be required for normal TDP-43 degradation, such that preventing cleavage would favor the accumulation of TDP-43, possibly to greater detriment [124]. Current evidence therefore argues that inhibiting fragmentation of TDP-43 may not be a sound approach for preventing ALS pathogenesis.

Misfolding, Aggregation, and Insolubility of TDP-43

The role of TDP-43 aggregation in pathogenesis is one of the most controversial topics in ALS research; thus, preventing aggregation as a therapeutic strategy is equally controversial. Several animal studies have found that mutant TDP-43 causes toxicity in the absence of visible aggregates [59], and preventing visible TDP-43 aggregates from forming failed to reduce toxicity in a cellular model [159]. It should be noted, however, that visible aggregates are only the endpoint of an aggregation pathway that includes a range of TDP-43 species from misfolded monomer to oligomer to mature aggregate [76]. Unfortunately, sensitive methods for detecting misfolded or early aggregated isoforms are seldom employed, so it is difficult to rule out their existence in studies that fail to find aggregates by light microscopy. Certainly, the vast majority of ALS-linked TDP-43 mutations are found in the prion-like C-terminal domain and serve to promote misfolding, which strongly implies that disease pathogenesis is linked, if not to visible inclusions then at least to TDP-43 misfolding [16, 39, 64, 67].

Misfolded mutant TDP-43 shows a reduced ability to transport RNA appropriately [18, 19], constituting a loss of function. Subsequent to misfolding, the formation of oligomers and aggregates of TDP-43 in the cytoplasm may recruit native TDP-43 or its interactors [160]. This constitutes a gain of function, which acts in a dominant-negative fashion, thus essentially also causing loss of function [9, 155]. By restoring proper TDP-43 folding and/or clearing early misfolded TDP-43, we predict that both loss and gain of function toxicity could be abrogated. It is also possible that late TDP-43 aggregates acquire novel toxic functions such as impairment of the proteasome or blockade of axonal transport, but few studies...
have addressed this. If large aggregates are not a toxic species then strategies that prevent the sequestration of toxic misfolded species into macroaggregates might be detrimental.

There are several intrinsic cellular mechanisms that can act to either prevent or resolve protein misfolding, namely the chaperone system, autophagy, and the UPS. The chaperone system maintains proper protein folding during synthesis and thereafter, or delivers misfolded substrates for degradation [161]. In the case of TDP-43, the chaperone heat shock protein (Hsp)90 enhances solubility (i.e., folding) [162], while HspB8 promotes autophagic clearance of aggregated TDP-43 [163]. Potentiated forms of the disaggregate Hsp104 can mediate TDP-43 refolding [161]. Monomeric misfolded TDP-43 is likely handled by the UPS [76].

Augmentation of chaperones or protein degradation pathways has been protective in several models of ALS and FTD [161, 162]. Because TDP-43 misfolding causes loss of function, as well as providing a substrate for aggregation and gain-of-function/dominant-negative toxicity, we see early misfolding events as one of the most attractive therapeutic targets in ALS with TDP-43 proteinopathy.

Phosphorylation and Ubiquitination of TDP-43

In ALS and FTD patient tissue, hyperphosphorylation and ubiquitination are signatures for pathological TDP-43, as they preferentially label TDP-43 that is cleaved, aggregated, and detergent-resistant [164–166]. Phosphorylation appears to precede ubiquitination, and phospho-TDP-43-specific antibodies detect a greater proportion of TDP-43 inclusions in patient tissue [164, 166]. However, the role of post-translational modifications in promoting or preventing TDP-43 toxicity, and the likely therapeutic benefit of targeting them, remains hotly debated.

There are 29 phosphorylation sites on TDP-43 for casein kinase 1 alone [167], the best studied of which are residues 409/410. Casein kinase 2 may also phosphorylate TDP-43 [13, 164]. TDP-43 phosphorylation at 409/410 is not a prerequisite for aggregation [80, 151, 168]. However, when phosphorylation is detected it is almost always associated with misfolding and insolubility of TDP-43. In addition, interventions that modify TDP-43 phosphorylation have been demonstrated to alter its toxicity. Unfortunately, the reported studies conflict over whether TDP-43 phosphorylation mitigates or exacerbates aggregation and toxicity [13, 169–172]. Thus, at present, TDP-43 phosphorylation is of uncertain value as a therapeutic target but can be considered a good marker for gauging the efficacy of therapeutics that aim to modify TDP-43 misfolding.

