NEURODEGENERATIVE DISEASE

RNA binding proteins and the pathological cascade in ALS/FTD neurodegeneration

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Advanced genetic approaches have accelerated the identification of causative genes linked to the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Most of the disease-related proteins encoded by these genes form aggregates in the cellular machineries that regulate RNA and protein quality control in cells. Cross-talk among the signaling pathways governing these machineries leads to pathological cascades mediated by the accumulation of mutant RNA binding proteins. We outline the molecular basis of ALS and FTD pathogenesis and discuss the prospects for therapeutic strategies to treat these diseases.

INTRODUCTION

Charcot (1) reported the first case of amyotrophic lateral sclerosis (ALS) in 1869, describing it as an “abominable” neurological disease characterized by progressive loss of motor neurons (Fig. 1). ALS tends to strike individuals in their 50s and 60s, and most die within 2 to 5 years of disease onset. Establishing therapeutic strategies for treating intractable diseases, including neurodegenerative diseases such as ALS, requires the identification of disease-related molecules, the unraveling of pathogenic pathways, and the targeting of pathogenic proteins through development of new therapies. A major discovery in 2006 was the identification of mutant TAR DNA binding protein 43 (TDP-43) as a pathological hallmark of ALS and of the related neurodegenerative disease, frontotemporal dementia (FTD) (2, 3). Patients with FTD have early onset dementia that mainly affects the frontal and temporal lobes of the brain, resulting in deficits in behavior and language. Mutant TDP-43 accumulates in the motor neurons of ~90% of patients with the sporadic form of ALS. In 2009, mutations were found in another RNA binding protein encoded by the FUS/TLS (fused in sarcoma/translated in liposarcoma) gene, and these mutations were found to be causative in familial ALS (4, 5).

To date, mutations in more than 20 genes have been shown to cause ALS and FTD, including mutations in SOD1, which was the first ALS-related gene to be identified. These genes account for about two-thirds of familial ALS cases and ~11% of sporadic ALS cases who have no family history of the disease (6). The identification of multiple disease-related molecules helps unravel the complex molecular network that underlies the pathogenesis of ALS and FTD. Notably, most of these disease-related mutant proteins form aggregates in two intracellular machineries: the RNA and protein quality control systems (Fig. 2). The dysfunction of these two machineries may play a critical role in the neurodegeneration seen in ALS and FTD (7). An important question is how these RNA and protein quality control systems communicate with each other, how their dysfunction leads to neurodegeneration, and whether these two pathways converge to form a single pathogenic pathway in neurons. Here, we focus on the cellular and biochemical features of ALS/FTD-related molecules in RNA and protein quality control systems, and we discuss possible pathogenic mechanisms and how these could be exploited to drive the development of new therapeutic strategies for treating these diseases.

RNA BINDING PROTEINS WITH PRION-LIKE DOMAINS

Seven ALS-related molecules are RNA binding proteins in which mutations are known to cause ALS or FTD. These include TDP-43, FUS, TATA box–binding protein–associated factor 15 (TAF15), Ewing sarcoma breakpoint region 1 (EWSR1), the heterogeneous nuclear ribonucleoproteins (hnRNPs) hnRNPA1 and hnRNPA2B1, and T-cell intracytoplasmic antigen (TIA1). Most of these mutant RNA binding proteins form characteristic deposits in affected regions of central nervous system (CNS) tissue from patients with ALS or FTD (Fig. 3). TDP-43 was first isolated as an RNA binding protein in inclusions comprising ubiquitinated proteins in CNS tissue from ALS and FTD patients, resulting in these diseases being termed TDP-43 proteinopathies (2, 3). TDP-43 was originally identified as a factor that binds to the transcription response region of the long terminal repeat in the HIV-1 virus genome. TDP-43 is crucial for maintaining the normal splicing of various mRNAs, including pre-mRNAs with long introns and noncoding RNAs (8–10).

The FUS/TLS gene was originally identified as part of a fusion gene with the transcription factor C/EBP-homologous protein in tumors called liposarcomas. FUS was the second ALS-related gene encoding an RNA binding protein identified in familial ALS (4, 5). Together with the highly homologous Ewing’s sarcoma protein (EWS) and TAF15, FUS/TLS forms the FET (FUS, EWS, and TAF15) protein family that plays roles in DNA repair, transcription, alternative splicing, translation, and RNA transport (11).

