Mini-Symposium

Phenotypic Suppression of ALS/FTD-Associated Neurodegeneration Highlights Mechanisms of Dysfunction

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A fundamental question regarding the etiology of amyotrophic lateral sclerosis (ALS) is whether the various gene mutations associated with the disease converge on a single molecular pathway or act through multiple pathways to trigger neurodegeneration. Notably, several of the genes and cellular processes implicated in ALS have also been linked to frontotemporal dementia (FTD), suggesting these two diseases share common origins with varied clinical presentations. Scientists are rapidly identifying ALS/FTD suppressors that act on conserved pathways from invertebrates to vertebrates to alleviate degeneration. The elucidation of such genetic modifiers provides insight into the molecular pathways underlying this rapidly progressing neurodegenerative disease, while also revealing new targets for therapeutic development.

Key words: **ALS; FTD; genetic modifiers; RBPs; stress granules; disease models**

Introduction

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) have long been considered distinct neurodegenerative diseases whose precise pathogenesis remains elusive. Each is associated with a loss in specific neuronal populations. The loss of neurons in the frontal and/or temporal lobe of the brain in FTD produces changes in personality, language, and memory; whereas in ALS, functional disruptions in both upper and lower motor neurons first manifest as muscle weakness, with a rapid, progressive, and complete loss of voluntary movement in the arms and legs, followed by reduced respiratory muscle function and failure, usually 2–5 years after diagnosis. Despite these differences in clinical presentation, there is much genetic, clinical, and pathological overlap between ALS and FTD, suggesting that they may be a single disease with a range of phenotypic manifestations. For example, ~50% of people with ALS exhibit cognitive and/or behavioral deficiencies, and 15%–20% reach a diagnosis of FTD (Marin et al., 2017; Nguyen et al., 2018). To date, no treatments for FTD exist, and the few ALS therapies provide only a minimal extension in lifespan of a few months. There is an urgent need to develop new therapeutics that slow the loss of motor function and prevent further neurodegeneration. To achieve such goals, we need a better understanding of what causes ALS and what cellular pathways are compromised in diseased individuals. Treatments could then target the causative factor and/or the resulting dysfunctional cellular pathway(s) that precede motor neuron death.

ALS does not arise from a single insult or one specific genetic mutation. Approximately 5%–10% of patients suffer from familial ALS (fALS), which is caused by a mutation in one of >25 different genes that act in a variety of cellular processes. A handful of these genes (*C9ORF72*, *SOD1*, *TARDBP*, *FUS*, *PFN1*) harbor the majority of fALS mutations. Genetic perturbations, such as the expansion of a hexanucleotide repeat in the *C9Orf72* locus, result in abnormal RNA production and/or the accumulation of dipeptide repeat protein

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products (DPRs) resulting from RAN translation. ALS mutations can also disrupt superoxide dismutase (*SOD1*) activity, the normal function of RNA splicing factors (*TARDBP* and *FUS*) or of a cytoskeletal regulator (*PFN1*). The challenge before us is to determine how mutations in these genes all result in the same disease phenotype. Do they converge on a single downstream molecular pathway whose disruption triggers the neurodegeneration typical of ALS and FTD? Or do they affect distinct pathways, any one of which independently leads to the common disease phenotype?

Animal models provide a means to study aspects of complex diseases using tractable, well-characterized systems. In particular, invertebrates, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, allow rapid, large-scale genetic and pharmacological studies (Sin et al., 2014; Fernández-Hernández et al., 2016; Deal and Yamamoto, 2018; Ma et al., 2018). Not only do >40% of the *C. elegans* genome (7569 genes) and 75% of the *Drosophila* genome (15,500 genes) have human orthologs (Pandey and Nichols, 2011; Kim et al., 2018), these model organisms share the same fundamental cellular processes governing neuronal function and survival. The functional conservation of the motor circuit between *Drosophila* and mammals further emphasizes their value in the rapid identification of disease-relevant pathways and genetic modifiers, which can then be studied in rodent models and in human-derived-induced pluripotent stem cell (iPSC) models of ALS/FTD. The identification of gene variants that suppress neurodegeneration in animal models of ALS can provide critical molecular insights into the mechanistic basis of ALS/FTD. Are there phenotypic suppressors that alleviate degeneration across all cases of ALS and FTD, regardless of cause? If so, such suppressors are valuable in the identification of targets for developing new, more effective therapeutics. This review addresses efforts to identify suppressors using different experimental approaches and different genetic models of ALS/FTD, through the examination of diverse cellular mechanisms ranging from stress response to local protein synthesis, uniting around the concept of identifying factors that modify the disease phenotype. In each case, we explore specific issues related to our overarching questions: does ALS/FTD arise from one common defect, or from a defect in any one of many cellular processes, each of which has the ability to orchestrate degeneration of motor neurons, and/or cortical neurons?

