Abstract

The increase in average human life expectancy has concurrently resulted in a higher incidence of neurodegenerative diseases like amyotrophic lateral sclerosis (ALS). ALS is the most common adult-onset motor neuron disease which eventually leads to death 3-5 years after diagnosis. It is a highly heterogeneous disease, characterized by selective degeneration of motor neurons in the motor cortex, brainstem, and spinal cord. While most ALS cases are sporadic (i.e. sALS), 5- 10% of ALS patients have a family history (i.e. fALS). However, the molecular mechanisms underlying ALS remain elusive. The most common genetic cause of fALS are expanded hexanucleotide (GGGGCC) repeats in the first intron of *C9ORF72*. Other frequent mutations related to ALS are found in the ubiquitously expressed RNA-binding proteins (RBPs) TDP-43 and FUS. These mutations result in the formation of insoluble cytosolic inclusions and cytosolic mislocalization of the nuclear proteins TDP-43 and FUS. These inclusions contain characteristic components of stress granules (SGs), membraneless ribonucleoprotein organelles that form upon exogenous stress by liquid-liquid phase separation (LLPS). SGs play a critical role in the metabolism and translation of mRNA. Dysregulation of the assembly and disassembly of these highly dynamic organelles has been implicated in several neurodegenerative diseases, including ALS.

In this study, we characterise changes in the SG proteome of SGs isolated from induced pluripotent stem cells (iPSCs) and iPSC-derived motor neurons harboring ALS-related mutations in TDP-43, FUS and *C9ORF72* (i.e. C9-ALS). We found that EXOC1, a component of the exocyst complex, was less abundant in SGs isolated from iPSC-derived motor neurons harboring ALS mutations TDP-43$^{WT/M337V}$, FUS$^{P525L/P525L}$ and expanded hexanucelotide repeats in *C9ORF72* (~6000-8000 bp repeat expansion). Interestingly, blocking the exocytosis pathway or the exocyst complex in control cell lines mimicked the disturbed SG dynamics observed in mutant cell lines. Furthermore, knockdown of EXOC1 in control cell lines, resulted in perturbed SG recovery. Our results suggest that exocytosis serves as a pathway for SG clearance that is dysfunctional in motor neurons harboring different ALS-related mutations. Therefore, dysfunctional exocytosis of SGs may be a disease-related mechanism linking different types of ALS.