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Pathological mechanisms underlying TDP-43 driven neurodegeneration in FTLD–ALS spectrum disorders

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Aggregation of misfolded TAR DNA-binding protein 43 (TDP-43) is a striking hallmark of neurodegenerative processes that are observed in several neurological disorders, and in particular in most patients diagnosed with frontotemporal lobar degeneration (FTLD) or amyotrophic lateral sclerosis (ALS). A direct causal link with TDP-43 brain proteinopathy was provided by the identification of pathogenic mutations in *TARDBP*, the gene encoding TDP-43, in ALS families. However, TDP-43 proteinopathy has also been observed in carriers of mutations in several other genes associated with both ALS and FTLD demonstrating a key role for TDP-43 in neurodegeneration. To date, and despite substantial research into the biology of TDP-43, its functioning in normal brain and in neurodegeneration processes remains largely elusive. Nonetheless, breakthroughs using cellular and animal models have provided valuable insights into ALS and FTLD pathogenesis. Accumulating evidence has redirected the research focus towards a major role for impaired RNA metabolism and protein homeostasis. At the same time, the concept that toxic TDP-43 protein aggregates promote neurodegeneration is losing its credibility. This review aims at highlighting and discussing the current knowledge on TDP-43 driven pathomechanisms leading to neurodegeneration as observed in TDP-43 proteinopathies. Based on the complexity of the associated neurological diseases, a clear understanding of the essential pathological modifications will be crucial for further therapeutic interventions.

INTRODUCTION

Over the past years, it became clear that frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) constitute the opposite ends of a disease continuum of overlapping disease phenotypes (1, 2). FTLD is a presenile dementia characterized by selective atrophy of the frontal and anterior temporal lobes of the brain (3). Clinically, patients develop progressive behavioral changes, language impairment and/or executive dysfunction (4, 5). ALS is an incurable, severely disabling condition in which both upper and lower motor neurons degenerate (6–8). Disease progression is characterized by progressive muscle weakening evolving into paralysis with respiratory failure leading to death within 1–5 years after disease onset (8, 9). The comorbidity of ALS and FTLD syndromes in patients is estimated to occur in ~50% of the patients (10–13). Currently, there is no effective treatment available for either FTLD or ALS.

In addition to the overlapping clinical symptomatology, the common pathological hallmark in the majority of these patients consists of TAR DNA-binding protein 43 (TDP-43), the major protein within ubiquitinated cytoplasmic inclusions. TDP-43 aggregates are present in a spectrum of distinctive neurodegenerative disorders suggesting a key role for TDP-43 in disease pathogenesis (14–16). Furthermore, secondary TDP-43 accumulation occurs in multiple other neurodegenerative disorders (1, 17) and is also observed in brains of control subjects over 65 years (18). Since the discovery of TDP-43 in 2006 (14, 15), major efforts have been directed to unravel its physiological functions in normal and disease brain. To date, TDP-43 is known as a highly conserved, nuclear RNA-binding protein

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(RBP) involved in transcription and splicing regulation (19–22) (reviewed in 23). Subsequently, identification of causal mutations in *TARDBP*, the gene encoding TDP-43 (24–26) (reviewed in 27), mechanistically linked neurodegeneration to the occurrence of TDP-43 aggregates. Causal *TARDBP* mutations were first observed in families segregating autosomal dominant ALS identifying *TARDBP* as a new ALS gene. Few mutations were also described in FTLD–ALS or FTLD patients (28–31), although their pathogenicity was not always convincing. In addition to *TARDBP*, mutations were identified in other genes that had been associated with TDP-43 pathology. Mutations are observed at variable frequencies in ALS, FTLD–ALS or FTLD patients in progranulin (*GRN*) (32, 33), angiogenin (*ANG*) (34), heterogeneous nuclear ribonucleoprotein A1 and A2/B1 (*hnRNPA1* and *hnRNPA2/B1*) (35), optineurin (*OPTN*) (36), ubiquilin 2 (*UBQLN2*) (37), sequestosome 1 (*SQSTM1*) (38, 39) and valosin-containing protein (*VCP*) (40, 41) (Table 1). A large number of these genes is thought to be implicated in RNA processing and protein degradation pathways, suggesting that impairment of these processes might be central to the disease cascade (42, 43). The majority of the encoded proteins have been identified as components of the pathological inclusions, emphasizing the heterogeneous molecular basis of both FTLD and ALS. The discovery of a pathogenic GGGGCC (G_4C_2) repeat expansion mutation in the gene *C9orf72* further stressed the involvement of altered RNA pathways in FTLD/ALS pathogenesis (Table 1). Not only became *C9orf72* the most frequently mutated gene in both ALS and FTLD, the sharing of the (G_4C_2) repeat expansion mutation linked both disorders into one disease continuum of overlapping clinical symptoms and TDP-43 pathology (44–46). Understanding the molecular basis of C9orf72 associated diseases might provide important insights into common biological mechanisms.