The influence of ubiquitination on TDP-43 proteinopathy is less well studied. TDP-43 is modified with polyubiquitin chains that are predominantly K48- or K63-linked [7, 76, 173]. However, it is unclear precisely which TDP-43 conformers are ubiquitinated, and what the effects of TDP-43 ubiquitination are. Ubiquitination involving UBE2E ubiquitin ligases causes TDP-43 to shift to the insoluble fraction, but does not promote its degradation [174]. In contrast, Parkin ubiquitination of TDP-43 promotes its cytosolic translocation either with or without an enhancement in degradation [7, 175]. Studies preventing the removal of ubiquitin chains have yielded conflicting answers as to the possible therapeutic utility of targeting TDP-43 ubiquitination. Inhibition of the deubiquitinase USP14 promotes TDP-43 clearance through retention of ubiquitin chains [98]. However, knockdown of the deubiquitinase UBPY exacerbated the toxicity of TDP-43 in Drosophila, despite ubiquitin chain retention [174]. The promiscuity of deubiquitinases may limit the therapeutic usefulness of inhibitors in patients; however, augmentation of proteolysis itself, which declines with age [176], remains a potential strategy in ALS, as well as other neurodegenerative diseases.

Conclusions and Future Perspectives

The contributions of genetic and environmental factors to the etiology of ALS and FTD are complex and interwoven. The increasing accessibility of genotyping and the recent demonstration of safe gene silencing using antisense oligonucleotides may render the targeting of individual gene mutations feasible, but currently this is not the case, except perhaps for C9ORF72 patients. Similarly, dysregulated RNA processing almost certainly lies at the heart of pathogenesis, but its widespread downstream effects argue against rescue of selected transcripts being useful in patients. Therapeutic strategies would best be directed at a common target proximal to the deficit, which, for most cases, is the misfolding of TDP-43 that is central to its loss of function and gain of function/dominant-negative toxicity. Specifically, enhancers of chaperone-dependent TDP-43 folding, as well as activators of the UPS and autophagy, have shown most promise in model systems. Ongoing refinement of model systems in which to test therapeutics, and recognition of the roles of non-neuronal cell types will be important in bringing these compounds to preclinical and clinical testing. In addition, unbiased large-scale compound screening efforts and the identification of novel causative genes may yield additional insights into disease mechanisms and the role of TDP-43.

Acknowledgments. This publication is dedicated to the patients and families who contribute to our research. This work was supported by grants from the Motor Neurone Disease Association UK, American ALS Association, the Heaton-Ellis Trust, European Union [NeuroNE Consortium; European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement number 259867], and, in New Zealand, Sir Thomas and Lady Duncan Trust, Coker Family Trust, and the Hugh Green Foundation. E.L.S. is funded by an Aotearoa Foundation
Fellowship through the Centre for Brain Research in New Zealand. H.-J.C. is funded by a Strategic Grant Award from the Wellcome Trust and Medical Research Council UK. C.E.S. receives salary support from the National Institute for Health Research (NIHR) Dementia Biomedical Research Unit and Biomedical Research Centre for Mental Health at South London and Maudsley National Health Service (NHS) Foundation Trust and King’s College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

Conflict of Interest The authors have no conflicts of interest to declare. Full conflict of interest disclosure is available in the electronic supplementary material for this article.

