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MND AUSTRALIA INTERNATIONAL RESEARCH UPDATE

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Fresh perspectives on protein management

2018 has begun with researchers making leaps in the identification of key molecules and mechanisms which underlie the damage that occurs in motor neurones (MNs) in motor neurone disease (MND). In particular, there has been notable progress in our understanding of the importance of cellular protein regulation in preventing MND. Read on to learn about the latest discoveries.

Protein clumps and busted molecular machines in motor neurones

A well-known feature of neurodegenerative diseases is the presence of clumps of proteins in the nerves and brains of patients, sometimes referred to as plaques and inclusions. These clumps of proteins take different forms depending on the disease. In MND, the range of protein inclusions found in patients' MNs is exceptionally diverse and varies with the genetic background of individuals. The most common genetic cause of MND is a defect in the *C9ORF72* gene, which results in the production of five different types of abnormal toxic proteins called dipeptide repeat proteins (DPRs). One of these, Poly-GA, is the most commonly found DPR in inclusions from MND patient brains. Qiang Guo and his colleagues in Germany and the USA looked more closely at these Poly-GA inclusions using a state-of-the-art technique called cryoelectron tomography. This involved flash-freezing MNs and using a beam of electrically-charged atoms to carve out slices of each MN containing inclusions. The researchers built up 3D images of the microscopic inclusions and saw the Poly-GA molecules were present as flat, twisted "ribbons" tangled up with huge numbers of tiny tubes. Qiang's group were amazed to identify these tiny tubes as proteasomes. Proteasomes are involved in protein degradation (see box below) and this finding confirms previous reports demonstrating an involvement of proteasomes in MND. In fact, Qiang's group measured a 30-fold higher concentration of proteasomes in the Poly-GA inclusions compared to other parts of the cell, which is an astounding build-up. What was also remarkable was that a significant proportion of the proteasomes were active, with their protein targets trapped inside them in the process of being chopped up. It seems the MNs were trying to break down and remove the Poly-GA inclusions by recruiting a massive population of the cells' proteasomes, which subsequently get overwhelmed by the sheer mass of inclusions. The consequences of proteasome impairment on the cell are extensive. It can lead to a catastrophic collapse of the cell's ability to regulate protein recycling, resulting in irreversible damage and cell death. Qiang's ground-breaking research provides much-needed information on the link between protein inclusions and protein degradation in MND, and will guide further studies into how this can be targeted therapeutically

Recycling systems in the cell

Our cells are populated by tens of thousands of proteins. Proteins are the molecular workhorses of cells, and each different type of protein has a specific role to play in the cell's life. Proteins are usually dissolved in the gel-like substance that fills cells. However, when damaged they can become insoluble and lose their ability to function. Moreover, they can develop into disease-associated clumps, known as inclusions. Cells have mechanisms in place to monitor the functionality of their proteins and regulate protein turnover. When proteins reach the end of their functional life in the cell, or become damaged, they get directed to either of two recycling systems; the ubiquitin-proteasome system (UPS) or the autophagy pathway. In both systems, the target proteins get broken down into their building blocks, which are then recycled to make new proteins. Protein recycling is essential for cell health.

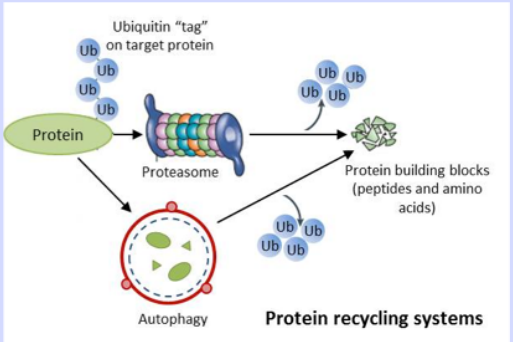

Image adapted from Lagier-Tourenne, Polymenidou and Cleveland. Hum. Mol. Genet. 2010; 19: R46-R64; Ling, Polymenidou and Cleveland. Neuron 2013; 79: 416-438; Wilhelm et al. Science 2014;344:1023-1028.

