MND AUSTRALIA INTERNATIONAL RESEARCH UPDATE

DECEMBER 2018

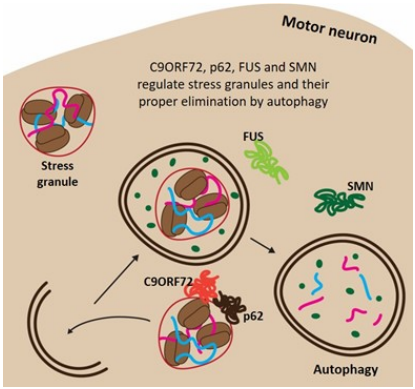
Isabella Lambert-Smith, Research Associate, Macquarie University MND Research Centre

A brilliant year of motor neurone disease research progression

The end of an industrious year in the motor neurone disease (MND) research world heralds a promising 2019 for research progress. The past few months have yielded welcome advances in our understanding of the links between different MND-linked genes and the mechanisms they facilitate that cause MND. These new findings will receive focused attention by the hard-working MND researchers around the world in the new year. Here's to an exciting start to 2019!

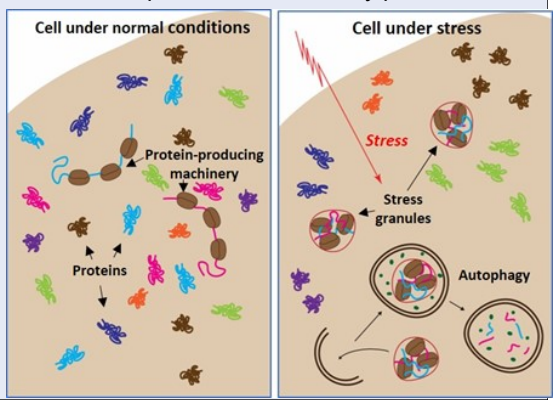
Convergence of MND-linked genes in regulating stress responses

Mutations in several proteins genetically linked to MND cause the abnormal formation of stress granules (SGs) in motor neurones (MNs) (see box below). This is linked with MNs having major problems eliminating the SGs by autophagy (see box). Maneka Chitiprolu and her colleagues in Ontario, Canada, investigated how this happens in MND caused by genetic abnormalities in the *C9ORF72* gene, the most prevalent inherited form of MND. Maneka firstly demonstrated that the protein encoded by *C9ORF72* associates with p62, a protein that monitors the cell's proteins and carries any that are old and damaged to the autophagy machinery for removal. P62 is also genetically linked with MND. When bound together, C9ORF72 and p62 can regulate the removal of SGs by autophagy. But then the picture gets fascinatingly complicated. The C9ORF72-p62 partnership requires two other MND-linked proteins to mediate the final steps of SG removal; FUS and SMN. These findings break new ground in demonstrating that four proteins genetically linked to MND (C9ORF72, p62, FUS, SMN) are needed by cells to regulate SGs and their elimination by autophagy. In the MNs of MND patients, insoluble clumps of aggregated proteins are always detected. These clumps, called inclusions, contain proteins such as FUS and p62 that are involved in SG degradation. Some studies have shown that abnormally persistent SGs can seed the formation of inclusions. Genetic abnormalities that disrupt SG degradation may thus be one of the key factors that lead to MND.



How do cells cope with stress?

Our cells have evolved countless strategies to continue functioning even when exposed to different stressors. The multitude of different proteins populating each cell have various jobs that they carry out to keep the cell functioning. But when faced with stress, the whole cell rapidly responds, almost as though it has a repertoire of well-choreographed dances for every possible situation. This involves altering the numbers and activity of each type of protein, depending on what is the protein's job. When damaged or under stress, cells can stop producing proteins that aren't necessary for the cell to survive. Messenger molecules that carry the information to make proteins get captured into structures called stress granules (SGs), where they're kept for the duration of the stress. This allows the cell to use its resources to eliminate the stress rather than produce unnecessary proteins. SGs usually get eliminated from cells through a process called autophagy. Autophagy, meaning "self-devouring" in ancient Greek, involves the cell pinching off parts of its contents and degrading them with special enzymes. Autophagy is not only essential for breaking down SGs but is also a major route of elimination of old and damaged proteins and organelles (the cell's 'organs').