TAF15 is a component of the transcription factor IID complex that is essential for RNA polymerase II–mediated transcription. Sequencing of the TAF15 gene in patients with sporadic or familial ALS revealed several variants that are not found in healthy individuals (12, 13). In FTD with FUS pathology, TAF15 coaccumulates with FUS-positive inclusions and shows a reduction in nuclear staining, suggesting mislocalization to the cytoplasm (14).

The genomic structure and sequence of EWSR1, which is translocated in Ewing’s sarcoma, show similarities to FUS, suggesting that these two genes may have been derived from a common ancestor. Familial forms of neurodegenerative diseases caused by EWSR1 mutations remain to be identified, but two missense variants in EWSR1 in patients with sporadic ALS that are not seen in healthy controls have been reported (15). EWSR1 forms cytoplasmic inclusions in CNS tissue from FTD patients with FUS pathology, suggesting that it may modulate pathological processes in FTD (14).

The ribonucleoproteins hnRNPA1 and hnRNPA2B1 regulate alternative splicing, metabolism, and transport of nuclear pre-mRNA transcripts. Missense mutations in hnRNPA2B1 and hnRNPA1 have been
CHARACTERISTICS OF PROTEINS WITH PrLDs

PrLDs involving self-propagating pathological conformations exhibit two critical features: They are rich in glutamine and asparagine (Q/N) (19, 21), and they contain a glycine/serine-tyrosine-glycine/serine (G/S-Y-G/S) motif (22). PrLDs in ALS-related RNA binding proteins are enriched in asparagine, glutamine, tyrosine, serine, and glycine residues but contain few charged or hydrophobic residues (Fig. 3). The percentage of these five amino acids in the PrLDs and the position of the PrLDs in the proteins are as follows: TDP-43, 65.2% (277 to 414); FUS, 84.8% (1 to 237); hnRNPA1, 84.6% (233 to 272); hnRNPA2B1, 86.83% (266 to 303); EWSR1, 57.5% (1 to 280); TAF15, 80.26% (1 to 152); TIA1, 59.8% (292 to 386) (19, 23). Many stress granule proteins harbor PrLDs that are essential for physiological function and have been conserved across evolution (19). The assembly of cytoplasmic RNA granules is driven by membraneless droplets formed by liquid-liquid phase separation (22), which is mediated by a low complexity sequence in PrLDs. Liquid-liquid phase separation rapidly increases the local concentration of proteins and recruits and enhances their interactions with constituent molecules in stress granules. Under pathological conditions or during aging, this liquid-like state can change into an inappropriate aggregated state that is linked to the pathological inclusions seen in the CNS tissue from ALS and FTD patients. Analyses using circular dichroism and nuclear magnetic resonance spectroscopy have shown that ALS-causing mutations in the PrLD of TDP-43 are predicted to remodel the energy landscape of self-assembly, leading to the formation of amyloid fibrils and irreversible protein aggregation (24). Conceivably, this toxic conversion is accelerated by ALS-linked mutations clustered in the PrLDs of TDP-43, FUS, hnRNPA2B1, or hnRNPA1 or through an increase in protein concentration mediated by cytoplasmic mislocalization or impaired clearance by the protein degradation machinery (25–27).

STRESS GRANULE FORMATION

Stress granules are cellular structures that package mRNA and RNA binding proteins into membraneless organelles during cellular stress and may function as an RNA quality control system, stabilizing and editing mRNA and arresting translation to prevent the accumulation of misfolded proteins (28). The ALS-linked mutations in TDP-43, FUS, hnRNPA2B1, and TIA1 lead to the formation of aberrant stress granules both in vitro and in vivo (18, 23, 29–31). Pathological findings have confirmed that both cytoplasmic TDP-43 and FUS inclusions in ALS and FTD brain tissue are frequently positive for stress granule markers, such as TIA1 and eukaryotic initiation factor 3 (32–34). Recently, induced pluripotent stem cell–derived neurons derived from patients with FTD caused by mutations in the C9orf72 gene also showed evidence of abnormal stress granule formation (35).