Disclosure forms provided by the authors are available with the online version of this article.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314:130–133.
2. Ling SC, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron 2013;79:416–438.
3. Arai T, Hasegawa M, Akiyama H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006;351:602–611.
4. Ou SH, Wu F, Harrich D, Garcia-Martinez LF, Gaynor RB. Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. J Virol 1995;69:3584–3596.
5. Winton MJ, Igaz LM, Wong MM, Kwong LK, Trojanowski JQ, Lee VM-Y. Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. J Biol Chem 2008;283:13302–13309.
6. Buratti E, Brindisi A, Giombi M, Tisminetzky S, Ayala YM, Baralle FE. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: an important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. J Biol Chem 2005;280:37572–37584.
7. Hebron ML, Lonskaya I, Sharpe K, et al. Parkin ubiquitimates TAR-DNA binding protein-43 (TDP-43) and promotes its cytosolic accumulation via interaction with histone deacetylase 6 (HDAC6). J Biol Chem 2013;288:4103–4115.
8. Gregory RJ, Yan K-P, Amuthan G, et al. The microprocessor complex mediates the genesis of microRNAs. Nature 2004;432:235–240.
9. Xu ZS. Does a loss of TDP-43 function cause neurodegeneration? Mol Neurodegener 2012;7:27.
10. Polymenidou M, Lagier-Tourenne C, Hutt KR, Huelga SC, Moran J, Liang TY, et al. Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. Nat Neurosci 2011;14:459–468.
11. Ayala YM, De Conti L, Avendano-Vazquez SE, et al. TDP-43 regulates its mRNA levels through a negative feedback loop. EMBO J 2011;30:277–288.
12. Tollervey JR, Curk T, Rogejl B, et al. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat Neurosci 2011;14:452–458.
13. Carlonagno Y, Zhang Y, Davis M, et al. Casein kinase II induced polymerization of soluble TDP-43 into filaments is inhibited by heat shock proteins. PLoS One 2014;9:e90452.
14. Al-Sarraj S, King A, Troakes C, et al. p62 positive, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol 2011;122:691–702.
15. Bose JK, Huang CC, Shen CK. Regulation of autophagy by neuro-pathological protein TDP-43. J Biol Chem 2011;286:44441–44448.
16. Barmada SJ, Skibinski G, Korb E, Rao EJ, Wu JY, Finkbeiner S. Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral sclerosis. J Neurosci 2010;30:639–649.
17. Diaper DC, Adachi Y, Shutt D, et al. Loss and gain of Drosophila TDP-43 impair synaptic efficacy and motor control leading to age-related neurodegeneration by loss-of-function phenotypes. Hum Mol Genet 2013;22:1539–1557.
18. Alami NH, Smith R, Carrasco MA, et al. Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. Neuron 2014;81:536–543.
19. Liu-Yesucevitz L, Lin AY, Ebata A, et al. ALS-linked mutations enlarge TDP-43-enriched neuronal RNA granules in the dendritic arbor. J Neurosci 2014;34:4167–4174.
20. Colombrita C, Zennaro E, Fallini C, et al. TDP-43 is recruited to stress granules in conditions of oxidative insult. J Neurochem 2009;111:1051–1061.
21. Lindquist S. Regulation of protein synthesis during heat shock. Nature 1981;293:311–314.
22. Mackenzie IR, Bigio EH, Ince PG, et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. Ann Neurol 2007;61:427–434.
23. Maekawa S, Leigh PN, King A, et al. TDP-43 is consistently colocalized with ubiquitinated inclusions in sporadic and Guam amyotrophic lateral sclerosis but not in familial amyotrophic lateral sclerosis with and without SOD1 mutations. Neurology 2009;72:672–683.
24. Mackenzie IRA, Bigio EH, Ince PG, et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. Ann Neurol 2007;61:427–434.
25. Tan CF, Eguchi H, Tagawa A, et al. TDP-43 immunoreactivity in neuronal inclusions in familial amyotrophic lateral sclerosis with or without SOD1 gene mutation. Acta Neuropathol 2007;113:535–542.
26. Tateishi T, Hokonohara T, Yamasaki R, et al. Multiple system degeneration with basophilic inclusions in Japanese ALS patients with FUS mutation. Acta Neuropathol 2010;119:355–364.
27. Vance C, Rogejl B, Hortaouyi T, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 2009;323:1208–1211.
28. Suzuki N, Aoki M, Warta H, et al. FALS with FUS mutation in Japan, with early onset, rapid progress and basophilic inclusion. J Hum Genet 2010;55:252–254.
29. Kwong LK, Neumann M, Sampathu DM, Lee VM, Trojanowski JQ. TDP-43 proteinopathy: the neuropathology underlying major forms of sporadic and familial frontotemporal lobar degeneration and motor neuron disease. Acta Neuropathol 2007;114:63–70.
70. Nishimura AL, Shum C, Scotter EL, et al. Allele-specific knockdown of ALS-associated mutant TDP-43 in neural stem cells derived from induced pluripotent stem cells. PLoS One. 2014;9:e91269.