MND Research Shorts

- *Researchers in Korea have identified that genetic activation of the parkin protein rescues fruit flies modelling MND caused by abnormalities in the TAF15 gene. Interestingly, genetic defects in parkin are one of the most common known causes of Parkinson's disease. In TAF15-MND flies, increased levels of parkin reduced the amount of aggregated TAF15 clumps and was able to improve the health of the flies.*
- *Genetic defects in C9ORF72 account for a major proportion of inherited MND cases. They cause some of the machinery inside MNs to carry out a very unusual process in which tiny, toxic 'dipeptide repeat' proteins (DPRs) are produced. Researchers collaborating across the USA and France have discovered exactly how the cellular machinery produces these DPRs, opening an opportunity to figure out how to therapeutically target this process.*
- *MND is a particularly complex disease so developing effective treatments first requires researchers to understand the causal cellular mechanisms underlying the vulnerability of MNs. Researchers in the USA have identified many of the interactions that occur between proteins linked with MND and the remaining thousands of proteins and organelles populating each MN. Some of these unusual interactions may prove to be novel therapeutic targets.*
- *Amongst the web of toxic mechanisms that lead to MN death is an abnormal interaction between mutant SOD1 protein and the Derlin-1 protein. Derlin-1 normally resides in a membranous organelle, the ER, but in MND the interaction between SOD1 and Derlin-1 triggers MN death. A drug screen study carried out by researchers in Japan identified several drugs that inhibited this aberrant interaction and* alleviated *MND symptoms in MND models. Further studies are needed to test if targeting this interaction with drugs is feasible and effective in humans.*

mnd Australia

Enhancing removal of toxic MND-linked proteins to improve motor neurone health

In diseases like MND in which insoluble clumps of aggregated proteins form in the affected cells, strategies to accelerate the removal of the toxic proteins might be beneficial. Yan-Ming Wei and Bo Han in Shanxi, China, investigated a protein that is essential for regulating one of the cell's major systems for breaking down old and damaged proteins, autophagy (see page 1 box). They wanted to investigate this protein, called Beclin-1, in neurones modelling MND caused by a defect in the *SOD1* gene. In MND patients carrying this *SOD1* mutation, the mutant SOD1 protein forms into insoluble clumps of protein.

When the researchers increased the levels of Beclin-1 in neurones, the amount of insoluble, aggregated SOD1 was reduced. When they increased the levels of Beclin-1 but also inhibited the autophagy protein degradation pathway, it diminished the effect of Beclin-1, showing that autophagy was essential for this beneficial reduction in SOD1 levels.

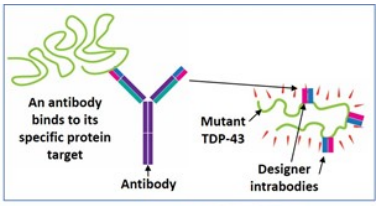
These results excitingly show that enhancing autophagy by increasing the levels of the key autophagy regulator, Beclin-1, may present a possible therapeutic strategy for MND by reducing the amount of aggregated SOD1.

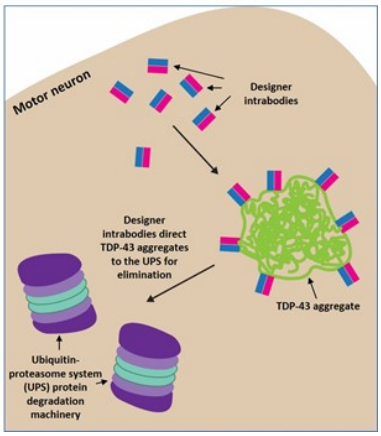
Recruiting chaperone proteins alleviates the accumulation of toxic TDP-43 fragments in motor neurones and muscle cells

