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## Criminals and law enforcers in the nervous system

More than any other organ system in the body, the motor nervous system that controls the muscles and bodily movements requires strict control of the proteins and other molecules that populate it. In this final quarter of 2016, researchers around the world have discovered several of the criminals that are involved in motor neurone disease (MND), wreaking havoc in motor neurones (MNs). There has also been much progress in the discovery of the law enforcers in MNs that help keep potential criminals in-check. In this report, we explore new ideas for therapeutics that aid the law enforcers in MNs and newly identified criminal molecules we can work on targeting through drug development.

## A Parkinson-related gene protects motor neurones from SOD-1

We now know that there are several different genes that are linked to MND. Specific changes, or mutations, in these genes somehow cause MNs to stop functioning properly and eventually die. Mutations in the SOD1 gene were the first to be identified in people with MND. These mutations cause the SOD1 protein to be unstable and form aggregates – protein clumps – in MNs. It's important for cells to be able to remove unstable SOD1 molecules, but in people with SOD1 mutations their MNs become unable to properly eliminate them. One of the two major systems that cells use to remove unwanted proteins, called the ubiquitin-proteasome system (UPS), is known to become dysfunctional in MNs because mutant SOD1 molecules clog it up. So in order to combat this problem, Cheryl Yung and her colleagues in Georgia, USA, decided to investigate the different mechanisms used by cells to manage mutant SOD1. They discovered that an E3 enzyme (see the box below) called parkin selectively identifies and targets mutant SOD1 for removal by the autophagy-lysosome system, directing it away from the clogged-up UPS. Interestingly, mutations in the parkin gene cause early-onset Parkinson's disease, another neurodegenerative disease that affects a different type of neurone to the MNs that are affected in MND. In their mutant SOD1 mouse model of MND, the researchers found that parkin only targeted *mutant* SOD1, leaving normal SOD1 molecules untouched. By removing mutant SOD1, parkin was able to protect MNs from its toxic effects. The researchers had increased the levels and activity of parkin in the MNs of their model mice, so perhaps with further work parkin can become a target for gene therapy in people with SOD1-linked MND.



## MND Research Shorts

- *Inflammation in the nervous system (neuroinflammation) and high levels of inflammation-promoting cytokine proteins occur in MND patients. Researchers in China investigated the changes that occur in the cell's cytokine network in MND in order to understand how they relate to disease progression. Their results suggest that the interactions between cytokines change throughout disease. Further investigation will help us identify how to target these changes to help treat MND.*
- *Researchers in Korea have discovered MND-specific changes in protein production that are associated with neuroinflammation. These changes occurred in the immune system, revealing its role in MND.*
- *There is urgent need for the discovery of blood biomarkers that allow specialists to diagnose MND before symptom onset and to monitor a patient's progression through disease. Researchers in Germany have identified that the levels of the NF-L protein in the blood of MND patients corresponds accurately with whether their form of MND is slow or fast progressing, and may thus aid in diagnosis and prediction of outcome.*
- *The brain and spinal cord are notoriously difficult to access with drugs. But researchers in the USA have discovered that antioxidant cerium oxide nanoparticles (CeNPs) have unusual access to the MNs of SOD1-MND model mice. The antioxidant activity of these tiny CeNPs eased the toxicity caused by mutant SOD1. Even when treatment was started after the onset of muscle weakness, it prolonged survival. Thus CeNPs may be developed into an effective antioxidant therapy for MND.*

## MND and the removal of garbage proteins

Our cells have two major systems for removal of unwanted proteins, the ubiquitin-proteasome system (UPS) and the autophagy-lysosome system. In both of these systems, a cascade of special enzymes direct little proteins, called ubiquitin, to unwanted or old proteins to target them for removal. This cascade begins with an E1 enzyme that activates the ubiquitin, followed by an E2 enzyme that works with an E3 enzyme to bind ubiquitin to its target protein. There are hundreds of different E3 enzymes in our cells, which allow the ubiquitin tagging of proteins to be very specific – which is very important so the right proteins are removed! In MND and other diseases linked to specific genes, the problem begins when the gene is damaged, or mutated. The resulting protein it produces is then unable to assume its correct shape, which it normally does by folding itself in precise ways. The genetic mutations linked to MND cause their encoded proteins to become unable to fold into their proper shape. Our cells use the UPS and autophagy to eliminate these mutant proteins, however in the case of MND, it seems that the MNs become overwhelmed by these proteins and are unable to remove them, leading to protein aggregation and accumulation.