In contrast to FTLD, a large proportion of ALS patients do not have documented family histories of disease but express disease due to a complex interplay of genetic and environmental risk factors. Genome-wide genetic and animal model screens have identified several putative susceptibility genes and modifiers of TDP-43 toxicity that were strongly associated with ALS including ephrin type-A receptor 4 precursor (*EPHA4*) (47), RNA lariat debranching enzyme (*DBR1*) (48), elongator protein 3 (*ELP3*) (49) and intermediate-length polyQ expansions in ataxin 2 (*ATXN2*) (Table 1) (50, 51).

The common TDP-43 pathology in ALS and FTLD patients suggests that pathways disrupting TDP-43 integrity might be shared between patients with a different clinical, pathological and genetic etiology. The question remains, however, which disease processes are essential to drive TDP-43-related pathogenesis. In this review paper, we highlight the research outcomes that contributed to valuable insights into TDP-43-related pathomechanisms. As suggested by the molecular genetic findings, multiple pathways related to RNA processing, repeat expansions, protein aggregation and proteostasis are likely contributing to the multifactorial nature of FTLD/ALS disorders.

EMERGING ROLE FOR ALTERED RNA PROCESSING AND RBPS

Processing of RNA molecules in the nervous system is an elaborate and remarkably complex network to maintain a functional environment in neurons. Dysfunctional RNA metabolism has been related with certain neurodegenerative diseases (52–54). Hence, mutations in *TARDBP* and *FUS* indicate that RBPs might exert a central role in the pathogenesis of FTLD/ALS-related disorders. TDP-43 and FUS/TLS are typically accumulating in the majority of these disorders (55, 56). Likewise, genetic studies identified mutations in additional RBPs such as *TAF15*, *EWSR1* and in *hnNRPA1* and *hnRNPA2/B1*, demonstrating that RBPs might contribute generally to ALS and/or FLTD (35, 57–59) (Tables 1 and 2).

Table 1. Genetics of the FTLD and ALS spectrum related to TDP-43 proteinopathy

	Gene	Protein	Locus	Inheritance	Clinical phenotype	Mutation type	Ref.
RNA metabolism	*TARDBP*	TDP-43	1p36	Auto. Dom.	ALS, ALS–FTD, rare FTD	Missense, nonsense	(24, 25)
	ANG	Angiogenin	14q11	Auto. Dom.	ALS, ALS–FTD	Missense	(34)
	hnRNPA1	Heterogeneous nuclear ribonucleoprotein A1	12q13	Auto. Dom.	ALS, IBMPFD	Missense	(35)
	hnRNPA2/B1	Heterogeneous nuclear ribonucleoprotein A2/B1	7p15	Auto. Dom.	ALS, IBMPFD	Missense	(35)
Repeat expansions	*C9orf72*	Chromosome 9 open reading frame 72	9p21	Auto. Dom.	ALS, ALS–FTD, FTD	GGGGCC expansion	(44–46)
	ATXN2	Ataxin 2	12q24	Auto. Dom. risk factor	ALS	CAG expansion	(50)
Protein homeostasis	*VCP*	Valosin-containing protein	9p13	Auto. Dom.	FTD (IBMPFD), ALS	Missense	(40, 41)
	UBQLN2	Ubiquilin 2	Xp11	Auto. Dom. X-linked	ALS, ALS/ dementia	Missense	(37)
	OPTN	Optineurin	10p13	Auto. Dom. Auto. Rec.	ALS, ALS–FTD	Missense, nonsense, deletion	(36)
Growth factor	*PGRN*	Progranulin	17q21	Auto. Dom. (FTLD) Modifier (ALS)	FTD	Missense, nonsense, deletion, frameshift, splice site	(32, 33)

Auto. Dom., autosomal dominant; Auto. Rec., autosomal recessive; FTD, frontotemporal dementia; IBMPFD, inclusion body myopathy with early-onset Paget disease and frontotemporal dementia data from AD & FTD mutation database (http://www.molgen.ua.ac.be/FTDMutations Accessed 29 July 2013) (164).