71. Miller TM, Pestronk A, David W, et al. An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. Lancet Neurol 2013;12:435–442.

72. Fecto F, Yan J, Vemula SP, et al. SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch Neurol 2012;68:1440–1446.

73. Watts GD, Wymer J, Kovach MJ, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 2004;36:377–381.

74. Deng HX, Chen W, Hong ST, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 2011;477:211–215.

75. Maruyama H, Morino H, Ito H, et al. Mutations of optineurin in familial amyotrophic lateral sclerosis. Nature 2010;465:179–184.

76. Watts GD, Wymer J, Kovach MJ, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 2004;36:377–381.

77. Tresse E, Salomons FA, Vesa J, et al. VCP/p97 is essential for autophagy and is disrupted in VCP disease. J Cell Biol 2004;24:8055–8068.

78. Seibenhener ML, Babu JR, Geetha T, Wong HC, Krishna NR, Wooten MW. Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. Mol Cell Biol 2004;24:8055–8068.

79. Pankiv S, Clausen TH, Lamark T, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 2007;282:24131–24145.

80. Kleijnen MF, Shih AH, Zhou P, et al. The hPLIC Proteins may provide a link between the ubiquitination machinery and the proteasome. Mol Cell 2000;6:409–419.

81. N'Diaye EN, Kajihara KK, Hsieh I, Morisaki H, Debnath J, Brown EJ. PLIC proteins or ubiquilins regulate autophagy-dependent cell survival during nutrient starvation. EMBO Rep 2009;10:173–179.

82. Wild P, Farhan H, McEwan DG, et al. Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. Science 2011;333:228–233.

83. Scotter EL, Vance C, Nishimura AL, et al. Differential roles of the ubiquitin proteasome system and autophagy in the clearance of soluble and aggregated TDP-43 species. J Cell Sci 2014;127:1263–1278.

84. Caccamo A, Majumder S, Deng JJ, Bai Y, Thornton FB, Oddo S. Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability. J Biol Chem 2009;284:27416–27424.

85. Urushitani M, Sato T, Bamba H, Hisa Y, Tooyama I. Synergistic effect between proteasome and autophagosome in the clearance of polyubiquitinated TDP-43. J Neurosci Res 2010;88:784–797.

86. Wang X, Fan H, Ying Z, Li B, Wang H, Wang G. Degradation of TDP-43 and its pathogenic form by autophagy and the ubiquitin-proteasome system. Neurosci Lett 2010;469:112–116.

87. Zhang YJ, Xu YF, Cook C, et al. Abrupt clearance of TDP-43 enhances aggregation and cellular toxicity. Proc Natl Acad Sci U S A 2009;106:7607–7612.

88. Wojcik C, Yano M, DeMartino GN. RNA interference of valosin-containing protein (VCP/p97) reveals multiple cellular roles linked to ubiquitin/proteasome-dependent proteolysis. J Cell Sci 2004;117:281–292.

89. Lamark T, Johansen T. Autophagy: links with the proteasome. Curr Opin Cell Biol 2009;22:192–198.

90. Thomas M, Alegre-Abarrategui J, Wade-Martins R. RNA dysfunction and aggrephagy at the centre of an amyotrophic lateral sclerosis/frontotemporal dementia disease continuum. Brain 2013;136:1345–1360.

91. Lamark T, Johansen T. Aggrephagy: selective disposal of protein aggregates by macroautophagy. Int J Cell Biol 2012;2012:736905.

