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Autophagy meets FUS-positive Stress Granules

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Abstract

Mutations in fused in sarcoma/translocated in liposarcoma (FUS/TLS or FUS) are linked to familial cases of amyotrophic lateral sclerosis (ALS). Mutant FUS selectively accumulates into discrete cytosolic structures known as stress granules under various stress conditions. In addition, mutant FUS expression can alter the dynamics and morphology of stress granules. Although the link between mutant-FUS and stress granules is well established, the mechanisms modulating stress granule formation and disassembly in the context of ALS are poorly understood. In this issue of *Neurobiology of Aging*, Ryu and coworkers uncover the impact of autophagy on the potential toxicity of mutant-FUS positive stress granules. The authors provide evidence indicating that enhanced autophagy activity reduces the number of stress granules, which in the case of cells containing mutant-FUS positive stress granules, is neuroprotective. Overall, this study identifies an intersection between the proteostasis network and alterations in RNA metabolism in ALS through the dynamic assembly and disassembly of stress granules.

Keywords

ALS; FUS; Stress Granules; Autophagy

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by motor neuron loss, muscle weakness and paralysis (Bosco and Landers, 2010). The presence of protein inclusions containing specific misfolded proteins is a histopathological hallmark of the disease and signs of proteostasis alterations are extensively described in ALS human tissue and mouse models of the disease (Matus, et al., 2013). In recent years, fused in sarcoma/translocated in liposarcoma (FUS/TLS or FUS) and Tar DNA Binding Protein 43

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(TDP-43), two RNA metabolism-associated proteins, were identified as major components within intracellular protein inclusions in ALS autopsy tissue, thereby opening new perspectives in the field (Mackenzie, et al., 2010). In this issue, Ryu *et al.* explored the consequences of ALS-associated mutations in FUS within the context of cellular stress response, namely the impact of this ALS-linked protein on stress granule assembly and disassembly. Stress granules (SGs) are dynamic, mRNA/protein foci that form in response to acute stressors, such as oxidative stress, endoplasmic reticulum (ER)-stress and heat shock, but that disassemble once the stress is mitigated. The exact functions of stress granules are not completely understood, but these foci are thought to regulate protein expression during stress in an attempt to re-establish cellular homeostasis (Kedersha, et al., 2013). A recent screen in yeast uncovered macroautophagy (here refereed to as autophagy) as a key pathway governing stress granule disassembly (Buchan, et al., 2013), but the role of this pathway in stress granule assembly and disassembly within neurons, the affected cell-type in ALS, had not yet been investigated. The current study confirmed that the autophagy pathway is a regulatory mechanism that modulates SG formation and clearance in mammalian neurons. Importantly, autophagy appears to provide a protective effect in neurons with mutant FUS-positive stress granules. Most disease-related protein aggregates are targeted to autophagy, a catabolic route that degrades cellular components through the lysosomal pathway. These findings build on the beneficial consequences of targeting autophagy in neurodegenerative disease that have been demonstrated as a strategy for removing neurotoxic misfolded protein species (Nixon, 2013).

Protein misfolding and impairment of the proteostasis network translate into distinct perturbations to motoneuron physiology in ALS, impacting synapse function, axonal transport, redox control, proteasome function, among other cellular processes (Pasinelli and Brown, 2006). One of the salient features of ALS is the occurrence of protein folding stress at the endoplasmic reticulum (ER), which is observed as one of the earliest alterations in mouse models of the disease, prior to motor neuron denervation (Saxena, et al., 2009). ER stress triggers adaptive reactions, including the unfolded protein response (UPR) and activation of the autophagy pathway, both of which reduce the unfolded protein load help to reestablish cell proteostasis (Hetz and Mollereau, 2014). Interestingly, in the context of ALS, ER stress can also induce the formation of SGs that contain mutant TDP-43 (Walker, et al., 2013) and mutant FUS (Bosco, et al., 2010). SG markers have been detected within pathological inclusions formed by these proteins in human disease, leading to the hypothesis that SGs may in fact represent precursors to protein-aggregates detected at the end-stage of disease (Wolozin, 2012).

FUS and TDP-43 are DNA- and RNA-binding proteins that participate in a wide range of nuclear and cytoplasmic processes including transcription, mRNA splicing, microRNA processing, among other functions (Lagier-Tourenne, et al., 2010). These proteins are predominantly expressed in the nucleus, and continuously shuttle between the nucleus and the cytosol (Ayala, et al., 2008, Dormann, et al., 2012, Dormann, et al., 2010, Winton, et al., 2008). Mutations in the genes encoding these proteins induce drastic changes to their subcellular distribution, leading to their accumulation in the form of insoluble aggregates in the cytosol (Sreedharan and Brown, 2013). This abnormal accumulation is proposed to

contribute to ALS through a loss-of-function mechanism, in addition to a gain-of-neurotoxic activity triggered in the cytosol (Ling, et al., 2013). Here, Ryu *et al.* studied the distribution of wild-type and ALS-linked mutant FUS into SGs under conditions of oxidative stress and validated previous findings indicating an active recruitment of mutant FUS into these RNA/protein foci (Sama, et al., 2014). In addition to these ALS-associated proteins, SGs contain translation initiation factors, small ribosome subunits, RNA-binding proteins and repressed mRNA (Buchan and Parker, 2009). Under stress conditions that generally induce SG formation, global translation is reduced, thereby conserving energy. SGs are thought to function in the triage of repressed mRNAs, allowing for the focused translation of those proteins necessary to overcome stress (Kedersha and Anderson, 2002).