Diverse cellular pathways contribute to TDP-43 proteinopathy in *C. elegans* models of ALS/FTD

Mutations in *TARDBP*, encoding the DNA/RNA binding TDP-43 protein, cause <1% of ALS cases, but ~95% of ALS and ~50% of FTD (FTLD-TDP) cases exhibit cytoplasmic or nuclear aggregates containing the protein TDP-43 (Mackenzie and Neumann, 2016; Prasad et al., 2019). Because TDP-43 dysfunction can cause disease, understanding the biology driving this process is of critical importance. TDP-43 proteinopathies, such as ALS and FTLD-TDP, have been modeled by expressing human TDP-43 in neurons of *C. elegans*. Expressing WT hTDP-43 causes relatively mild behavioral or functional phenotypes, including uncoordinated locomotion, modest progressive neurodegeneration, shortened lifespan, and accumulation of pathologically truncated, ubiquitinated, phosphorylated, and insoluble TDP-43; expression of mutant hTDP-43 causes more severe presentation of these phenotypes (Ash et al., 2010; Liachko et al., 2010; Zhang et al., 2011; Vaccaro et al., 2012). The increased toxicity of mutant TDP-43 is also observed in vertebrate models, indicating that *C. elegans* can be used to investigate conserved mechanisms of TDP-43 biology (Tan et al., 2017).

TDP-43 is a DNA- and RNA-binding protein and has diverse roles regulating transcription, pre-mRNA splicing, micro-RNA processing, mRNA stability and transport, and stress granule (SG) assembly (Prasad et al., 2019). In human disease, aggregated TDP-43 exhibits post-translational modifications, including ubiquitination, SUMO-ylation, phosphorylation, and truncated protein species. These modifications are consistently observed across ALS/FTLD spanning multiple genetic causes and are typical of the majority of sporadic cases of disease. Phosphorylation of TDP-43 decreases TDP-43 protein turnover, promotes mislocalization from the nucleus to the cytoplasm, and increases protein aggregation. *C. elegans* models accumulate prominent phosphorylated TDP-43, which increases with age and correlates with neuronal loss and functional impairment (Liachko et al., 2010). Studies in both *C. elegans* and *in vitro* systems have identified conserved TDP-43 kinases and phosphatases responsible for this post-translational modification of TDP-43 (Hasegawa et al., 2008; Liachko et al., 2013, 2014, 2016; Li et al., 2017; Gu et al., 2018). These include the kinases CK1, CDC7, TTBK1, and TTBK2, which directly phosphorylate TDP-43 at serines 409 and 410 and promote its relocalization from the nucleus to cytoplasm, where aggregates are most commonly found. Notably, these kinases colocalize with TDP-43-positive inclusions in cortical and spinal cord tissue from ALS and FTLD-TDP patients, whereas the expression of TTBK1/2 protein is elevated in FTLD-TDP patients (Liachko et al., 2013, 2014; Taylor et al., 2018). TDP-43 is also phosphorylated at threonines 153 and 155 by MEK, a kinase in the MAPK/ERK signaling family, although it is unclear whether these epitopes are involved in disease (Li et al., 2017). The importance of tightly regulating TDP-43 phosphorylation has also been suggested by studies showing that genetic loss of calcineurin, a phosphatase that removes the pathological C-terminal phosphates on TDP-43, increases motor dysfunction, and accelerates neurodegeneration in *C. elegans*-expressing hTDP-43, and pharmacological inhibition or siRNA-mediated reduction in calcineurin expression promotes accumulation of phosphorylated endogenous TDP-43 in human cultured cells (Liachko et al., 2016). Phosphorylation of TDP-43 may be a consequence of disruptions in cellular homeostasis; therefore, intervening to decrease or eliminate TDP-43 phosphorylation, either by inhibiting TDP-43-targeted kinases or activating TDP-43 phosphatases, is an attractive strategy for treating TDP-43 proteinopathies, such as ALS and FTLD-TDP.