Table 2. Cellular protein functions and molecular pathology of FTLD- and ALS-related genes

	Protein	Abbreviation	Suggested protein function	Molecular pathology	Ref.
RNA-binding proteins	TAR DNA-binding protein 43	TDP-43	Transcription and splicing regulation microRNA biogenesis RNA transport and stabilization (*Member of hnRNP protein family*)	TDP-43	(14, 15)
	Fused in sarcoma/translocated in liposarcoma	FUS/TLS	Transcription and splicing regulation microRNA processing Maintenance of genomic integrity (*Member of FET proteins*)	FUS/TLS	(165, 166)
	TATA-binding protein-associated factor 15	TAF15	RNA Polymerase II component Transcription initiation (*Member of FET proteins*)	FUS/TLS	(57)
	Ewing sarcoma breakpoint region 1	EWSR1	Transcriptional repressor (*Member of FET protein family*)	FUS/TLS	(58)
	Angiogenin	ANG	RNA processing and tRNA modification Vascularization Assembly of stress granules	TDP-43	(34, 167)
	Heterogeneous nuclear ribonucleoprotein	hnRNPA1 hnRNPA2/B1 hnRNPA3	Packing and transport of mRNA (*Member of hnRNP protein family*)	n.d.	(35, 95)
Repeat expansions	Chromosome 9 open reading frame 72	C9orf72	Unknown protein function (*Related to DENN proteins*)	TDP-43, UPS	(44–46)
	Ataxin 2	ATXN2	Regulator of EGFR trafficking	TDP-43	(50, 168)
Protein homeostasis	Valosin-containing protein	VCP	Membrane fusion Protein degradation (ER, proteasome and autophagy-associated)	TDP-43	(40, 41)
	Ubiquilin 2	UBQLN2	Proteasome-mediated protein degradation	TDP-43	(37)
	Sequestosome 1 — p62	SQSTM1	Autophagic degradation Regulator of NF-κB signaling pathway Involved in immune response	n.d.	(38)
	Optineurin	OPTN	Golgi maintenance Exocytosis Vesicular trafficking	TDP-43	(36)
Growth factor	Progranulin	GRN	Multifunctional growth factor Inflammation Wound repair	TDP-43	(32, 33, 169)

FET proteins, *F*US—*E*WSR1—*T*AF15 DNA/RNA-binding proteins; EGFR, epidermal growth factor receptor; ER, endoplasmatic reticulum; DENN protein, *d*ifferentially *e*xpressed in *n*ormal and *n*eoplastic cells; n.d., not determined; UPS, ubiquitin proteasome system.

As a member of the hnRNP family, TDP-43 is involved in multiple steps of gene expression regulation, including RNA splicing and transport (22, 60, 61) (Fig. 1). Loss of spliceosome integrity was reported in motor neurons of ALS patients (62). A mouse model expressing mutant TDP-43 induced significant splicing alterations accompanying motor neuron disease in the absence of TDP-43 aggregation or nuclear clearing (63). To unravel the biological functions of TDP-43, TDP-43 RNA targets were characterized in cultured cells, mouse brain, and in human brain of FTLD and ALS patients. TDP-43 was shown to bind to ≈30% of the mouse transcriptome, highlighting the versatility and importance of TDP-43 for splicing regulation (64, 65). Preferentially, TDP-43 bound long clusters of UG-rich sequences, mostly at intronic regions. Pre-mRNAs with exceptionally long introns (>100 kb) are a characteristic feature of brain-enriched transcripts which might explain neuronal vulnerability observed in patients (64, 66). Also, proteins encoded by the RNA targets were enriched for genes involved in synaptic function, neuronal development and RNA metabolism. A number of these proteins are implicated in neurological diseases including sortilin (*SORT1*), FUS/TLS and GRN, providing a speculative connection between disease mutations and pathology (64, 65, 67). Expression of a muscle-specific actin binding protein filamin C, one of the identified genes regulated by TDP-43, was increased in frontal cortex of FTLD patients (68).

Similar to other hnRNPs, TDP-43 auto-regulates its expression levels through a negative-feedback loop involving alternative polyA site selection and exosome or miRNA-regulated degradation mechanisms (Fig. 2) (69–71). The C-terminal region of TDP-43, harboring most of the pathogenic disease mutations, is required for self-regulation (Fig. 1) (69). Tight regulation of TDP-43 expression levels was further illustrated in animal models where human TDP-43 overexpression reduced endogenous protein levels. Because of the lack of TDP-43 aggregates in these models, down-regulation of TDP-43 has been suggested to be toxic (72–74). However, this is questionable taken the strong homology (i.e. 96% sequence identity) of human and mouse TDP-43 (20) and the toxicity of mouse TDP-43 overexpression (75, 76). TDP-43 overexpression might directly alter splicing or stability of RNA targets as shown by dose-dependent toxicity in mice (63, 76, 77). Increased TDP-43 levels have rarely been described in patients (78, 79), but cell stress and pathogenic ALS mutations might indirectly increase TDP-43 levels (80–82).

