MND Australia
International Research Update

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New perspectives on motor neurone disease (MND)

After decades of intense research, we have discovered much about MND and are ever closer to figuring out how to effectively treat it. In recent months, several research groups around the world have thought outside the square of our current ideas of MND and the approaches we take to study it.

In this report, we will explore how their novel approaches have shed light on several surprising aspects of MND, ranging from the involvement of virus DNA in the development of disease to innovative MND models, and promising therapeutic strategies.

SOD1's greed for copper

The most common model that researchers use for studying MND is based on mice that carry a genetic mutation in the *SOD1* gene. However, even after 20 years of testing, no effective therapeutic strategy has been discovered. This is of course an extremely frustrating situation, but Jared Williams and collaborators in the USA, Uruguay and Australia, decided to approach this problem from a different angle. They began exploring the form of MND with the most rapid disease progression ever observed; this form involves increased levels of a very important protein, the copper chaperone for SOD1 (CCS). Mice with this form of MND (SOD1xCCS mice) die eight times faster than mice without increased CCS. Paradoxically, CCS is needed for the normal functioning of SOD1 as it inserts a copper ion into the SOD1 protein, completing the maturation of SOD1. Believing that this apparent paradox was due to SOD1 being rather greedy for copper and causing copper depletion in the spinal cord, Jared's group treated SOD1xCCS mice with CuATSM, a drug that is known to deliver copper into the spinal cord within minutes. Amazingly, CuATSM treatment extended the life of these mice by an average of 18 months. When treatment was stopped, the mice developed MND-related symptoms and died within three months, but restoration of treatment could rescue these mice even after they developed symptoms. As all MND patients also have increased levels of CCS, there is great potential for SOD1-MND patients to respond to CuATSM treatment as positively as SOD1xCCS mice.

The importance of metals in the human body

Metal ions, particularly those of iron, magnesium and zinc, have been important factors in the normal functioning of all kinds of cells and organisms for billions of years. Ions are forms of elements and compounds that have an electric charge, and when we talk about metal ions in the context of the human body we often refer to them as minerals. Many of the proteins and enzymes in our cells would not function without the addition of specific minerals, as is the case with SOD1, which requires both copper and zinc to help it carry out its work in cells.

The most abundant mineral in the human body is calcium, which may come as no surprise as we grow up being told how important it is in our diet. Our body needs calcium to maintain strong bones as well as for normal muscle movement, the functioning of hormones and enzymes, and for nerves to carry messages between the brain and every part of our body.

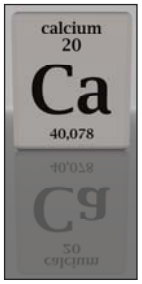
MND Research Shorts

- *The first approved drug for multiple sclerosis, Fingolimod, has been found to protect motor neurones (MNs) in a mouse MND model. Researchers in Rome, Italy, discovered that it protected MNs from inflammatory proteins and extended the life of mice already exhibiting symptoms. Fingolimod is yet to undergo clinical trials, but this study highlights its potential as a therapeutic for MND.*
- *MND involves not only MNs, but muscle and surrounding support cells too. With this in mind, another group in Rome developed a tool for studying MND in which they generated muscle tissue by reprogramming skin cells from patients. This functional muscle tissue can be grown in the laboratory along with MNs and other support cells to accurately recreate the neuromuscular environment in the body, and then be used to screen for drugs that target MN-muscle interactions in MND.*
- *Researchers in Pennsylvania have discovered that treatment with statins, which are used to manage cardiovascular disease, can worsen disease progression in people with MND.*
- *Different groups of MNs in the body do not degenerate simultaneously in MND. A study in Pennsylvania has shown that accumulations of TDP-43 deposits, found in the brain and spinal cord of 90% of MND patients, lead to degeneration of MNs only in the spinal cord and those that innervate the tongue. This demonstrates that despite TDP-43 being present in every cell of the body, only these specific MNs were affected. Knowing the specific MNs that are intrinsically vulnerable in MND will allow the development of therapies that directly target them.*

Bubbling up with excitement

The role of MNs is to communicate between the brain and all the muscles throughout the body, allowing us to control the way our body moves. MNs signal to other MNs by releasing a chemical, glutamate (Glu), into the small space (the synapse) between the MNs, which then excites and activates the neighbouring MN. However, there is strong evidence showing this excitatory transmission is abnormal in MND, based on high levels of Glu in the synapses, and that this increased excitation eventually kills MNs. At the end of each MN (MN terminal), Glu is carried from the packaging factory of the cell (the Golgi complex) to the cell surface in structures that can be thought of as bubbles. This bubble-like process is called exocytosis, and it occurs at a much higher level in MND than normal, both at late stages of disease and even before symptoms develop. It is thus potentially a very important mechanism in the development of MND. Tiziana Bonifacino and a team of researchers in Milan and Genoa in Italy sought to understand why and how this occurs. Using a SOD1 MND mouse model and examining the mice before symptoms developed, they found that the collections of Glu-carrying bubbles at MN terminals were increased, and that this was caused by a number of molecular changes. There were increased levels of calcium in the MN terminals and of complexes of proteins (SNARE complexes), and inhibition of a protein called GSK-3, all of which promote the release of Glu from the bubble-like structures into the synapses. So in order to correct the toxic over-excitation of MNs in MND, these molecular changes may need to be targeted early in the disease process through novel pharmacological approaches.