In the cytoplasmic RNA binding protein Ataxin2, intermediate-length polyglutamine expansions are associated with an increased risk for ALS (36). Ataxin2 is also a component of stress granules and is reported to affect stress granule formation and to modify the subcellular distribution and toxicity of TDP-43 (36, 37). Mutations inprofilin-1, an actin-binding protein (38), have also been implicated in ALS, and such mutations cause impairments in stress granule formation or clearance (39). Collectively, these findings suggest that aberrant stress granules caused by ALS-associated mutations lead to dysfunction of the RNA.
aggregated β sheet proteins in the cytoplasm, including TDP-43, resulted in dysfunctional nuclear pore complexes and impaired nucleocytoplasmic protein transport, potentially contributing to TDP-43 mislocalization and proteopathy (43).

LOSS OF FUNCTION OR TOXIC GAIN OF FUNCTION?
A major question that remains to be resolved is whether neuronal dysfunction and neurodegeneration in ALS and FTD are caused by a toxic gain of function by mutant RNA binding proteins in cytoplasmic aggregates or by a loss of function of mutant RNA binding proteins that are depleted from the nucleus (Fig. 4). TDP-43–deficient mice or conditional knockout mice are embryonic lethal or die postnatally (44, 45), indicating that TDP-43 is essential for viability. Sporadic ALS is characterized by TDP-43–positive inclusions in the cytoplasm and also by the depletion of intact TDP-43 protein in the nucleus (2, 46). Therefore, one hypothesis concerning ALS pathogenesis is that cytoplasmic inclusions in affected motor neurons act as a sink that removes TDP-43 protein from the nucleus, leading to motor neuron dysfunction and degeneration (2, 46, 47).

The FUS protein is pivotal for DNA repair, RNA splicing, RNA translation, and RNA transport (11), suggesting that the loss of function of FUS could contribute to functional deficits and neuronal loss. FUS knockout or knockdown in the hippocampus of mice results in FTD-like behavioral phenotypes, such as hyperactivity and reduced anxiety-related behaviors (48, 49), but does not result in the ALS phenotype, suggesting that FUS loss of function is possibly associated with the FTD phenotype.

The gain-of-function hypothesis is supported by several pieces of evidence in the FUS-related ALS phenotype. First, Kino et al. (49) and Sharma et al. (50) reported that loss of FUS in motor neurons in mice does not lead to motor deficits. Second, a haploinsufficiency mutation in FUS, which leads to nonsense-mediated mRNA decay, was identified in a family affected by essential tremor who did not have an ALS phenotype (51). Third, transgenic mice expressing exogenous FUS lacking a nuclear localization signal (NLS) (∆NLS-FUS) developed a progressive ALS-like motor phenotype associated with the formation of ubiquitin-positive FUS aggregates in the cytoplasm and neuronal loss but with no changes in the expression and nuclear localization of the endogenous FUS protein. In addition, transcription and the specific RNA splicing regulated by FUS were not affected, indicating that the mislocalization of FUS was sufficient to induce the ALS phenotype (50, 52, 53). Fourth, overexpression of human wild-type FUS in mice causes progressive motor neuron degeneration similar to that seen in ALS patients (54). Fifth,
Fig. 3. Structure of ALS-associated RNA binding proteins. The glycine/serine-tyrosine-glycine/serine (G/S-Y-G/S) motif and the arginine/glycine/glycine repeat (RGG) motif, which are predicted to have prion-like properties, are shown. Thick black line shows the prion-like domain (PrLD) and the amino acid positions that it occupies. Mutations identified in ALS/FTD, distal myopathy, or both diseases are shown by red, blue, and green tick marks, respectively. Seven of the ALS-related RNA binding proteins contain a PrLD, but Matrin 3 does not. RRM, RNA recognition motif; GRD, glycine-rich domain.

MOLECULES CONTRIBUTING TO PROTEIN QUALITY CONTROL

Many ALS-associated mutations have been identified in proteins that regulate the proteasome and autophagy pathways of protein degradation and clearance (Fig. 2). These proteins include Sequestosome-1 (SQSTM1)/p62, optineurin, TANK-binding kinase 1 (TBK1), ubiquilin2 (UBQLN2), adenosine 5’-triphosphate–driven chaperone valosin-containing protein (VCP)/p97, and cyclin F (CCN F). Many of these mutant proteins (SQSTM1/p62, optineurin, UBQLN2, and VCP/p97) are components of the pathogenic inclusion bodies seen in CNS tissue from ALS patients; mutations in these proteins are predicted to impede the protein quality control machinery (58–65).