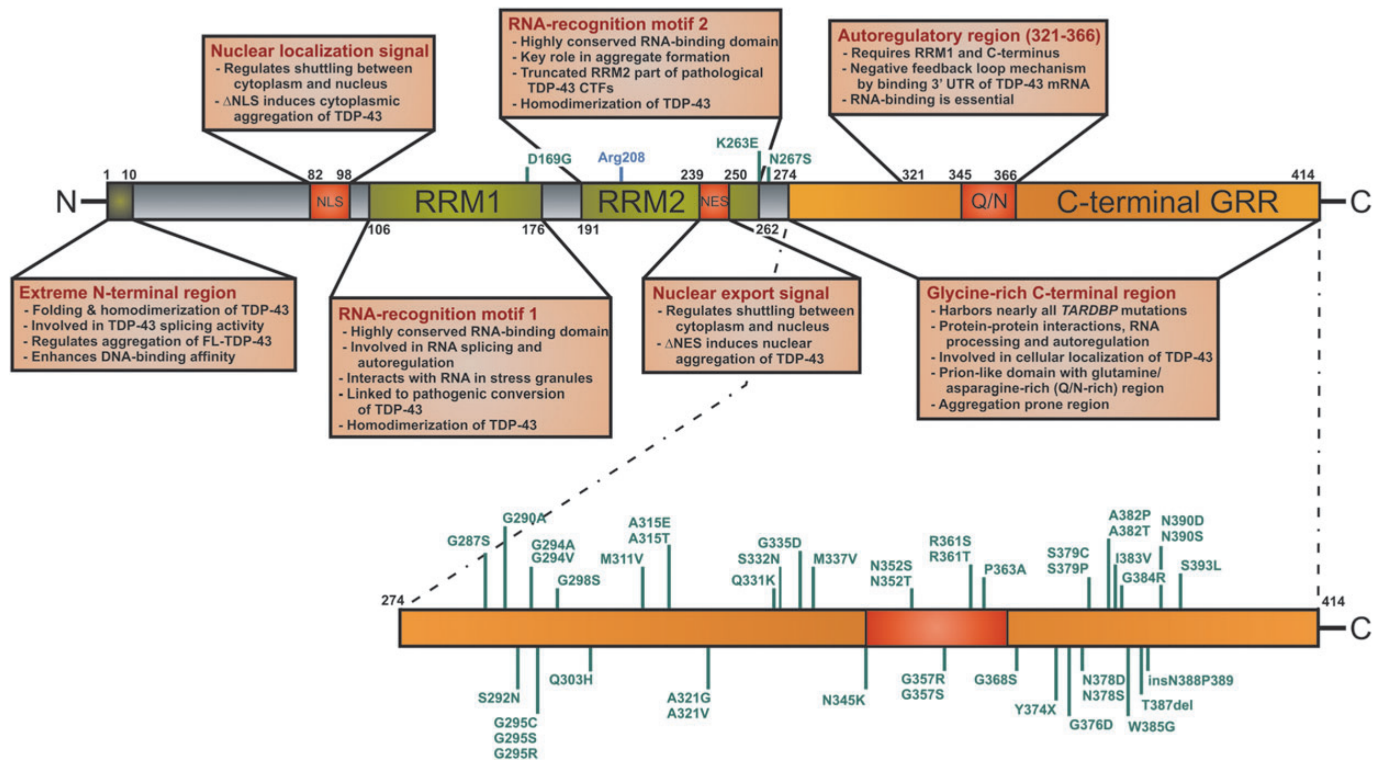

Figure 1. Schematic representation of TDP-43 with its protein domain structures and localization of disease-associated mutations. TDP-43 comprises an NLS and NES, respectively, 2 RNA-recognition motifs (RRM1 and RRM2) and a C-terminal glycine-rich region (GRR). Numerous mutations in *TARDBP* have been identified in sporadic and familial ALS patients and rarely in FTLD patients. Mutations are predicted to enhance aggregation. Furthermore, experimental evidence also suggested that different domain structures of TDP-43 are involved in the aggregation process. Abbreviations: del, deletion; ins, insertion; FL-TDP, full-length TDP-43; GRR, glycine-rich region; UTR, untranslated region.