Calcium imbalance in motor neurones activates a toxic chain of events



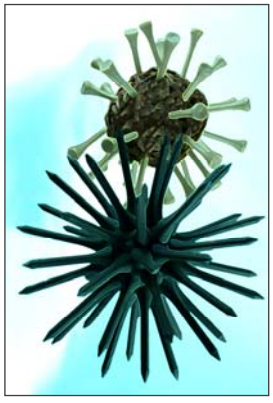
A mutation in the *C9orf72* gene accounts for a considerable portion (40%) of familial MND cases. Understanding how this mutation causes MND, and how this can be resolved, will thus make a difference in the lives of many in the MND community. It is currently believed that an imbalance of calcium inside MNs plays an important role in the disease process of MND. However a direct link between this imbalance and the *C9orf72* mutation has not yet been investigated. Choosing to address this, Ruxandra Dafinca and collaborators in Oxford, UK and Ibarai, Japan, developed a model to study C9orf72-MND in which they used MNs derived from the skin cells of patients with the mutation. The ability to generate MNs and diverse other cell types from adult skin cells is a recently developed technology that allows researchers to model and study disease in a more true-to-life way than ever before. With their model, Ruxandra's group discovered that a serious calcium balance did exist in the diseased MNs, and this activated a pathway in cells that led to programmed cell death. Programmed cell death is a normal mechanism used by cells that are damaged beyond repair, but MNs are a type of cell that are unable to regenerate themselves, and thus when they die they are not replaced. This activated pathway involved calcium imbalance specifically in the endoplasmic reticulum, which is the cell's centre for manufacturing proteins and fats and packaging them for redistribution around the cell. With this discovery, the next step will be to identify where in the cell this toxic chain is initiated so that we can figure out how to target it.

Sprouting like a tree: new protein promotes recovery of diseased motor neurones

Degeneration of MNs in MND involves a loss of the connections between the MNs and muscle cells (the neuromuscular junctions, NMJs) in a dying-back process. In the normal functioning of NMJs, a protein called neuregulin-1 (Nrg-1) is essential, and another protein that interacts with it, ErbB4, has been found to be mutated in some people with MND. Thus Renzo Mancuso and his colleagues in Spain, the UK and USA, conducted a study to examine the role of Nrg-1 in the functioning of NMJs in a SOD1-MND mouse model. Assessing the effect of high levels of Nrg-1, they found it promoted increased sprouting of the branch-like axons that extend from MNs. This sprouting of axons is just like a tree sprouting new branches, and it is a natural mechanism used by MNs to compensate for muscle denervation. However, in MND it is much reduced. So the discovery that Nrg-1 rescues this sprouting mechanism in diseased MNs opens a window for novel MND therapies that promote functional recovery rather than simple preservation of the damaged cells.

Do viruses play a role in MND?

When we think of viruses, we usually think of infections such as the common cold. But the involvement of viruses in human life goes much deeper. Our genome is actually a composite of human and viral genes; 8% of our DNA is occupied by viral genetic material. Human endogenous retrovirus-K (ERVK) is one of the most recent entrants into our genome, and the levels of the protein it encodes have been linked to various inflammatory and neurological diseases. Mouse models of MND, in fact, have shown that the ERVK protein may contribute to MN degeneration. ERVK in MNs has been correlated with TDP-43, a protein that is found in abnormal forms in the majority of people with MND. The high prevalence of abnormal deposits of TDP-43 in diseased MNs is a clear sign that these cells have lost the ability to control the production and recycling of their proteins. Mamneet Manghera and fellow researchers in Manitoba, Canada, identified the need to explore the link between TDP-43, dysfunctional cellular protein control, accumulation of ERVK protein and MN degeneration. To do this, they used a cell model in which they induced cultured human MNs and other non-neuronal cell types to produce a mutated form of TDP-43. They found that the mutant TDP-43 promoted the accumulation of ERVK in the MNs, but not in other types of cells. This finding was confirmed in human post-mortem tissue from patients with MND, showing that accumulation of viral proteins, in this case ERVK, in MNs is a novel aspect of TDP-43-linked MND. Further studies may consequently find that supressing ERVK activity in MND improves disease symptoms.



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