92. Kopito RR. Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 2000;10:524–530.

93. Colby DW, Cassidy JP, Lin GC, Ingram VM, Wittrup KD. Stochastic kinetics of intracellular huntingtin aggregate formation. Nat Chem Biol 2006;2:319–323.

94. Jarrett JT, Lansbury PT, Jr. Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 1993;73:1055–1058.

95. Wood SJ, Wypych J, Steavenson S, Louis J-C, Citron M, Biere AL. α-Synuclein fibrillogenesis is nucleus-dependent: Implications for the pathogenesis of Parkinson's disease. J Biol Chem 1999;274:19509–19512.

96. King OD, Gitler AD, Shorter J. The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. Brain Res 2012;1462:61–80.

97. Johnson JO, Mandrioli J, Benatar M, et al. Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuro 2010;68:857–864.

98. Lee BH, Lee MJ, Park S, et al. Enhancement of proteasome activity by a small molecule inhibitor of UPS14. Nature 2010;467:179–184.

99. Wang IF, Guo BS, Liu YC, et al. Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43. Proc Natl Acad Sci U S A 2012;109:15024–15029.

100. Lagier-Tourenne C, Polymenidou M, Hutt KR, et al. Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. Nat Neurosci 2012;15:1488–1497.

101. Honda D, Ishigaki S, Iguchi Y, et al. The ALS/FTLD-related RNA-binding proteins TDP-43 and FUS have common downstream RNA targets in cortical neurons. FEBS Open Bio 2013;4:1–10.

102. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded C9orf72 hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011;72:245–256.

103. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011;72:257–268.

104. Lee YB, Chen HJ, Peres JN, et al. Hexanucleotide repeats in ALS/FTD form length-dependent RNA foci, sequester RNA binding proteins, and are neurotoxic. Cell Rep 2013;5:1178–1186.

105. Cooper-Knock J, Walsh MJ, Higginbottom A, et al. Sequestration of multiple RNA recognition motif-containing proteins by C9orf72 repeat expansions. Brain 2014;137:2040–2051.

106. Lagier-Tourenne C, Baughn M, Rigo F, et al. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc Natl Acad Sci U S A 2013;110:E4530–E4539.

107. Donnelly CJ, Zhang PW, Pham JT, et al. RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. Neuron 2013;80:415–428.

108. Troakes C, Maekawa S, Wijesekera L, et al. An MND/ALS phenotype associated with C9orf72 repeat expansion: abundant p62-positive, TDP-43-negative inclusions in cerebral cortex, hippocampus and cerebellum but without associated cognitive decline. Neuropathology 2012;32:505–514.

109. Bretschneider J, Van Deerlin VM, Robinson JL, et al. Pattern of ubiquilin pathology in ALS and FTLD indicates presence of C9ORF72 hexanucleotide expansion. Acta Neuropathol 2012;123:825–839.
110. Mann DM, Rollinson S, Robinson A, et al. Dipeptide repeat proteins are present in the p62 positive inclusions in patients with frontotemporal lobar degeneration and motor neurone disease associated with expansions in C9ORF72. Acta Neuropathol Commun 2013;1:68.

111. Ash PE, Bieniek KF, Gendron TF, et al. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptide species to c9FTD/ALS. Neuron 2013;77:639–646.

112. Mori K, Weng SM, Arzberger T, et al. C9orf72 FTLD/ALS-variant analysis identifies TUBA4A mutations associated with expansions in C9ORF72. Acta Neuropathol Commun 2014;2:70.

113. Kwon I, Xiang S, Kato M, et al. Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. Science 2014;345:1139–1145.

114. Mizielska S, Gronke S, Niccolì T, et al. C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. Science 2014;345:1192–1194.

115. Yamakawa M, Ito D, Honda T, et al. Characterization of the dipeptide repeat protein in the molecular pathogenesis of c9FTD/ALS. Hum Mol Genet 2014 Nov 14 [Epub ahead of print].

116. May S, Hornburg D, Schludi MH, et al. The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science 2013;339:1335–1338.