A recent genomewide RNA interference (RNAi) screen has been conducted using *C. elegans*-expressing human fALS-linked mutant TDP-43 pan-neuronally. The screen surveyed 16,767 genes, covering >86% of the *C. elegans* genome (Kamath et al., 2003). TDP-43-induced uncoordinated locomotion was suppressed by 46 RNAi targets, 24 of which have human homologs. These genes have known functions in diverse areas of cell biology, including energy production, cellular metabolism, extracellular matrix, cytoskeleton, ion transport, proteostasis, and signaling, highlighting the complexity of pathways contributing to TDP-43 proteinopathy. One suppressor identified *hse-5*, a heparan sulfate epimerase that catalyzes the inversion stereochemistry of D-glucuronic acid to L-iduronic acid. Elucidating the mechanism underlying *hse-5*-mediated suppression, as well as that of other identified suppressors, is future important work.

Disruption of RNA processing: a common denominator in ALS and FTD

The groundbreaking discovery of TDP-43 as a component of ubiquitinated inclusions in neurons of patients with sporadic

ALS and FTD (Arai et al., 2006; Neumann et al., 2006) was the first evidence pointing to a role for altered RNA processing in these disorders (Lagier-Tourenne et al., 2010). Indeed, TDP-43 is mislocalized in the vast majority of ALS patients and ~45% of patients with FTD. This finding was rapidly followed by the identification of mutations and/or the mislocalization of several RNA-binding proteins (RBPs) in ALS and FTD, including Fused in sarcoma (FUS) (Kwiatkowski et al., 2009; Vance et al., 2009), Ewing's sarcoma (EWSR1) (Couthouis et al., 2012), TATA-binding protein-associated factor 15 (TAF-15) (Couthouis et al., 2011), heterogeneous ribonucleoparticle proteins A2B1 and A1 (hnRNPA2B1 and hnRNPA1) (Kim et al., 2013), Matrin 3 (MATR3) (Johnson et al., 2014), and the T cell-restricted intracellular antigen-1 (TIA1) (Mackenzie et al., 2017). These RBPs have been implicated in several steps of RNA metabolism, including RNA splicing, transport, stability, or translation, and their disruption leads to widespread defects in RNA processing (Nussbacher et al., 2019).

Genomewide approaches have provided major insights into the multiple ways these RBPs influence the processing of their RNA targets, with hundreds of expression or splicing alterations identified in ALS/FTD models and patient tissues (Nussbacher et al., 2015). Importantly, several splicing defects consistent with a loss of TDP-43 function were identified in brain tissues of ALS/FTD patients (Polymenidou et al., 2011; Prudencio et al., 2012; Shiga et al., 2012; Ling et al., 2015), likely as a result of TDP-43 relocating from the nucleus to the cytoplasm. Indeed, a striking nuclear clearance of TDP-43 is widely observed in both sporadic and familial cases, including in patients with expansions in the *C9orf72* gene. Notably, TDP-43 pathology, rather that DPR aggregates, correlates with neurodegeneration in postmortem tissues from C9orf72 patients (Chew et al., 2015, 2019; Vatsavayai et al., 2016; Solomon et al., 2018). Considering the widespread disruption of RNA processing associated with TDP-43 pathology, a major challenge is to determine the relative contribution to disease of TDP-43 versus DPR aggregates.