MND research shorts

The MND-associated proteins FUS and TDP-43 have roles in both the nucleus (where DNA is stored) and in the main cell compartment (the cytoplasm). One of the critical roles of FUS inside the nucleus is helping repair DNA damage. A study carried out by researchers in Germany has demonstrated that defects in FUS impair signalling pathways that respond to DNA damage (DDR signalling). It also caused FUS to migrate in excess to the cytoplasm and form inclusions, as well as MN death. As impaired DDR signalling was key in this toxic pathway, researchers hope targeting DDR signalling may lead to novel therapeutic strategies for MND.

One of FUS's normal roles in the cytoplasm is regulating tiny structures called stress granules (SGs) that help cells cope with stress. When defective, however, it is believed FUS's interaction with SGs changes and it forms disease-associated inclusions. Researchers in Germany, Italy and the US, have discovered that inducing autophagy (see box below) in MND models prevents this SG-inclusion transformation and increases survival. Furthermore, they identified a number of anti-depressants and anti-psychotics already on the market that also induce autophagy. These drugs could potentially be repurposed for MND treatment.

The largest genetic studies of MND have been carried out on people of European ancestry. These studies have advanced our understanding of MND, however, it is likely there are MND-causing genetic defects of ancient origin that are shared across ethnic groups. To address this, a huge collaborative project was carried out by scientists in Australia, China, the Netherlands, UK and USA. They explored the genomes of thousands of individuals with MND across different ethnicities, and found significant association of two novel genes with MND. These genes, *GPX3* and *TNIP1*, interact with other known MND genes. Further investigation of their roles in MND will now need to be carried out.

Star-shaped cells called astrocytes in the brain and spinal cord usually support MNs. However, in MND these cells become reactive and cause inflammation. A study by US researchers has revealed that a family of proteins, BH3-only proteins, contribute to this reactive, inflammatory behaviour, providing a potential new therapeutic target for MND.

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A new helper in the protein recycling system

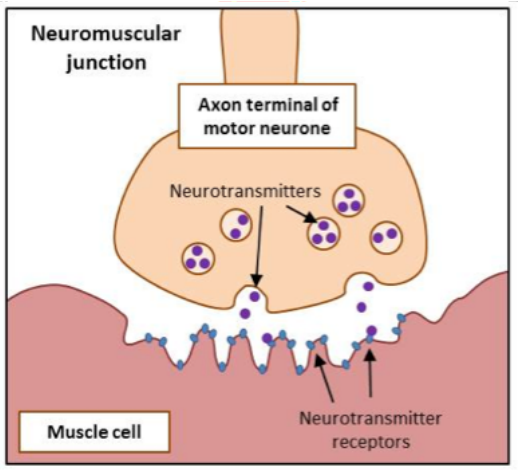
While the proteasome functions at the end of the UPS by chopping up and recycling unwanted proteins, the rest of the work is carried out by tiny molecules of ubiquitin and hundreds of off-siders that help coordinate ubiquitin's function. The proteins that accumulate in inclusions in MNs in MND are tagged by chains of ubiquitin molecules. This ubiquitin "signature" is one of the hallmarks of MND and several other neurodegenerative diseases. The function of ubiquitin molecules is to attach to unwanted and damaged proteins to lead them to the proteasome for degradation. As Qiang Guo's work on Poly-GA inclusions highlighted, it seems that MNs are unable to properly degrade aberrant disease-associated proteins, leading to the accumulation of ubiquitin-tagged protein inclusions. Hiroyuki Uechi and a team of researchers in Sendai and Tokyo, Japan, set out to understand how ubiquitin's off-siders actually organise and process proteins targeted for degradation. They carried out a study to identify other regulators of the UPS, and discovered a new protein called CG5445. The results suggest CG5445 prevents the build-up of ubiquitin-tagged disease proteins by keeping disease proteins soluble (see box). Not only that, CG5445 can promote the clearance of disease proteins. In fact, when the levels of CG5445 were increased in MNs, there was a reduction in the toxicity caused by the MND disease protein TDP-43. Hiroyuki's work demonstrates CG5445 may be involved in a previously unknown mechanism that helps cells hold disease proteins in a soluble state and reduce their toxicity until they are able to be degraded by the proteasome, preventing the build-up of inclusions in MNs.

