MND AUSTRALIA INTERNATIONAL RESEARCH UPDATE

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Hi I'm Rosie, and I'm writing to you from the University of Tasmania's Menzies Institute for Medical Research. I am in the lab of Prof. Tracey Dickson and am currently investigating whether the interneurons, a cell type that applies the brakes on neuronal excitability, have a key role in brain circuitry in motor neuron disease (MND). Last year I was fortunate to be the recipient of the 2019 Bill Gole Postdoctoral Research Fellowship supported by MNDRIA, and this year it is my pleasure to keep you up-to-date with the latest research in MND from universities and journals across the world through the MND Australia International Research Update.

In this mid-year update we talk about the latest findings from researchers across the globe who are united in the fight against MND. In particular, we discuss recent developments that have changed our understanding of the disease and that highlight new therapeutic targets. There have been great steps taken towards determining the cause of both SOD1 and C9ORF72-related toxicity, which underpin the most common genetic causes of MND.

MicroRNAs, a new frontier in MND research

One of the most important discoveries in the 1990s was the existence of microRNAs. These are small RNA-like molecules that do not translate into a biologically active protein, but instead are able to alter gene expression and control cell activity. The discovery of microRNAs redefined our understanding of gene regulation and may now redefine our understanding of MND. Researchers at Washington University and Johns Hopkins University have identified a microRNA produced in excess, specifically by dead or dying motor neurons. This microRNA-218 was consequently taken up by neighbouring astrocytes causing them to become dysfunctional.

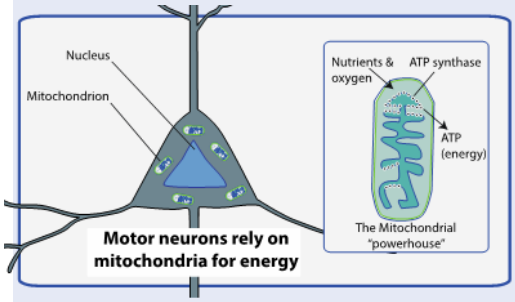
Astrocytes are another key component of the nervous system playing a number of important roles. Motor neurons rely on astrocytes to provide essential nutrients and to clear other molecules for their health and function. To demonstrate that this microRNA-218 damaged astrocytes, scientists blocked its production in a mouse model of ALS and were able to prevent damage to astrocytes. This finding led authors to suggest that microRNA may be a therapeutic target for MND.

In May this year, researchers at Columbia University and the Institute of Molecular Biology in Taiwan added to our knowledge of the therapeutic potential of microRNAs in MND. Building upon a series of studies, they were able to show that a cluster of microRNAs (miR-17/92) can confer resistance to MND-associated degeneration. By overexpressing microRNAs they were able to significantly rescue human motor neurons carrying the SOD1 mutation, which had been produced from patient stem cells. Furthermore, using a virus to deliver the microRNAs into the cells, they were able to improve motor deficits and modestly extend survival of a SOD1 MND mouse model. The relevance of microRNAs in MND is only just beginning to be revealed, but signs are promising that these small nucleotide sequences may shed considerable light on disease processes and lead the way to identifying novel therapeutic targets.

C9ORF72 messes up the mitochondrial power house of the cell

The expanded GGGGCC repeat expansion mutation in C9ORF72 is the most common genetic cause of both sporadic and familial MND. Scientists are making rapid progress to understand what makes these run-on repeats toxic. The C9ORF72 gene accounts for 40% of inherited forms of the disease and 6 percent of sporadic cases. People who have the C9ORF72 mutation have an abnormally long GGGGCC repeat expansion in the gene. Typically, individuals can carry up to 25 hexanucleotide GGGGCCC repeats in the C9ORF72 gene without developing disease, but the number of repeats in the DNA of ALS patients can be four hundred to several thousand. The result of this expansion is the production of unintended proteins, called dipeptide repeats, which are not normally found in people, and are thought to be a source of the toxicity to neurons.

To better understand how these dipeptide repeats lead to neuron death, a team of researchers led by Dr Fen-Biao Gao of the University of Massachusetts made a model that allowed poly(GR) repeats to slowly accumulate in mouse neurons. They identified that mitochondrial function is disrupted in cells in the presence of C9ORF72. Mitochondria are the cells' powerhouse. They take oxygen and simple sugars from food and convert it into a form of energy that cells can use. The process called oxidative phosphorylation is essential for neurons to maintain cellular function and underlies brain information processing. What they found was mitochondria are damaged early, well before significant neurodegeneration or behavioural symptoms. This suggests a drug that can increase mitochondrial function may be able to rescue neuronal function.