The wide range of TDP-43 targets makes it difficult to pinpoint the exact disease culprit(s). Also, it is inconceivable that a single TDP-43 target acts as a sole trigger of neurodegeneration. Therefore, determining the key targets and their altered function in disease are a major goal.

C9ORF72 IS A MAJOR DISEASE GENE

FTLD and ALS constitute the opposite ends of a broad disease continuum, but also co-occur in the same patient or segregate within the same family. A substantial fraction of the families with concomitant FTLD and ALS were explained by the co-segregation of a G_4C_2 repeat expansion mutation in *C9orf72* located at chromosome 9p21 (44–46, 83–85). In unaffected, non-expanded repeat carriers the repeat length does not exceed 24 units, while in patients the pathological expansions range from 700 to 4400 repeat units (86, 87). At present, little or no information exists about the physiological functions of the C9orf72 protein. Nevertheless, several G_4C_2-related pathomechanisms have been suggested, involving both loss-of-function and toxic gain-of-function, which might not be mutually exclusive.

Based on homology searches, C9orf72 appears distantly related to DENN (differentially expressed in normal and neoplastic cells) domain-containing proteins which are regulators of membrane trafficking (88). DENN domain-containing proteins and impaired trafficking have been linked to Alzheimer's disease (AD) and ALS (89, 90). Primary model systems have demonstrated that the pathological expansion of the G_4C_2 repeat is sufficient to induce neurodegeneration (91). As the G_4C_2 repeat is located between two transcription initiation sites, C9orf72 expression might be disrupted by aberrant binding of regulatory elements or altered methylation. CpG hypermethylation near the repeat and loss-of-transcription have been reported (44, 46, 87, 92). The reduced expression, however, has not yet been confirmed on the protein level due to lack of proper C9orf72 antibodies. Supporting evidence was obtained for decreasing transcriptional activity when intermediate repeat alleles with increasing numbers of repeat units were expressed in cultured cells (93).

Entrapment of RBPs and other RNA molecules by the expanded G_4C_2 repeat was suggested to induce RNA toxicity through the formation of RNA foci (46). However, this was not a universal finding in all C9orf72 studies (94). Nonetheless, hnRNPA3 was shown to bind the G_4C_2 motif and was deposited in the enigmatic TDP-43-negative neuronal inclusions (95). Another interesting candidate is hnNRPA2/B1 which is known to directly interact with TDP-43 and forms RNA foci by binding C/G-rich repeats in certain ataxias (61, 96). Mutations in *hnRNPA2/B1* were described that cause ALS and related disorders (35).

Apart from the pathognomonic TDP-43 inclusions, TDP43 negative/p62-positive inclusions were also observed in the hippocampus and cerebellum of C9orf72 G_4C_2 expansion carriers (97–99). Two studies reported the co-localization of aggregation-prone dipeptide repeat proteins (DPRs) with these TDP-43 negative/p62-positive inclusions (100, 101). These