Calming down overactive molecules helps motor neurones manage their energy

In addition to the MND-causing mechanisms explored so far in this report, failure of the MN's energy production system and inability of the cells to detoxify free radicals contribute significantly to MN degeneration. Tiny molecular machines called mitochondria are typically referred to as the "powerhouses" of the cell. Researchers are beginning to better understand the mechanisms mitochondria use to regulate energy production and the cell's antioxidant system, and work out ways they can be targeted therapeutically. Armed with the knowledge that mitochondria are frequently observed to be fragmented and dysfunctional in diseased MNs, Amit Joshi and his fellow researchers at Stanford University in the US studied a molecular interaction believed to be responsible for looking after mitochondria. They found the interaction between the proteins Drp1 and Fis1 was getting out of hand in diseased MNs and causing mitochondria to be broken apart. Amit tested the effects of using a chemical compound that inhibits this Drp1/Fis1 interaction and found it resulted in a marked reduction of toxic free radicals and improvements in the structure and function of mitochondria. When they treated MND mice with this compound, their motor skills were improved and their lifespans were extended, suggesting targeting Drp1/Fis1 could be effective with other therapeutics in MND treatment strategies.

Disorganisation and miscommunication between motor neurones and muscles

Neuromuscular junctions (NMJs) are specialised connections between MNs and muscles. At each NMJ, the MN releases a chemical called a neurotransmitter into the small space that separates the MN from its muscle cell. After travelling across this space, the neurotransmitter binds to a receptor protein that sits on the outside of the muscle cell. The most abundant neurotransmitter in the nervous system is called glutamate. After the signal has been relayed, the neurotransmitter must be cleared from the space, otherwise the receiving muscle cell will be overstimulated, resulting in toxicity and irreversible damage (a mechanism called excitotoxicity). A few years ago, researchers in Italy reported that TDP-43 is needed at the NMJs to organise and look after the receptors. It appeared that defects in TDP-43's function could cause paralysis due to a loss of NMJs. Giulia Romano in this group wanted to identify the responsible molecules, and carried out a series of experiments to determine if there were any changes in the levels of different proteins between MND model fruit flies carrying the normal TDP-43 protein and those carrying a defective form. Interestingly, flies carrying the defective TDP-43 form had lower levels of an enzyme called glutamic acid decarboxylase (Gad1) and increased levels of the neurotransmitter, glutamate. Gad1 metabolises glutamate and thus is important for clearing it after the electrochemical signal has been relayed from the MN to the muscle cell. Genetically restoring Gad1 in neurones was enough to recuperate the flies' ability to move normally, and restored the structure of their NMJs and glutamate levels. The flies with defective TDP-43 also recovered when they were treated with chemical compounds that inhibit glutamate receptors. This shows that Gad1 promotes NMJ organisation and prevents excitotoxicity by limiting excessive accumulation of glutamate, opening the way to a possible therapeutic strategy.



The unique vulnerability of motor neurones

Multiple mechanisms cause MND and differ between people depending on their genetic background. Even defects within the same gene can cause MN degeneration through more than one mechanism. This is certainly the case with genetic defects in *C9ORF72*. On the previous page, we discovered that Qiang Guo's research revealed a critical involvement of proteasome impairment in C9ORF72-associated MND. An international team of researchers have discovered another disease-causing effect of C9ORF72 defects. Led by Bhuvaneish Selvaraj, they measured the activity of genes in patient-derived MNs and showed that the *C9ORF72* defects led to increased levels of a glutamate receptor called AMPAR. With more AMPARs on the surface of muscle cells, the muscle cells are able to bind to more glutamate molecules and become overstimulated, resulting in excitotoxicity. Bhuvaneish's team also found that this effect was unique to MNs and absent from other types of nerve cells. Along with Giulia Romano's findings, this discovery highlights the prevalence of excitotoxicity as one of the underlying mechanisms causing MN-specific degeneration in MND.

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