MND Australia International Research Update

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The complicated life of motor neurones

As we study motor neurones (MNs) and learn more about them we discover just how complex a cell they are. For one thing, they are the oldest and largest cells in our bodies. The MNs we are born with are those we have for life. They can even be up to a metre long, as is the case with the MNs that extend from the spinal cord to muscles of the foot in people with long legs. Part of their complexity is likely due to their large size and old age, as it takes a lot more energy and coordination of the molecules inside them to keep them going. This also makes them more vulnerable to dysfunction and disease. But as we see in this report, researchers are coming a long way in nutting out their complexity and understanding the specifics of how they become diseased.

Exposing the social life of MND proteins

The proteins in cells can be thought of as tiny social entities. At any one time there are millions of interactions going on, and it is through this "socialising" that cells are able to function at all. However, when some of these proteins start displaying unusual social behaviour, the dysfunction they cause in the cell can lead to disease. The genetic abnormalities, called mutations, that are known to cause MND are the original culprits behind most of this unruly protein behaviour. But we don't yet know the full range of proteins that are guilty and how they are interacting; what exactly are they doing?

Addressing this, Anna Blokhuis and collaborators across the Netherlands, Italy, the UK and Japan carried out a large study to identify the proteins interacting with MND-associated mutant versions of ATXN2, C9orf72, FUS, OPTN, TDP-43 and UBQLN2 in neuronal cells. Using several techniques to isolate these proteins, they identified numerous novel interacting partners of the MND proteins. Some of these partners, in fact, were shared between the different MND proteins. For example, shared partners of ATXN2, FUS and TDP-43 had roles in RNA metabolism while OPTN and UBQLN2 partners were related to protein degradation.

To confirm that these overlapping "social networks" are important for the disease process of MND, Anna and her collaborators examined fragile X mental retardation protein (FMRP), one of the common interactors of ATXN2, FUS and TDP-43, using a FUS-linked MND model. FMRP normally controls activity at the junction where neighbouring neurones communicate (the synapse). Anna's team found that FMRP stuck to clumps of mutant FUS and other defective proteins, and was bound to FUS directly. Additionally, the dysfunction caused in MNs by mutant FUS was rescued by the administration of extra FMRP. So it appears that FMRP, a VIP (Very Important Protein) in MNs, is the victim of the miscreant mutant FUS. Restoring normal FMRP activity therapeutically may be a way to combat the damage caused by mutant FUS and TDP-43.

As for the other interacting partners identified by Anna's team, they remain to be further investigated in future studies.

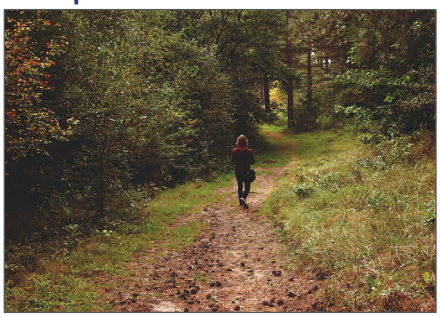
How are proteins "pulled out" of biological samples and studied?

Because proteins are so tiny, and are mixed together like soup within biological samples such as blood and urine, scientists have developed all sorts of ingenious ways of isolating and studying them. This includes making use of the antibody-antigen interaction that occurs in the immune system (antigens are sections of proteins that are capable of triggering an attack by the immune system). Because this interaction is highly specific, like paired-up jigsaw pieces, scientists have found a way of producing antibodies that specifically target and bind to the proteins they want to isolate from biological samples. This manipulation of antibodies is referred to as **immunostaining**, and it can be used for staining specific proteins in cells when imaging them using microscopy, pulling proteins out of samples, and measuring the amount of protein present in samples.

MND Research Shorts:

- *Astrocytes, the star-shaped support cells of MNs, are often found to exacerbate toxicity in MNs in MND experimental models. Using astrocytes containing MND-linked mutant SOD1 protein, researchers in Uruguay and the USA tested the effect of exposing them to nitro-fatty acids. This caused the astrocytes to produce antioxidants that protected their neighbouring MNs, introducing the therapeutic potential of nitro-fatty acids.*
- *Because hyperexcited MNs can become toxic, hyper-excitability is implicated in the early phases of MND development. Calcium, which is essential for signal transmission between MNs, is believed to behave unusually in this early phase, causing the hyperexcitement. Researchers in the USA have discovered abnormal behaviour in the pore-forming cell surface proteins that control calcium entry and exit from MNs in MND model mice. This gives us great insight into the early changes that occur in MND.*
- *A study carried out in Ireland has revealed that caspase 6, an enzyme involved in the normal programmed death of old or damaged cells, is protective in mice that contain MND-linked mutant SOD1. Interestingly, its beneficial effect only occurred in diseased mice; in unaffected mice it carried out its usual role as executioner.*
- *Researchers in Italy have discovered a new link between sporadic and familial MND. MEF2C and MEF2D, proteins important for muscle and neural development and maintenance, showed altered levels in blood taken from MND patients. Further investigation will determine the reliability of using these proteins for biochemical diagnostic tests.*