The human RNA encoding Stathmin-2, a neuronal microtubule-associated protein also known as SCG10, was recently shown to be the most affected transcript upon TDP-43 loss (Klim et al., 2019; Melamed et al., 2019). TDP-43 disruption induces aberrant splicing and premature polyadenylation within the first intron of *STMN2*, producing a nonfunctional truncated mRNA. Altered *STMN2* expression was found in TDP-43-mutant or -depleted neurons directly converted from fibroblasts and iPSC-derived motor neurons, as well as in motor cortex and spinal motor neurons from sporadic and C9orf72 ALS/FTD patients. Stathmin-2 is one of four mammalian stathmins known to directly bind dimers of α/β Tubulin and to affect microtubule dynamics (Belmont and Mitchison, 1996; Chauvin and Sobel, 2015). Stathmin-2 has been suggested as an axonal-maintenance factor (Shiga et al., 2012) and as an essential component for axonal regeneration (Mason et al., 2002). Upon axonal injury, Stathmin-2 is upregulated and recruited to growth cones of regenerating axons (Shin et al., 2014) with a demonstrated requirement for neuromuscular junction stability in *Drosophila* (Graf et al., 2011). Antisense oligonucleotides targeting either *STMN2* or *TARDBP* transcripts were recently shown to preclude regeneration following axotomy of iPSC-derived human motor axons (Melamed et al., 2019). Remarkably, although reduction in TDP-43 is associated with hundreds of RNA alterations, restoration of Stathmin-2 levels was sufficient to rescue axonal regenerative capacity in motor neurons lacking TDP-43.

The potential consequences of other TDP-43-mediated RNA defects and their contribution to disease remain to be demonstrated, and further investigations are required to better understand the role of Stathmin-2 in axonal biology and renervation/denervation of synapses, especially neuromuscular junctions. Nevertheless, the weight of evidence supports rescue of Stathmin-2 levels as a potential therapeutic approach in neurodegenerative diseases affected by TDP-43 proteinopathy.

ALS/FTD mutations in the RNA-binding protein FUS suppress local axonal protein synthesis

The gene encoding another RBP, FUS, like *TARDBP* (TDP-43), is the site of ALS/FTD-causing mutations (Kwiatkowski et al., 2009; Vance et al., 2009). RBPs have been shown to play key roles in highly polarized neurons, including the selective transport of mRNAs along axons and their localization at synapses allowing for local synthesis of proteins (Donlin-Asp et al., 2017). A wealth of data has led to a consensus that protein synthesis occurs in mature dendrites and growth cones and that this synthesis is critical for memory, learning, and synaptic plasticity (Biever et al., 2019). Whereas there is ample evidence that invertebrate axons can synthesize proteins (Piper and Holt, 2004; Twiss and Fainzilber, 2009) and that local translation is required for axonal growth during development and repair following injury, there is no consensus local translation contributes to neuronal homeostasis and degeneration in mature mammalian axons. In mammals, some axons, including those of motor neurons, can extend >1 m with an estimated volume of up to 160 million μm^3 (>5000 times the volume of typical cell). This poses a challenge for protein homeostasis in distal synaptic structures, such as at neuromuscular junctions, whose loss is one of the earliest events observed in ALS patients.

It has recently been shown that ALS/FTD *FUS* mutations suppress local axonal protein synthesis through activation of an integrated stress response in mouse hippocampi, which in turn leads to impaired neuronal activity and synaptic deficits (López-Erauskin et al., 2018). Using humanized mice in which ALS/FTD-causing *FUS* mutations provoke age-dependent progressive motor and cognitive deficits, the inhibition of local translation in sciatic nerves was observed before development of disease and was accompanied by increased axonal accumulation of mutant FUS. This work provided *in vivo* evidence for local intra-axonal translation in adult mice and uncovered a role of FUS in local protein synthesis, extending its cellular functions beyond its known roles in RNA processing, microRNA biogenesis, and mRNA transport (Butti and Patten, 2018). It also raises the possibility that, like deficits in translation observed in C9orf72 and TDP-43-associated ALS/FTD (Kanekura et al., 2016; Cestra et al., 2017; Balendra and Isaacs, 2018; Lehmkuhl and Zarnescu, 2018), impaired local translation may contribute to, or even cause, the age-dependent neurodegeneration associated with mutations in *FUS*. Future efforts will build on these findings to define the local "*translatome*" (mRNAs that are actively translated) and proteome (newly synthesized proteins) in healthy and diseased motor axons. Together, these efforts have the potential to further our understanding of how mature axons and their synapses with muscles are lost in ALS.