Mutant TDP-43 has been reported to accumulate into SG in various ALS models (Colombrita, et al., 2009, Liu-Yesucevitz, et al., 2014, Yasuda, et al., 2013). The induction of ER stress by mutant TDP-43 was recently reported to drive its accumulation into SG, suggesting a mechanistic connection between the UPR and the proteostasis network (Walker, et al., 2013). Mutant FUS also triggers ER stress (Farg, et al., 2012) and cytosolic inclusions have been reported in close association with the ER and fragmented Golgi (Farg, et al., 2013). Recent findings have shown that the expression of ALS-linked mutant forms of FUS (i.e. FUS^{R495X} and FUS^{R521G}) abnormally incorporate into SGs formed under conditions of oxidative stress, ER-stress and heat shock *in vitro* and in zebrafish models, whereas wild-type FUS is largely excluded from these structures under the same conditions (Bosco, et al., 2010). Hyperosmolar stress is the only stimuli reported to drive endogenous, wild-type FUS into SGs, suggesting that FUS may also play a physiological role in stress response under certain conditions (Sama, et al., 2014, Sama, et al., 2013). In the context of ALS, mutant-FUS proteins were shown to interfere with the dynamics of SG assembly/disassembly and to alter SG morphology, suggesting that the association of mutant FUS with stress granules represents a toxic event that impairs stress response (Baron, et al., 2013). Remarkably, similar observations were recently reported in cells expressing ALS-linked forms of TDP-43 and Profilin-1 (Figley, et al., 2014, Liu-Yesucevitz, et al., 2014), suggesting that alterations in the biogenesis and/or morphogenesis of SGs may operate as a common factor driving the pathogenesis of different ALS genes. In addition, another recent study suggested that TDP-43 mutations might alter the bidirectional trafficking of RNA granules along microtubules in neurons (Alami, et al., 2014).

The study by Ryu *et al.* provides an interesting link between autophagy and the generation of SG containing FUS proteins. First, the authors confirmed that expression of ALS-linked FUS^{R521C} impairs the disassembly of SG under oxidative stress conditions. They also observed that FUS-positive SGs colocalize with the classical autophagy marker LC3. Moreover, they show that autophagy manipulation can directly impact SG formation and clearance. Stimulation with rapamycin, an autophagy activator, reduced the number of SGs, including those that were FUS^{R521C} positive. Importantly, upregulation of autophagy attenuated the neurotoxic consequences of mutant FUS expression under oxidative stress conditions that induced SG assembly in neurons. Specifically, neurite fragmentation and cell death were ameliorated in these cells. Together, this data supports the notion that

accumulation of mutant FUS into SG may be detrimental to motoneurons (Wolozin, 2012), and that the autophagy pathway can be manipulated to buffer this process.

These findings further our understanding of the connection between two key stress pathways, autophagy and SG assembly, at the intersection between protein misfolding and mRNA metabolism alterations in ALS. This study opens a new perspective in the field, providing an integrative view of the protein and mRNA homeostatic network that converges onto the generation of SGs. On one hand, the authors' findings support a gain of toxic function for mutant FUS, involving the sequestration of mutant FUS into SGs in a manner that could alter SG biology, and also links this phenomena with a well established pathway involved in the degradation of protein aggregates in ALS. The consequences of mutant FUS retention in SGs under stress conditions remains to be determined. Since autophagy has been explored as a therapeutic target for ALS in certain models with successful results (Castillo, et al., 2013, Wang, et al., 2012, Hetz et al., 2009), the reduction of SG formation may also be assessed as a possible driver of neuroprotection in those studies. Interestingly, recent drug screenings have identified small molecules that can reduce the accumulation of TDP-43 cytosolic aggregates, which may offer new avenues for therapeutic intervention (Boyd, et al., 2014, Burkhardt, et al., 2013). Moreover, the PERK/eIF2 α axis of the UPR has been associated with SG formation, and inhibition of a kinase involved in ER-stress, PERK, was shown to have a neuroprotective effect in a *Drosophila* model of TDP-43 proteinopathy (Kim, et al., 2014). In summary, SGs are emerging as a relevant interface between RNA and protein homeostasis in neurodegenerative diseases offering novel targets for therapeutic intervention.

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Figure 1. The biology of stress granules in ALS

Mutations in FUS and TDP-43 in ALS triggers the mislocalization of the protein from the nucleus to the cytosol. In stress conditions, including ER and oxidative stress, protein translation is reduced and stress granules are formed. FUS and TDP-43 are found within cytosolic protein inclusions called stress granules, an observation that may be relevant to the neurotoxic mechanisms triggered by ALS-linked mutations in these proteins. In addition, mutant FUS and TDP-43 alter the dynamics of stress granule formation and disassembly and overall SG morphology. Activation of autophagy may have neuroprotective roles in ALS by inducing the clearance of stress granules that contain aberrant ALS-linked proteins.