Mutations in VCP/p97 associated with ALS, FTD, and myopathy (60, 61) contribute to the dysfunction of the ubiquitin-dependent protein quality control system and the clearance of stress granules by the autophagy pathway (66). ALS-associated mutations in VCP can also lead to the accumulation and persistence of stress granules, possibly resulting in the dysfunction of the RNA quality control system due to the aberrant dynamics of stress granules.

Both p62 and optineurin are ubiquitin-binding proteins, and mutations in these molecules are linked to familial ALS (62, 63). Optineurin and p62 are autophagy receptors that recognize ubiquitinated cargo and tether the cargo to the autophagy machinery by directly binding to microtubule-associated protein light chain 3 (67, 68). Cell biological evidence shows that mutant optineurin acts as a dominant-negative trap through the formation of wild-type/mutant optineurin complexes that interfere with autophagy-mediated degradation of TDP-43 (69).

The protein kinase TBK1, in which a haploinsufficiency mutation causes ALS/FTD (64, 65), phosphorylates p62 on Ser403 (70) and optineurin on Ser177 (68), enhancing their binding affinity to microtubule-associated protein light chain 3 and boosting autophagic clearance. Haploinsufficiency of TBK1 has been implicated in decreased clearance of aggregated proteins, leading to proteinopathy and the development of ALS/FTD.

UBQLN2 is a multidomain member of the ubiquitin-like/ubiquitin-associated family of proteins (71) that are involved in the proteasome and autophagy pathways. Mutations in the unique proline-X-X repeat region cause dominantly inherited ALS/FTD that is X-linked (59). Biochemical and cellular analyses have revealed that these ALS-associated mutations impede protein degradation by reducing the delivery of ubiquitinated proteins to the proteasome and autophagy machineries (59, 72–74), presumably leading to neurotoxicity.

Cyclin F is a member of the cyclin family, which constitutes one of the four subunits of the E3 ubiquitin protein ligase complex called SKP1–cullin–F-box and functions in phosphorylation-dependent ubiquitination (75). Mutations in the CCN F gene encoding cyclin F have been identified in a large ALS-FTD kindred. These mutations cause abnormal ubiquitination and accumulation of ubiquitinated substrates.

ALS/FTD ASSOCIATED WITH C9orf72 MUTATIONS

The most common causative gene mutation in the molecular pathogenesis of ALS and FTD is a hexanucleotide repeat expansion in the untranslated region of the gene C9orf72 (77, 78). Patients with ALS/FTD associated with C9orf72 mutations (referred to here as C9ALS/FTD) present with abnormal pathological aggregation of TDP-43. Forced expression of C9orf72 with repeat expansions in the mouse brain leads to abnormal TDP-43 aggregation in the cytoplasm of neurons and motor deficits. The C9orf72 repeat expansion thought to be located upstream in the pathogenic cascade of TDP-43 proteinopathy (79, 80). The protein encoded by the C9orf72 gene is unknown but is predicted to function as a guanine nucleotide exchange factor that activates Rab guanosine triphosphatases (GTPases) in silico, indicating a role for C9orf72 in Rab GTPase–dependent membrane trafficking. Accumulating evidence suggests that C9orf72 colocalizes with Rab proteins and regulates endocytosis, autophagy, and stress granule formation (81–83).

Three pathogenic mechanisms have been proposed for ALS/FTD caused by C9orf72 mutations. The first is the decreased expression (haploinsufficiency) of C9orf72 protein due to the repeat expansion. The second is the abnormal

Including TDP-43, indicating a dominant disruption in protein homeostasis (58).

Controversy exists regarding whether it is a dysfunction of RNA metabolism or the protein control system that is the primary cause of ALS and FTD. Notably, much evidence has shown that optineurin, UBQLN2, and p62 are direct regulators of TDP-43 clearance and degradation (69, 73, 76). Dysfunctional protein quality control due to mutations in the causal genes associated with ALS and FTD is assumed to lead to the accumulation of TDP-43 and other RNA binding proteins harboring a PrLD, resulting in RNA dysregulation. Although further biochemical and cellular studies are necessary to elucidate the detailed molecular mechanisms of ALS and FTD, we propose that these two pathogenic mechanisms involving the protein and RNA quality control systems form a single pathway leading to the development of ALS/FTD (Fig. 2).