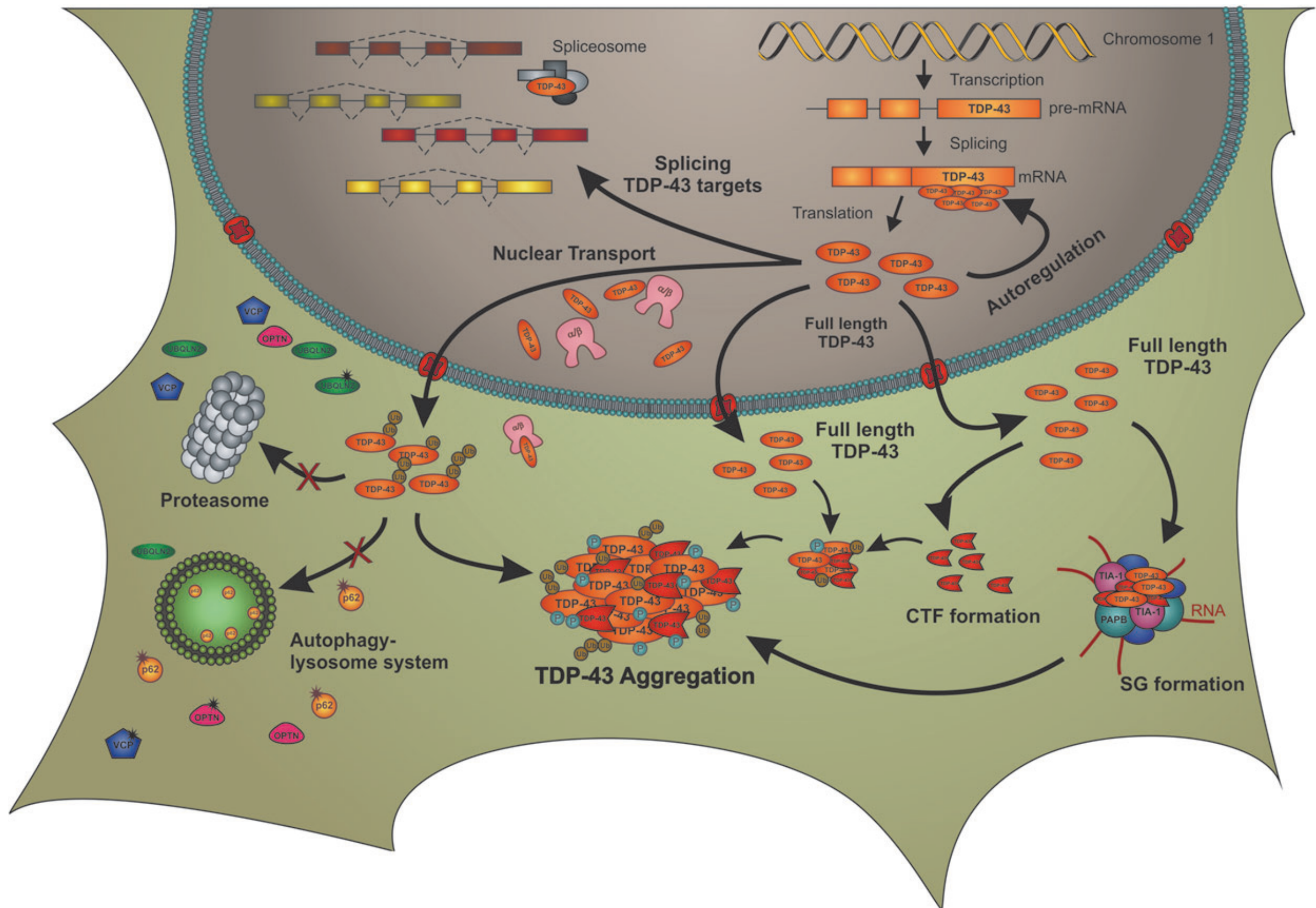

Figure 2. Overview of putative mechanisms involved in TDP-43 proteinopathy observed in ALS and FTLD patients. Physiological TDP-43 shuttles between the nucleus and the cytoplasm to exert its cellular functions ranging from gene expression regulation at the transcription and splicing level to mRNA transport and stabilization. Upon cellular stress, TDP-43 is reversibly directed to the cytoplasm and accumulates in SGs. Mutations in several genes (Table 1) have been observed in ALS and FTLD patients with TDP-43 proteinopathy, demonstrating that TDP-43 has a key role in the neurodegeneration process. TDP-43 proteinopathy is characterized by pathological modifications including aggregation, C-terminal cleavage into CTFs, hyperphosphorylation and ubiquitination of TDP-43. Furthermore, extensive research on TDP-43-related pathomechanisms suggests that different putative mechanisms might contribute to TDP-43 aggregation, including impaired protein degradation, alterations of TDP-43-associated splicing events, nuclear transport defects, loss of TDP-43 autoregulation and enhanced self-interaction of TDP-43. Abbreviations: α/β, importin α/β; Ub, ubiquitin; P, phosphorylation; Star *, mutant protein.

DPRs are generated by translation of the expanded G_4C_2 repeat through repeat-associated non-ATG (RAN) translation (102). Since hairpin formation is essential to initiate RAN translation, formation of stable RNA G-quadruplexes by the G_4C_2 repeat might trigger initiation of translation of the expanded repeat sequences (103, 104).

While different putative mechanisms have emerged for C9orf72, the key step will be to confirm a pathogenic nature for these mechanisms. Appropriate disease models as well as specific antibodies will be crucial to clarify disease pathogenesis in C9orf72 repeat mutation carriers.