Uncovering the origins of neurotoxicity in SOD1 aggregates

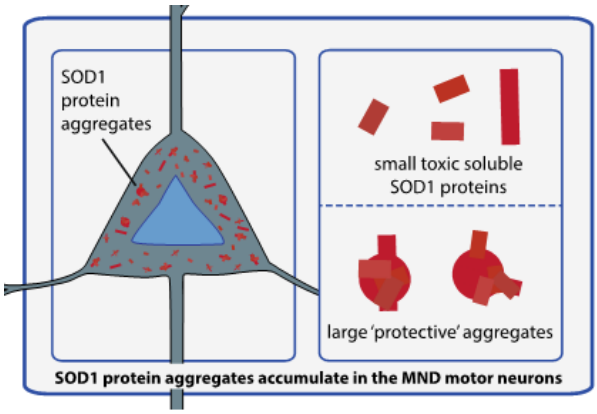
Protein aggregates and misfolded proteins are a hallmark feature of disease-affected motor neurons in MND. These abnormal protein clumps accumulate in the motor neurons of people with the disease, and can travel from cell-to-cell in affected regions of the central nervous system, causing the disease to progress. Since first identified, researchers have been striving to determine how these proteins contribute to disease, and how they play a role in initiation and progression of motor neuron loss.

A mechanism deciphered.

A few years ago, researchers at the Howard Hughes Medical Institute at UCLA identified that toxicity of SOD1 is likely caused by soluble oligomer-like SOD1 protein structures, not the large insoluble aggregates typically observed to accumulate and previously thought to drive disease. In April this year, Fernando Viera and his team at the ALS Therapy Development Institute (ALSTDI) in Massachusetts made the observation that regions of the central nervous system least affected by the disease had the most amount of insoluble aggregated SOD1. Furthermore, the accumulation of aggregated SOD1 predicted a slower disease progression. In comparison, the soluble misfolded SOD1 form appeared more enriched in vulnerable motor neuron populations.

Targeting toxic soluble SOD1 to help brain cells.

Dr Viera sees a promising opportunity in how this mechanism could be applied in therapy, suggesting that accumulation of SOD1 protein aggregates may be a protective mechanism that keeps brains cells alive longer, by sequestering toxic soluble SOD1 oligomers into less harmful insoluble aggregates. A therapeutic approach might focus on stabilising SOD1 into aggregates (see image). Most recently, researchers at the University of North Carolina have been attempting such a solution. Led by Professor of Biophysics Nikola Dokholyan, they were able to show that promoting the formation of fibrillar *insoluble SOD1 lessened neurotoxicity*, while increasing the levels of SOD1 oligomers was highly toxic to cells. This is potentially an important finding for MND, as previously aggregates of SOD1 protein were thought to be a culprit of cell death. These works suggest that promoting aggregation may be a protective mechanism, which can be used to “mop-up” these other toxic soluble oligomeric intermediates.

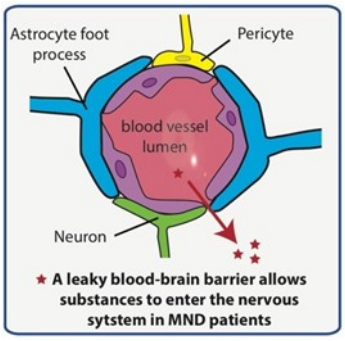


SOD1 protein aggregates accumulate in the MND motor neurons

MND Research Shorts

- Disease-associated C9ORF72 mutations cause cell death in MND and, while many research groups are trying to understand how this occurs, a laboratory group in Philadelphia has been taking a different approach. Lead by Nancy Bonini at the University of Pennsylvania, the team have been trying to tackle C9ORF72-toxicity before it even begins. Using a fruit fly model they were able to identify a new protein which controls the activation of the C9ORF72 gene. By knocking down this protein (decreasing its levels) , toxic protein production from the mutated C9ORF72 gene was prevented in the first place. This novel strategy is of great interest, as it could prevent C9ORF72-toxicity rather than try and treat it.

- The brain and spinal cord have their own security system, a mesh of interlocking blood vessels that allow the entry of essential nutrients and act as a barrier to prevent the access of other substances that might be potentially harmful. Accumulating evidence shows that these blood-brain and blood-spinal cord barriers become damaged in ALS patients. A study carried out in the USA tested whether human bone marrow stem cells could be used to repair the blood-spinal cord barrier in a mouse model of SOD1-linked MND. The strategy showed promise as it improved the structural integrity of blood vessels and increased motor neuron survival.



★ A leaky blood-brain barrier allows substances to enter the nervous sytstem in MND patients

- Inflammation of motor neurons or ‘neuroinflammation’ is a key aspect of MND pathology. Researchers from the USA and Sweden are paving the way to understanding not only how, but when and where, this inflammatory process begins to contribute to disease in MND. Developing a new technique that allows them to map and spatially resolve disease-driven changes, they have been able to identify the specific cells involved in each stage of disease progression. This strategy is very powerful, with lead author Silas Maniatis suggesting that dysfunction of a nervous system-specific immune cell-type called microglia contributes to the inflammatory disease process well before symptom onset, and is likely to be important early on in MND.

- In Australia, 60 percent of people with MND are male and 40 percent are female. The exact reason for this gender bias remains unknown. However, perhaps there are clues to be gained from the rare neurodegenerative disease, Kennedy’s disease. Affecting predominantly males, Kennedy’s is associated with a defect in the androgen receptor, which regulates the activity of the male hormone testosterone. This defect causes production of an abnormally large androgen receptor protein which behaves very differently to normal. A recent study carried at the University of Melbourne and La Trobe University discovered that blocking androgen receptor activity accelerated the disease onset and motor dysfunction in male, but not female, SOD1 MND mouse model. This may suggest a susceptibility of motor neurons to damaged androgen signalling.

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