Targeting stress response pathways and interactions within RNA granules to mitigate persistent protein aggregation

A common feature of ALS and FTD, as well as many other neurodegenerative diseases, is the presence of abnormal protein aggregates in neurons in the brain and spinal cord. Environmental

stresses can contribute to the formation of aggregates, and multiple forms of stress have been implicated in neurodegenerative disease pathogenesis, including oxidative stress, ER stress, and inflammation (Sprenkle et al., 2017). Most of these stresses activate the integrated stress response pathway leading to phosphorylation of the α subunit of the Eukaryotic initiation factor 2 and subsequent translational repression (Bosco, 2018). In cultured cells, stress-induced translational repression often coincides with the formation dynamic cytoplasmic foci called stress granules (SGs) (Kedersha and Anderson, 2002). SGs are membraneless organelles that contain hundreds of RBPs, components of the translation machinery, and repressed mRNA transcripts (Souquere et al., 2009; Sidrauski et al., 2015; Jain et al., 2016; Khong et al., 2017). The biological role of SGs under conditions of stress has not been fully elucidated (Protter and Parker, 2016); but within the last 10 years, the hypothesis that SGs are pathogenic in the context of neurodegenerative disease has emerged. This hypothesis is supported by observations that disease-linked variants associate with SGs in cell culture and frequently appear to alter their physical properties, including size, abundance, and/or dynamics. These effects have been demonstrated for the ALS/FTD-associated RBPs TDP-43, FUS, and hnRNPA2B1 (Liu-Yesucevitz et al., 2010; McDonald et al., 2011; Baron et al., 2013; Kim et al., 2013; Sama et al., 2014; Gomes and Shorter, 2019), and other ALS/FTD-associated proteins, including SOD1 (Gal et al., 2016), UBQLN2 (Alexander et al., 2018), MAPT (Vanderweyde et al., 2016), and DPRs encoded by the *C9orf72* nucleotide repeat expansion (Boeynaems et al., 2017). Moreover, SG-related proteins colocalize within pathological aggregates from postmortem CNS tissues from human ALS and FTD patients (Dormann et al., 2010; Liu-Yesucevitz et al., 2010; Alexander et al., 2018), supporting the idea that SGs could serve as precursors of inclusions found in neurodegenerative disease (Aulas and Vande Velde, 2015).

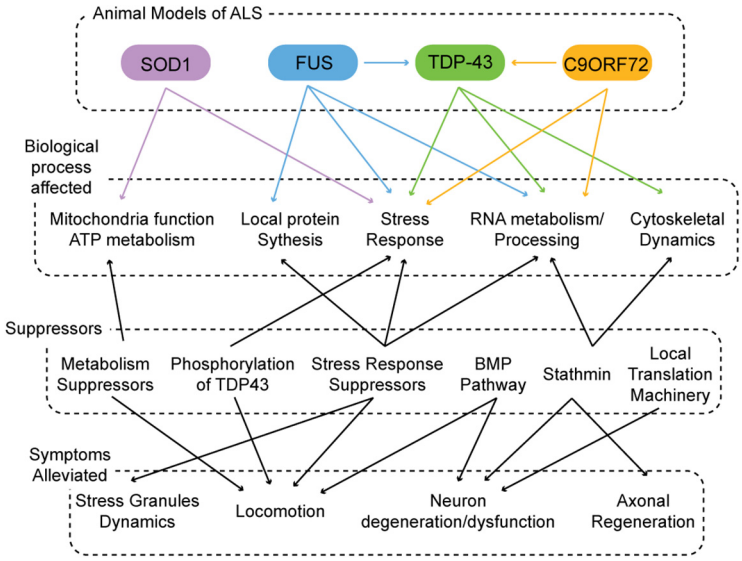

Figure 1. Suppressors of dysfunctional cellular pathways in ALS/FTD identify putative targets for therapeutic development. The etiology of ALS/FTD is complex, involving a number of independent cellular processes that lead to varied degenerative symptoms. Nevertheless, a single suppressor can reduce multiple symptoms, an encouraging outlook for therapeutic target identification by genetic modifier screens.