Catching MND early on its path



One of the main roadblocks to developing an effective treatment for MND is the frequent misdiagnosis in the early stages of disease. This has a two-fold effect. For one thing, it means that researchers have been unable to carry out biochemical studies using tissue from patients in the pre-symptomatic stages of disease, which would allow us to gain insight into the early biological events that initiate the disease process. Unable to identify these "biomarkers" of MND, clinicians cannot biochemically test individuals and diagnose MND. But much research effort is going into identifying suitable biomarkers in individuals diagnosed with MND. Yan Chen and his colleagues in Shanghai, China, carried out a study to determine the most effective biomarkers present in the cerebrospinal fluid (CSF), the clear watery fluid found in the brain and spinal cord. They compared the levels of proteins in the CSF of MND patients with the CSF of healthy individuals. To do this they used an immunostaining technique to isolate and pull out the proteins of interest from the CSF, which they then identified and quantified. Out of the cohort of different proteins in the CSF, Yan's group identified altered levels of two proteins; glutamate receptor 4 (GRIA4) and insulin-like growth factor II (IGF-2). Importantly, the levels of GRIA4 correlated with disease severity and thus could be useful as a reference for the timing of treatment. The levels of IGF-2 may also prove to be an effective biomarker to aid in the diagnosis of MND.

Metabolic changes in MND and Parkinson's disease may improve diagnosis accuracy

As well as the potential of using specific proteins in the CSF as biomarkers of MND, researchers are also looking into whether any metabolites show differences between MND patients and unaffected individuals. Metabolites are the substances involved in metabolism and formed during metabolic processes. As metabolism can be significantly affected or involved in the disease state, elucidating the specific metabolites that are altered in diseased individuals can be very informative.

Researchers in Umeå, Sweden, went about identifying robust metabolite biomarkers for MND, but additionally investigated patients with Parkinson's disease (PD), seeking to gain insight into the affected metabolic pathways that are similar and different between the two diseases. They collected CSF and blood plasma from individuals with MND or PD, and healthy individuals, and used a technique called nuclear magnetic resonance (NMR) spectroscopy to identify and quantify the levels of different metabolites. The results suggest that the creatine/creatinine pathway and the metabolism of some amino acids (the building blocks of proteins) are altered in both disease states. These changes suggest that altered energy metabolism is involved in both MND and PD. However, there was distinction between MND and PD patients in the specific metabolites of these pathways that were affected, reflecting important differences that may allow clinicians more ability to diagnose patients accurately in the future.

Motor neurones suffer indigestion too

One of the main cell defects believed to cause MND is impairment of autophagy, a normal mechanism used by cells to degrade old and damaged proteins.

Autophagy is the cell's digestive system, whereby digestive enzymes contained within a special membrane, the phagophore, break down old or abnormal proteins and recycle their components. Amongst several proteins that carry out autophagy is SQSTM1, and mutations in the gene encoding this protein are associated with MND. As cells do not have an oesophagus to transport substances through the digestive tract, they need proteins like SQSTM1 that carry protein targets to the autophagy machinery. When it reaches the phagophore it interacts with a membrane-anchored protein, LC3B, via a special interacting region called its LIR. Some of the MND-associated gene mutations are located in this LIR. Alice Goode and her team of researchers in Nottingham, UK and Tromsø, Norway investigated one of these LIR-located mutations and found that this altered form of SQSTM1 is unable to recognise LC3B. This means that this mutation restricts the critical step of SQSTM1 recruiting its protein cargo to the phagophore. Alice's team propose that while SQSTM1's delivery of proteins for autophagy is not completely stopped, over the very long lifetime of a MN this impairment causes a build-up of old, damaged proteins that can start to interfere with other cell activities, leaving MNs vulnerable to dysfunction and deterioration.

EnRAGEd motor neurones

Mutations of the SOD1 gene are responsible for 15-20% of familial MND cases. One of the ways mutant SOD1 proteins wreak havoc in MNs is through generating toxic molecules such as advanced glycation end products (AGEs). AGEs interact with a protein that sits on the surface of cells, called the receptor for AGEs (RAGE). This is a very appropriate name for this protein as it induces cell changes that are analogous to a person experiencing rage, i.e. stress and inflammation (going red with anger).

To further understand the role of RAGE in SOD1-linked MND, Judyta Juranek and her colleagues in New York, USA, made use of a SOD1-MND mouse model. By immunostaining spinal cord tissue from these mice, they found that the levels of RAGE were higher than in healthy unaffected mice. With this finding, they went on to test the effect of blocking RAGE activity using soluble RAGE (sRAGE), a natural chemical competitor for the proteins to which RAGE binds. The mice treated with sRAGE had a significantly slower disease progression rate and longer lifespan than untreated mice, showing the effectiveness of blocking RAGE from interacting with its target AGEs. In this case, as in the more familiar case of poor rage, or anger management, the healthier way to be is to reduce the levels of RAGE in our and our cells' lives. Thus treatment with sRAGE may prove to be an effective and novel therapeutic to intervene in MND.

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