**Fig. 4. A potential common pathogenic pathway for ALS/FTD.** ALS/FTD-associated RNA binding proteins have a PrLD, RRM, and NLS. These mutant RNA binding proteins become mislocalized from the nucleus to the cytoplasm due to disruption in nucleocytoplasmic trafficking caused by, for example, C9orf72 repeat expansions, pathological aggregation of proteins, or mutations in the NLS (A). This loss of function mediated by nuclear depletion may be involved in RNA dysregulation that results in neurodegeneration (B). The mislocalized RNA binding proteins assemble as cytoplasmic RNA granules (membraneless organelles formed by liquid-liquid phase separation) mediated by PrLDs (C), and the local concentration of proteins increases. Mutations in PrLDs lead to the formation of toxic protein aggregates and accelerate the formation of pathological inclusions (D). Meanwhile, the protein quality control system contributes by removing aberrant RNA granules and pathological inclusions. However, mutations in regulators of autophagy and the proteasome, as well as proteins containing dipeptide repeats, can disrupt the protein quality control system (E), leading to increased accumulation of RNA granules and pathological inclusions. In a toxic gain of function, the aberrant RNA granules and pathological inclusions (with a core composition of RNA binding proteins) trap RNAs and cause RNA dysregulation (F), leading to neuronal degeneration in ALS/FTD. Pathological protein aggregates may be transmitted from neuron to neuron in a “prion-like” manner (G). Complex interactions among these processes lead to the development of ALS/FTD.
aggregation of intranuclear RNA binding proteins with RNA containing repeat expansions, leading to RNA toxicity. The third is cytotoxicity due to synthesis and aggregation of dipeptide repeat proteins through repeat-associated non-ATG translation from the repeat expansion sequences (84, 85). These dipeptide repeats include those encoded in sense transcripts, such as polyglycine-alanine, polyglycine-proline, and polyglycine-arginine, and in antisense transcripts, such as polyproline-alanine and polyproline-arginine.

Decreased expression of the C9orf72 gene in brain tissue from ALS and FTD patients has been confirmed (77). However, in knockout mice, only macrophage dysfunction, and not motor neuron degeneration, has been observed. Therefore, loss of C9orf72 gene function is currently inconclusive as a cause of ALS/FTD (86, 87). In vitro and in vivo studies have shown that dipeptide repeat protein accumulation causes dysfunctional protein quality control (88–90), suggesting an association with abnormal accumulation of TDP-43. Two recent studies also report that dipeptide repeat proteins sequester nuclear proteins involved in regulation of nucleocytoplasmic transport, leading to neurodegeneration (90, 91). However, critically, the absence of a pathological correlation between dipeptide repeat protein aggregation and neurodegeneration does not support this possibility (92, 93).

Meanwhile, repeat expansion RNAs have been shown to directly inhibit nucleocytoplasmic transport (94, 95). A molecular mechanism in which toxic expanded RNA binds to and disrupts RanGAP, a key regulator of nucleocytoplasmic transport, has been postulated (93). This finding was confirmed by the analysis of C9ALS/FTD patient–derived induced pluripotent stem cells, which showed nucleocytoplasmic transport deficits (94). TDP-43 mislocalization is probably mediated by the disruption of nucleocytoplasmic transport due to repeat expansion RNA toxicity, resulting in a pathological cascade. Whether all toxicity caused by GGGGCC repeat expansions is mediated by TDP-43 proteinopathy, or whether other pathogenic pathways are also involved, requires further investigation.

**PHENOTYPES BEYOND THE ALS/FTD SPECTRUM**

ALS was originally thought to be a disease limited to upper and lower motor neurons (Fig. 1), but because of the identification of TDP-43 and FUS, a new disease spectrum that includes FTD (with dementia as a nonmotor symptom) has been proposed. In other words, ALS/FTD is now considered to be a spectrum disease with a common molecular pathogenic basis involving the dysfunction of the frontal lobe and loss of primary and secondary motor neurons in the spinal cord (Fig. 1). However, recent genomic studies have revealed that ALS-related mutations in protein control–related molecules, including p62, VCP, and optineurin, also play a role in entirely different diseases, such as IBM and PDB. This disease spectrum has now expanded to include multisystem proteinopathy (24).