In recent years, aberrant SG behavior has been proposed to integrate genetic and environmental factors to facilitate the formation of permanent insoluble inclusions. Both SG formation and permanent aggregation depend on liquid–liquid phase separation, a cellular process by which some proteins form highly concentrated liquid-like droplets that are demixed from the aqueous cellular milieu. Of particular relevance to ALS/FTD, mutations in *TARDBP*, *FUS*, and *HNRNPA2/B1*, as well as expansion of the hexanucleotide repeat in the *C9orf72* locus, interfere with liquid–liquid phase separation behavior and impact either the formation or disassembly of SGs (Murakami et al., 2015; Patel et al., 2015; Boeynaems et al., 2016; Lee et al., 2016; Martinez et al., 2016; Mackenzie et al., 2017; Markmiller et al., 2018). Importantly, therapeutic reduction of the SG-associated proteins Ataxin-2 and TIA1 components was recently shown to provide neuroprotective effects in animal models of ALS (Becker et al., 2017; Apicco et al., 2018).

Two important considerations remain. First, the molecular interactions governing the behavior of these granules is still incompletely understood, and a more complete understanding of how SG composition and behavior are affected by genetic, environmental, and aging-related factors is needed. Second, *in vivo* mammalian models of stress and disease are needed to rigorously test the hypothesis that SGs play a role in human neurodegenerative disease pathogenesis. To address the first of these questions, robust cellular models and APEX-mediated proximity labeling with high-content imaging was used to analyze the protein composition of SGs under different conditions to identify candidate targets that regulate SG biology and stress-induced protein aggregation (Markmiller et al., 2018). Reducing the levels of some candidate targets rescued degenerative phenotypes in three different *Drosophila* models of neurodegeneration (Markmiller et al., 2018). Additional work in ALS patient-derived iPSC-derived motor neurons showed that transient exposure to stress leads to persistent accumulation of insoluble TDP-43 and other ALS-associated RBPs, as well as long-lasting perturbations of global mRNA localization and increased neuronal cell death. A small-molecule screen by the same group identified a class of compounds that can prevent the RNA-dependent recruitment of TDP-43 to persistent aggregates and increases survival in transiently stressed ALS mutant iPSC-derived motor neurons (Fang et al., 2019). Last, experiments designed to systematically dissect the role of post-translational protein ubiquitylation in regulating RNA granules and protein aggregation came to the surprising conclusion that unconjugated ubiquitin, but not polyubiquitin, accumulates in SGs and that active polyubiquitylation is not necessary for SG dynamics (Markmiller et al., 2019).

To date, the majority of research on SGs has been performed in cell culture, and there is clear evidence that forced oligomerization of SG proteins is toxic to cells (Zhang et al., 2019). There are multiple factors to consider as we translate our knowledge of SGs from cell culture to *in vivo* models. One factor is the type of stress that can induce SG formation *in vivo*. Generally, nonphysiological stressors, such as sodium arsenite, sorbitol, and protein overexpression, are used to induce SG assembly in cultured cells (Sama et al., 2014). Whether granules formed by protein overexpression are *bona fide* SGs is unclear, as these granules exhibit different dynamic properties compared with SGs formed in re-

sponse to acute stress. Furthermore, disease-relevant stressors, such as glutamate-induced excitotoxicity, did not induce SG assembly in neurons; thus, not all types of stress necessarily result in SG formation (Tischbein et al., 2019). A recent study in *Drosophila* provided novel proof of concept that physical trauma can serve as an acute form of stress that triggers SG assembly *in vivo* (Anderson et al., 2018). Unpublished work from the D.A.B. laboratory supports the hypothesis that head trauma can also induce SG formation in mammals. Whether trauma-induced granules *in vivo* can precipitate larger pathological inclusions remains to be determined.