117. Davidson YS, Barker H, Robinson AC, et al. Brain distribution of dipeptide repeat proteins in frontotemporal lobar degeneration and motor neurone disease associated with expansions in C9ORF72. Acta Neuropathol Commun 2014;2:70.

118. Mackenzie IR, Arzberger T, Kremmer E, et al. Dipeptide repeat protein pathology in C9ORF72 mutation cases: clinicopathological correlations. Acta Neuropathol 2013;126:859–879.

119. Su Z, Zhang Y, Gendron TF, et al. Discovery of a biomarker and lead small molecules to target r(GGGGCC)-associated defects in c9FTD/ALS. Neurobiol Aging 2014;35:1212–1220.

120. Synofzik M, Born C, Rominger A, et al. Targeted high-throughput sequencing identifies a TARDBP mutation as a cause of early-onset FTD without motor neuron disease. Neurobiol Aging 2014;35:1212 e1–e5.

121. Johnson RT, Bradley W, Ritz B, Rocca WA, Shefner J, Wolfrom C. Amyotrophic lateral sclerosis in veterans: Review of the scientific literature. United States of America: The National Academies Press, 2006, Report No.: 9780309102544.

122. Mizielska S, Lashley T, Norona FE, et al. C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense RNA foci. Acta Neuropathol 2013;126:845–857.

123. Chio A, Calvo A, Dossena M, Ghiglione P, Mutani R, Mora G. ALS in Italian professional soccer players: the risk is still present and could be soccer-specific. Amyotroph Lateral Scler 2009;10:205–209.

124. Orrell RW, Lane RJ, Ross M. A systematic review of antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. Amyotroph Lateral Scler 2008;9:195–211.

125. Shen Y, Meng W, Glatz JF, et al. Muscle-specific EWSR1 isoform expression characterizes frontotemporal lobar degeneration in FUS mutation carriers. Hum Mol Genet 2014;23:157–170.

126. Ash PE, Bieniek KF, Gendron TF, et al. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptide species to c9FTD/ALS. Neuron 2013;77:639–646.

127. Smith BN, Vance C, Scotter EL, et al. Novel mutations support a role for human brain pericytes in neuroinflammation. J Neuroinflammation 2014;11:104.
brain but not in spinal cord of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Am J Pathol 2008;173:182–194.

150. Suzuki H, Lee K, Matsuoka M. TDP-43-induced death is associated with altered regulation of BIM and Bcl-xL and attenuated by caspase-mediated TDP-43 cleavage. J Biol Chem 2011;286:13171–13183.

151. Dormann D, Capell A, Carlson AM, et al. Proteolytic processing of TAR DNA binding protein-43 by caspases produces C-terminal fragments with disease defining properties independent of progranulin. J Neurochem 2009;110:1082–1094.

152. Zhang YJ, Xu YF, Dickey CA, et al. Progranulin mediates caspase-dependent cleavage of TAR DNA binding protein-43. J Neurosci 2007;27:10530–10534.

153. Fuentesalba RA, Udan M, Bell S, et al. Interaction with polyglutamine aggregates reveals a Q/N-rich domain in TDP-43. J Biol Chem 2010;285:26304–26314.

154. Furukawa Y, Kaneko K, Nukina N. Molecular properties of TAR DNA binding protein-43 fragments are dependent upon its cleavage site. Biochim Biophys Acta 2011;1812:1577–1587.

155. Yang C, Tan W, Whittle C, et al. The C-terminal TDP-43 fragments with disease defining properties independent of TAR DNA binding protein-43 by caspases produces C-terminal progranulin. J Neurochem 2009;110:1082–1094.

156. Che MX, Jiang YJ, Xie YY, Jiang LL, Hu HY. Aggregation of the C-terminal TDP-43 fragments are dependent upon its cleavage site. Biochim Biophys Acta 2011;1812:1577–1583.

157. Yang C, Tan W, Whittle C, et al. The C-terminal TDP-43 fragments have a high aggregation propensity and harm neurons by a dominant-negative mechanism. PLoS One 2010;5:e15878.