TDP-43 AGGREGATION VERSUS NEURODEGENERATION

Formation of TDP-43 pathology is a distinguishing feature in a wide range of neurodegenerative disorders including FTLD and ALS disorders, and to a lesser extent in, for example, Alzheimer's and Huntington disease (1, 17). However, the pathogenicity of TDP-43 aggregates and the accompanying protein modifications, including hyperphosphorylation, ubiquitination and cleavage into C-terminal fragments (CTFs), remain poorly understood (16). Few ALS and FTLD patients carry coding mutations in TDP-43 implying that in most patients the aggregation and concomitant nuclear depletion is of wild-type TDP-43. In these patients, TDP-43 proteinopathy is associated with mutations in other genes like *GRN* and *VCP* that are underlying the FTLD–ALS continuum (Table 1). Extensive research is ongoing on how these mutated proteins contribute to TDP-43 proteinopathy leading to novel insights in the molecular pathogenesis underlying neurodegeneration in these diseases.

Primary studies within different model systems found that at least one ALS mutation (p.A315T) in *TARDBP*, or a disruption of the nuclear localization signal (NLS) enhanced cytoplasmic mislocalization increasing toxicity in the absence of TDP-43

aggregates (72, 105–111). Given the diversity of RNA targets directly bound by TDP-43 (64, 65), it is not inconceivable that loss of nuclear TDP-43 has detrimental effects on neuronal function. In contrast to FUS/TLS, however, solid evidence for nuclear transport deficits related to TDP-43 mutations is lacking as none of the mutations cluster around the NLS or directly affect its sequence (112). Nonetheless, inhibition of the importin α/β pathway and altered transcription profiles of genes active in import processes were associated with sporadic FTLD/ALS and increased aging (Fig. 2) (113). Together, these data indicated that cytoplasmic mislocalization might contribute to disease pathogenesis, although additional hits are probably required such as cellular stress or genetic/environmental risk factors to induce full blown TDP-43 pathology (112).

Currently, most of the available data points towards increased aggregation propensity of TDP-43 in the presence of pathogenic *TARDBP* mutations, although variable results have been obtained for different mutations (Fig. 2) (110, 114, 115). Purified full-length TDP-43 is intrinsically aggregation prone and requires the C-terminal domain (114, 116, 117). The N-terminus and both RNA-recognition motifs of TDP-43 have been implicated in regulating inclusion formation (118–120). This suggested that RNA binding in addition to aggregation is a component of TDP-43 toxicity (Fig. 1) (121).

Further insights in the aggregation mechanisms were provided by structural analyses of TDP-43. More specifically, the C-terminal region harbors a glutamine/asparagine-rich (Q/N-rich) domain that shares similarities with yeast prions (Fig. 1) (122, 123). Prion proteins exhibit ordered, self-perpetuating aggregation and are thought to transmit from an affected cell to its progeny (124, 125). The importance of the Q/N-rich prion-like domain was further exemplified by the recurrent detection of this domain in other RBPs using algorithmic searches (57, 126, 127). Several of these RBPs, including TAF15, EWSR1, hnRNPA3, hnRNPA2/B1 and hnRNPA1 (Table 2), were also found associated with ALS and/or FTLD either by the identification of pathogenic mutations or as component of the proteinaceous inclusions (35, 57, 58, 95). These results indicate that perturbed RNA-binding might be crucial in the neurodegeneration process (42). Therefore, it is presumed that prionoid properties of TDP-43 might explain the progressive spread of TDP-43 pathology observed in ALS patients (128, 129). In contrast to known prions whose aggregates have amyloidogenic properties, conflicting results exist for pathological TDP-43 aggregates (130–133). One study suggested that the TDP-43 prion domain is involved in physiological mechanisms controlling splicing and protein stability (134). Regulated protein aggregation is reported for RBPs in the formation of RNA and stress granules (SGs) (135, 136). Hence, one of the remaining key questions is how regulated TDP-43 aggregation evolves into pathological structures. As a stress-responsive protein, TDP-43 is recruited to SGs following different forms of cellular stress (137–140). Cytoplasmic mislocalization was a common prerequisite for SGs recruitment of FUS/TLS and TDP-43 (141). Consequently, it was suggested that SGs might seed pathological aggregates in a prion-like manner under prolonged cellular stress conditions or by disease-associated mutations in SG-related proteins (136, 142). Additionally, disease-associated TDP-43 mutations might also impair protein stability inducing pathological cleavage and aggregation (134). It is important to note that at least two hits are required to establish CTF aggregation (143).