Most MND cases are characterised by the aggregation of the TDP-43 protein in affected cells, suggesting that clearance of TDP-43 aggregates could be a potential therapeutic strategy. These aggregates contain fragments of TDP-43 of two particular sizes, termed TDP-35 and TDP-25. Maria Elena Cicardi and her team of collaborators across Italy performed a comparative study between MNs and muscle cells to evaluate if these two cell types accumulate and clear different forms of TDP-43 in diverse ways. The TDP-43 fragments clumped up to a considerably greater extent in MNs than in muscle cells. In both cell types, all the TDP-43 fragments were cleared up by a 'garbage disposal' (protein degradation) system called the ubiquitin-proteasome system (UPS), but the TDP-25 fragment impaired autophagy, the other major route for protein degradation. Maria tried routing TDP-25 to the UPS or to the autophagy pathway by increasing the levels of 2 chaperone proteins that escort targeted proteins to these degradation pathways. These chaperones, called BAG1 and HSPB8, decreased the accumulation of the TDP-43 fragments in both cell types, demonstrating that promoting the chaperone -assisted clearance of MND-linked proteins is beneficial in motor neurones.

Designer proteins eliminate MND-linked clumps of TDP-43 in motor neurones

Maria Elena Cicardi's team aren't the only researchers investigating how to clear up TDP-43 aggregates in MNs. Yoshitaka Tamaki, and fellow researchers collaborating across Japan, designed and generated a small protein that recognises and binds to a section of TDP-43's structure to investigate if it could detect and eliminate TDP-43 aggregates. This small protein, called an intrabody, was derived from a special kind of protein called an antibody.





Antibodies are Y-shaped proteins our immune systems produce and use to neutralise bacteria, viruses and other foreign invaders. They specifically recognise proteins on the surface of the foreign invaders, and every different protein our immune system gets exposed to causes the production of its own unique antibody. Scientists have invented a way to exploit this specificity of antibodies, and now are able to generate antibodies that will bind to any protein they want to study in cellular and animal disease models. Yoshitaka's team, however, wanted the intrabody they generated against TDP-43 to be able to recognise mutant TDP-43 and direct it to the UPS for elimination. Their intrabody successfully bound to mutant TDP-43 and directed it to the UPS, clearing it away, with a subsequent improvement in the health of the MND model cells In which they tested it. These results suggest that a mutant-specific intrabody that carries a signal for the UPS is a promising strategy for mitigation of TDP-43 abnormality and toxicity in MND.

A new model of TDP-43-linked MND reveals key mechanisms occurring early in disease

Mouse models of MND caused by genetic defects in TDP-43 are usually generated by causing the MNs in mice to produce human mutant TDP-43 in addition to the normal production of their own TDP-43. They do this by injecting small DNA molecules containing the human TDP-43 gene into the mice' MNs, from which their cellular machinery then generates the protein. This means the levels of TDP-43 in mice are excessively high, which some researchers worry could cause toxicity in the mice that is unrelated to the specific disease mechanisms mutant TDP-43 causes in humans. To sidestep the potentially confusing effects of excessive TDP-43 levels, David Gordon and his colleagues in Oxford, UK, generated MND model mice in which they integrated the human TDP-43 gene into the genome of the mice rather than the usual injection of small DNA molecules containing TDP-43 into their MNs. This resulted in the mice' MNs generating TDP-43 at low, stable levels that led to slowly progressive weakness and reduced survival, more accurately simulating the disease course in humans. They then carried out studies using these mice, and found that there was abnormal assembly of stress granules (SGs; see box on page 1). This work provides evidence that MND may arise through defective SG function that causes toxicity in MNs of those carrying genetic defects in TDP-43. Importantly, the slow, more accurate disease progression that these mice model can facilitate the study of early pathways underlying MND.

MND Research Institute of Australia - the research arm of MND Australia
PO Box 430 North Sydney NSW 2059
Ph: 61 2 8287 4989 email: research@mndaustralia.org.au website: www.mndresearch.org.au

The *MND Australia International Research Update* is generously supported by MND Victoria