If SGs are found to promote disease pathology and/or worsen neurodegenerative disease outcomes *in vivo*, SGs may be appropriate therapeutic targets for these disorders. Small molecules, such as ISRIB, which prevent SG formation in culture, could be considered for suppressing SG-related phenotypes *in vivo* (Sidrauski et al., 2015). However, some cell-culture studies suggest that SGs protect cells from the toxic behaviors of proteins, such as FUS (Shelkovnikova et al., 2013) and TDP-43 (McGurk et al., 2018), and that these proteins exert toxicity independently of their association with SGs (Gasset-Rosa et al., 2019; Mann et al., 2019). Therefore, it will be important to carefully examine SGs *in vivo* under various conditions of acute and chronic stress, as well as disease models, to elucidate whether these species play a role in neurodegenerative disease pathogenesis.

Genetic suppressors of multiple models of ALS identify common dysregulated pathways

A preponderance of data shows that a wide range of molecular and cellular processes are affected by ALS-associated mutations. Studies on both patient populations and animal models have revealed that specific second-site gene perturbations can modify ALS phenotypes (Yanagi et al., 2019). Despite the fact that 40–50 such gene perturbations have been identified that modify more than one ALS gene, few have been further tested systematically across additional ALS/FTD models. A recent genomewide screen in *Drosophila* by the Artavanis–Tsakonas group identified specific genetic lesions that could modify both TDP-43 and FUS-associated degeneration, with many tested against a *C9orf72* G_4C_2 expansion model. The K.A.W. laboratory tested a subset of these genetic modifiers in a fourth ALS model, a knock-in of the SOD1-G85R lesion into the endogenous locus ($dSod1^{G85R}$) (Sahin et al., 2017), for suppression of degenerative phenotypes. More than 30 genes were identified that could modify all four ALS models. The affected genes fall into different functional categories, including energy metabolism, stress response, cell polarity, cytoskeletal regulators, and chromatin regulation. It has been shown that motor circuit dysfunction associated with $dSod1^{G85R}$ can be suppressed through the activation of BMP signaling in nonmotor neurons or in motor neurons (Held et al., 2019). ALS models where cell type-specific suppression can be assessed will provide important information about the cells in which modulation of ALS-associated phenotypes is possible by particular genetic modifiers.

The majority of genetic models of ALS/FTD involve the overexpression of a mutant human gene or the homologous host gene harboring a patient allele. More recently, the potential impact of gene dosage or levels of mutant protein is being considered as it may alter stoichiometries and molecular interactions in affected pathways. The use of a knock-in model has the advantage of providing a platform to identify and study suppressors of neurodegeneration in a genetic and homeostatic context that is closer to that seen in patients, as opposed to that in most overexpression models. While an exhaustive comparison of suppressors of knock-in versus overexpression models of ALS has not been done, it is of considerable interest that a significant number of gene modifiers were identified that suppress degeneration associated with both types of models. Interestingly, further genetic analysis of two modifiers whose gene products are known to physically interact (one affecting stress response and the other metabolism) indicates that they exhibit a genetic interaction that modifies their ability to rescue neurodegeneration phenotypes caused by some, but not all, ALS models tested. Moreover, the ability of the two modifier genes to rescue neurodegeneration-related phenotypes is inhibited by their functional interdependence in the case of one ALS model, whereas in the other these modifiers appear to act independently. Together, these results indicate that not all ALS models respond in the same way to modifiers.

In conclusion, the advances presented here provide valuable insights into biological processes involved in onset and/or progression of ALS/FTD (Fig. 1). They show that ALS/FTD is accompanied by a variety of different cellular dysfunctions and that the repair of just one dysfunction may be sufficient to restore function. Such studies will continue to shed light on molecules that underlie disease mechanism and provide targets for therapeutic intervention in ALS/FTD. As we move forward, considering the interactions between modifiers and components of identified pathways will inform us of the cellular basis of disease, as well as the effectiveness and mode of action of newly developed therapeutics.

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