Despite the increasing evidence for a role of aggregates in disease, transgenic animal models seem to undermine the importance of TDP-43 aggregation in disease. Although numerous models overexpressing both wild-type and mutant TDP-43 have been generated, cytoplasmic TDP-43 aggregates were rarely observed, despite the development of an aggressive ALS-like phenotype in nearly all models. This might imply that pathological aggregates are not a prerequisite for toxicity (63, 76, 144). Once formed, however, TDP-43 aggregates could hasten disease pathogenesis as has been observed in sporadic ALS patients (145). It has become clear that TDP-43 is a dosage-sensitive protein (76, 77) that plays an important role during development (146–148) and in adulthood (149, 150). Despite lack of clear evidence for TDP-43 overexpression in ALS and FTLD patients, certain pathogenic mutations in TDP-43 showed longer half-lives of mutant protein which correlated with an earlier disease onset (82, 151). Furthermore, stabilized proteins were shown to provoke toxicity through protein cleavage and insolubility together with proteasomal impairment and deregulation of mRNA levels (151).

Besides animal models, human induced pluripotent stem (iPS) cell lines have been generated from patients carrying a *GRN* or *TARDBP* mutation recapitulating different disease aspects (152, 153). Using this technology, neuronal cell models can be studied in the context of the same genetic background of the patient.

CO-OCCURRENCE OF IMPAIRMENTS IN PROTEIN DEGRADATION MACHINERY

The abundant accumulation of ubiquitinated proteins present in many neurodegenerative disorders strongly suggests that components of the protein degradation machinery are defective (154). Proteolysis is a complex process, which requires molecular chaperones, the ubiquitin proteasome system (UPS) and the autophagy–lysosome system (hereafter called ‘autophagy’) to monitor protein quality and protect cells from dysfunctional or misfolded proteins (unfolded protein response, UPR) (155). Evidence that these systems are impaired in FTLD–ALS spectrum diseases came from the identification of mutations in genes involved in proteostasis such as *UBQLN2*, *SQSTM*, *VCP* and *OPTN* (Table 1) (43, 156). These genes have different cellular functions but all share a link to protein degradation. More specifically, the majority operates as adaptor proteins linking ubiquitinated proteins to either the UPS or the autophagy system, necessary to initiate proteolysis. A detailed description of the individual gene functions falls outside of the scope of this review, but we summarized the essentials in Table 2.

Inhibition of either the UPS or autophagy results in increased TDP-43 aggregation and enhanced toxicity (Fig. 2) (157, 158). The autophagy receptor protein p62, encoded by the gene *SQSTM1*, has gained increasing interest, since it was identified as a component of TDP-43-negative, ubiquitin-positive inclusions in C9orf72-related patients (97–99). Overexpression of p62 has been shown to reduce TDP-43 aggregation in both an autophagy- and proteasome-dependent manner (159) Moreover, p62 directly interacts with TDP-43 and this interaction was

disrupted in FTLD brains (160). Motor neuron-specific disruption of the proteasome subunit Rpt3 in mice induced motor neuron loss and TDP-43 aggregation, whereas disrupted autophagy did not (161). These findings suggested that motor neurons might have a greater sensitivity to proteasome failure and that defective proteolysis might also play a causal role in disease. In addition to UPS and autophagy pathways, dysfunction of the UPR is also implicated in TDP-43 pathogenesis as mutant TDP-43 failed to up-regulate chaperons due to depletion of X-box-binding protein 1 (*XBP1*), a key component of the UPR (162) and regulator of stress resistance and longevity (163). Impaired proteostasis and concomitant accumulation of RBPs holds only for TDP-43, because FUS/TLS abnormalities in response to autophagy/proteasome failure are rarely observed (43).

Taken together, these findings indicate that both the UPS and autophagy play a key role in TDP-43 turnover. However, whether this role includes modifying or inducing TDP-43 pathomechanisms requires further examination.

CONCLUDING REMARKS

Substantial progress has been made in elucidating the biology of TDP-43 proteinopathy associated with neurodegeneration in FTLD and ALS, though the molecular and cellular mechanisms leading to disease remain largely elusive. Based on genetic and pathological evidence, different disease pathways are currently emerging which involve impaired RNA processing, protein homeostasis, TDP-43 autoregulation and enhanced self-interaction of TDP-43. Hence, disease etiology of ALS and FTLD is very likely to be multifactorial with different pathways converging into a common feature, i.e. TDP-43 misprocessing. The broad functionality of physiological TDP-43 impedes the identification of disease-related abnormalities and consequently also the development of valuable therapeutic treatments. Currently, no effective therapies are available that cure or delay disease progression of both FTLD and ALS. Animal models mimicking ALS and FTLD disorders are and will be very informative to gain novel insights into the TDP-43 biology and pathogenesis. Advances in iPS cell technology have been very instructive and may create new opportunities to study TDP-43-related neurodegeneration processes as well as therapeutic strategies.

Conflict of Interest statement. None declared.

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