158. Gregory JM, Barros TP, Meehan S, Dobson CM, Luheshi LM. The aggregation and neurotoxicity of TDP-43 and its ALS-associated 25 kDa fragment are differentially affected by molecular chaperones in Drosophila. PLoS One 2012;7:e31899.

159. Van Langenhove T, van der Zee J, Van Broeckhoven C. The molecular basis of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum. Ann Med 2012;44:817–828.

160. Tsuiji H, Iguchi Y, Furuya A, et al. Spliceosome integrity is defective in the motor neuron diseases ALS and SMA. EMBO Mol Med 2013;5:221–234.

161. Jackrel ME, DeSantis ME, Martinez BA, et al. Potentiated Hsp104 variants antagonize diverse proteotoxic misfolding events. Cell 2014;156:170–182.

162. Gregory JM, Barros TP, Meehan S, Dobson CM, Luheshi LM. The aggregation and neurotoxicity of TDP-43 and its ALS-associated 25 kDa fragment are differentially affected by molecular chaperones in Drosophila. PLoS One 2012;7:e31899.

163. Crippa V, Carra S, Rusmini P, et al. A role of small heat shock protein B8 (HspB8) in the autophagic removal of misfolded proteins responsible for neurodegenerative diseases. Autophagy 2010;6:958–960.

164. Hasegawa M, Arai T, Nonaka T, et al. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann Neurol 2008;64:60–70.

165. Inukai Y, Nonaka T, Arai T, et al. Abnormal phosphorylation of Ser409/410 of TDP-43 in FTLD-U and ALS. FEBS Lett 2008;582:2899–2904.

166. Neumann M, Kwong LK, Lee EB, et al. Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. Acta Neuropathol 2009;117:137–149.

167. Kametani F, Nonaka T, Suzuki T, et al. Identification of casein kinase-1 phosphorylation sites on TDP-43. Biochem Biophys Res Commun 2009;382:405–409.

168. Iguchi Y, Katsuno M, Takagi S, et al. Oxidative stress induced by glutathione depletion reproduces pathological modifications of TDP-43 linked to TDP-43 proteinopathies. Neurobiol Dis 2012;45:862–870.

169. Li HY, Yeh PA, Chiu HC, Tang CY, Tu BP. Hyperphosphorylation as a defense mechanism to reduce TDP-43 aggregation. PLoS One 2011;6:e23075.

170. Brady OA, Meng P, Zheng Y, Mao Y, Hu F. Regulation of TDP-43 aggregation by phosphorylation and p62/SQSTM1. J Neurochem 2011;116:248–259.

171. Choksi DK, Roy B, Chatterjee S, et al. TDP-43 Phosphorylation by casein kinase I epsilon promotes oligomerization and enhances toxicity in vivo. Hum Mol Genet 2014;23:1025–1035.

172. Salado I G, Redondo M, Bello ML, et al. Protein kinase CK-1 inhibitors as new potential drugs for amyotrophic lateral sclerosis. J Med Chem 2014;57:2755–2772.

173. Seyfried NT, Gozal YM, Dammer EB, et al. Multiplex SILAC analysis of a cellular TDP-43 proteinopathy model reveals protein inclusions associated with SUMOylation and diverse polyubiquitin chains. Mol Cell Proteomics 2010;9:705–718.

174. Hans F, Fiesel FC, Strong JC, et al. UBE2E ubiquitin-conjugating enzymes and ubiquitin isopeptidase Y regulate TDP-43 protein ubiquitination. J Biol Chem 2014;289:19164–19179.

175. Wengqiang C, Lonskaya I, Hebron ML, et al. Parkin-mediated reduction of nuclear and soluble TDP-43 reverses behavioral decline in symptomatic mice. Hum Mol Genet 2014;23:4960–4969.

176. Gray DA, Tsirigotis M, Woulfe J. Ubiquitin, proteasomes, and the aging brain. Sci Aging Knowledge Environ 2003;2003:RE6.

177. Majounie E, Renton AE, Mok K, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol 2012;11:323–330.