Mutations in the VCP gene have been implicated in IBM with PDB and FTD (IBMPFD) (61). Recently, VCP mutations (R191Q, L59G, and R155H), which had already been described in families with IBMPFD, were identified in patients with ALS (60). Moreover, mutations in p62, including P387L, P392L, G411S, and G425R, were also reported in patients with PDB, ALS, and FTD (96). A dominant mutation in optineurin has not been identified as a cause of PDB, but a genome-wide association study recently identified variants as genetic risk factors for PDB (97). p62, VCP, and optineurin all encode proteins that bind to ubiquitin, suggesting that the dysfunction of the protein quality control system is part of the pathogenic mechanism underlying PDB and IBM, as well as ALS/FTD.

Meanwhile, a D290V mutation in the RNA binding protein hnRNP A2/B1 has been identified in families showing clustering of IBMPFD, ALS, and FTD (23). This suggests that dysfunction not only of protein quality control but also of RNA quality control is involved in the molecular pathogenesis of PDB and IBM. Thus, a common molecular network may exist in PDB, IBM, and ALS/FTD, although they have very different disease phenotypes.

Mutations in Matrin 3, which is an RNA binding and DNA binding protein that interacts with TDP-43, have been identified in familial ALS (16). Matrin 3 has two RNA recognition motifs (RRM) but does not contain a PrLD (Fig. 3). An S85C mutation in the gene encoding Matrin 3, which also causes distal myopathy with vocal cord and pharyngeal weakness, has been reported (98). Interpretation of this phenotype has been confusing. In muscle biopsies from ALS patients, mutant Matrin 3 leading to the mislocalization of TDP-43 and the formation of perinuclear inclusions with TDP-43 has been observed. Therefore, this gene mutation is thought to be associated with motor neuron disease and myopathy. In contrast, in a German kindred with this same mutation, distal myopathy alone with no motor neuron involvement has been reported, indicating phenotypic diversity between ALS and myopathy (99). Similarily, WDM- and ALS/FTD-associated mutations have been identified in the PrLD of TIA1 (17, 18), suggesting that distal myopathy and ALS/FTD may constitute a disease spectrum with a common molecular pathogenic basis.

On the basis of these findings of ALS-related mutations conferring diverse disease phenotypes, our previous concept of ALS/FTD as a disease limited to motor neuron loss and frontotemporal involvement should be reconsidered. ALS/FTD is a diverse disorder affecting not only the nervous system but also other tissues including muscle and bone.

**SEEDING, AGGREGATION, AND PROPAGATION OF TDP-43**

Abundant molecular and cell biological evidence suggests that the misfolding of specific proteins, such as α-synuclein, β-amyloid, and tau, can result in aberrant proteins that act as seeds for aggregation and that can be transmitted from cell to cell in a self-dissemination pattern similar to that of prions (100). Therefore, prion-like propagation of aggregated proteins may be involved in the common pathogenic mechanism of various neurodegenerative diseases.

TDP-43 harboring a PrLD is predicted to show similar seeding, aggregation, and propagation properties. Clinical and neuropathological ALS-related motor deficits spread contiguously throughout the neuromuscular system (101). Recent neuropathological studies of postmortem brain tissue from ALS patients described a pattern of phospho–TDP-43 immunoreactivity that is in agreement with a systematic spread of TDP-43 pathology (102, 103). Two spread patterns are assumed: horizontal spread, that is, the transmission from cell soma to cell soma, and vertical spread, that is, the propagation from neuron to neuron across axon terminals.