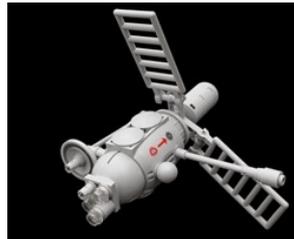
## Specialised removalists target mutant SOD1 and protect against toxicity

We've seen how important it is for MNs to be able to eliminate mutant SOD1 molecules, and Cheryl Yung's work has shown that parkin is able to help cells get rid of it using the autophagy-lysosome system. Another group in the Republic of Korea have also been trying to find a way to help cells remove mutant SOD1, and have discovered an alternative system that cells can use. Jin Sun Choi and his team investigated a family of proteins, called the inhibitor of apoptosis protein (IAP) family, that work to help cells avoid a special kind of cell death, apoptosis. Apoptosis occurs when cells are damaged beyond repair and need to sacrifice themselves in order to save their neighbouring cells. Avoiding apoptosis is particularly important for MNs affected by MND-related mutations because the cells seem to speed up the apoptosis process even when they are still functional. Surprisingly, the researchers found that IAPs would bind to mutant SOD1 selectively and actually help it get tagged and targeted for removal by the UPS. Furthermore, Jin's team showed that depleting the cell's number of IAPs exacerbated the toxicity caused by mutant SOD1. In a similar way to how specialised asbestos removalists work to specifically target and remove toxic asbestos from buildings, IAPs selectively target and remove toxic mutant SOD1. This is strong evidence that IAPs could be a therapeutic target for SOD1-linked MND.



## A novel drug scavenges dangerous levels of iron and free radicals in SOD1-linked MND

Iron is very important for the normal functioning of our bodies, particularly for the delivery of oxygen to our cells, and thus through our lives we are told it is an important nutrient we need in our diet. Our body is usually able to keep the levels and distribution of iron in balance in our different tissues. In people with MND, however, iron has been shown to be accumulated in the spinal cord. In order to try and restore the balance of iron in the central nervous system of MND patients, Sagit Golko-Perez and fellow researchers in Haifa, Israel, developed a multifunctional drug that acts as a scavenger of iron and the dangerous free radicals that are generated when iron is out of balance. This scavenger has previously been found by other researchers to have protective effects in mouse and rat models of Parkinson's disease. Sagit's group tested its effect in their mouse model of SOD1-linked MND, in combination with a high-calorie/energy-supplemented diet – which has recently been found to have a beneficial effect in MND patients by slowing down the progression of disease. Treatment with this scavenger improved the motor performance of the MND mice, increased their lifespan and prevented iron accumulation and MN loss in their spinal cords. These results support the use of a drug scavenger to aid in the treatment of people with this form of MND. It would roam through the nervous system like a satellite scavenger in space, tracking down free radicals and restoring the balance of iron in MNs in SOD1-linked MND.



## Partners in crime: PFN1 and TDP-43

A recently identified gene in which mutations occur in people with MND is PFN1. In order to figure out how PFN1 mutations cause MNs to dysfunction and die, Koji Matsukawa and a team of researchers in Tokyo, Japan, developed a model of PFN1-linked MND in fruit flies. A fruit fly disease model may sound strange, but they have proved extremely useful for many disease studies. With this model, Koji's team showed that mutant PFN1 itself did not cause any damage, but when mutations were also introduced into TDP-43, the mutant PFN1 exacerbated the toxicity caused by mutant TDP-43. This toxicity was associated with a shift of TDP-43 from its normal stomping ground, inside the cell nucleus, out into the rest of the cell. So it seems that MND-causing mutations in PFN1 may cause disease by acting as a partner in crime with TDP-43, exacerbating the degeneration caused by this major MND-causing protein. Further studies will unravel the means by which PFN1 causes this shift in TDP-43's territory and leads to degeneration.



## Rogue proteins lose their chaperones in sporadic MND

In 10% of cases people with MND have a family history of disease, which has enabled scientists to discover much about the causes of disease by studying the genetic mutations these people carry. However, in the remaining 90% of cases, there is no genetic history of disease, and thus the causes of this sporadic form of MND have remained elusive. Nevertheless, what we do know of both sporadic MND and hereditary MND, is that the affected MNs contain aggregated clumps of misfolded proteins, and thus the cell's ability to remove unwanted proteins is severely disrupted. In the two systems of protein removal used by cells, molecules called chaperones are busy little workers that, as their name suggests, chaperone proteins to help them fold into their correct shape and to travel to their proper location in the cell. With the crucial link between chaperones and protein quality control in mind, Simona Capponi and her colleagues in Italy and Belgium investigated the role of a candidate chaperone, HSPB1. They discovered a mutation in its gene in a cohort of 247 unrelated Italian MND patients. Isolating the mutant HSPB1 protein and examining its function, Simona found that it lost its chaperone activity, unable to escort rogue proteins away from the dangerous situations in which they get involved. As well as revealing a role for HSPB1 mutations in the dysfunction and degeneration of MNs in MND, the results of Simona's study highlight the importance of identifying rare but disease-causing gene variations in sporadic MND patients to understand how disease develops in these cases.