Nonaka et al. (104) demonstrated that insoluble TDP-43 aggregates in postmortem brain tissue from ALS and FTD patients were able to seed intracellular TDP-43 aggregates that were resistant to heat and proteinases in cultured cells. A microfluidic chamber study showed that neurons can transfer TDP-43 aggregates anterogradely from axon terminals to the next cell soma and transfer TDP-43 aggregates to axon terminals, followed by retrograde transport to the soma, indicating that TDP-43 is vertically transmitted across synapses (105).
DEVELOPING A DISEASE-MODIFYING THERAPY

Inclusions of mutant TDP-43 are detected in most cases of ALS (2, 3), and there is evidence for TDP-43 seeding and propagation (104, 105). Several mutations in ALS-related genes are involved in protein quality control and interfere with the degradation of TDP-43 (58, 69, 73). Together, these findings suggest that a single pathway may underlie the pathological cascade of ALS/FTD (Fig. 2). RNA dysregulation mediated by aberrant RNA binding proteins, such as TDP-43 and FUS, forms the core of the cascade, resulting in either a loss of function or a toxic gain of function for the mutant proteins (Fig. 4).

Cross-linking and immunoprecipitation analyses suggest that TDP-43 and FUS may target thousands of RNAs for quality control (9, 10, 106). These RNAs participate in the regulation of about 30% of the murine transcriptome; therefore, therapeutic targeting of each of these downstream RNAs is unrealistic (9, 10, 106). More reasonable molecular targets for the development of therapeutics are the aggregated RNA binding proteins themselves.

In an Alzheimer’s disease clinical trial, the key pathological feature of amyloid deposits was eliminated by passive immunotherapy using antibodies against amyloid-β peptide (107). More recently, immunotherapy has been reported to be effective not only against extracellular deposits of β-amyloid but also against intracellular aggregates of mutant tau and α-synuclein proteins (108, 109).

If a toxic gain of function due to aggregated RNA binding proteins is assumed to play a major role in disease pathogenesis, immunotherapy against ALS-related mutant RNA binding proteins may be a promising therapeutic strategy. Moreover, given that there is prion-like propagation of extracellular TDP-43 aggregates, elimination of the spread of TDP-43 aggregates may slow disease progression.

Studies in rodents and cell culture models have indicated that therapeutic antibodies are taken up by neurons and can clear intracellular protein aggregates (110, 111). Knockout of TDP-43 or FUS in mice is lethal, and TDP-43 loss of function is possibly involved in disease pathogenesis; thus, complete removal of TDP-43 or FUS would need to be selective. Selective conformation-specific antibodies directed against toxic aggregate species would need to be developed.

Because assessment of drug efficacy based on clinical symptoms of ALS/FTD would take too long, objective quantitative biomarkers of disease progression are essential. Molecular markers need to be developed that can be readily measured in cerebrospinal fluid or using positron emission tomography imaging of aggregated proteins in human patients.

Finally, multiple genes that do not seem to be associated directly with the RNA and protein quality control systems have also been identified as causative in ALS. Mutations in a second gene causing familial ALS, the SOD1 gene, lead to its aggregation in motor neurons, resulting in neurodegeneration, but without an RNA binding proteinopathy. A pathogenic mutation (R199W) in the enzyme D-amino acid oxidase that degrades D-serine cosegregates with disease in familial ALS (112). In the spinal cord, elevated D-serine, which is an activator/coagonist of the N-methyl-D-aspartate glutamate receptor, causes neuronal apoptosis and is thought to contribute to disease progression in ALS (113). Other causative molecular entities identified in familial ALS, the endoplasmic reticulum–resident chaperone protein SIGMAR1 and the vesicle-associated protein VAPB, may play a role in protein quality control in the endoplasmic reticulum (113, 114). Therefore, multiple pathways in addition to those in Fig. 2 must be involved in ALS pathogenesis. Further studies should investigate other pathogenic mechanisms to enable the development of an effective disease-modifying therapy.

In Charcot’s lecture “Leçons sur les maladies du système nerveux” (1) in 1880, he stated that “The prognosis of ALS at the present time is very gloomy. There is not, to my knowledge, any patient in whom all of the symptoms I have mentioned have resolved. Is there any end in sight for this condition? Only the future will tell.” Our understanding of ALS has rapidly increased over the past 10 years because of the breakthrough identification of TDP-43 as an ALS-associated gene. Now, 137 years after Charcot’s lectures on this disease, the identification of new therapeutic targets is pinpointing opportunities for developing new strategies for treating ALS/FTD.

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Sci Transl Med 9, eaah5436.
DOI: 10.1126/scitransmed.aah5436

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