

The majority of the presentations at this annual symposium are given in parallel sessions focussing on understanding the causes and mechanisms of motor neurone degeneration in the biomedical sessions, to the latest news on clinical research and management of MND in other sessions.

Dr Justin Yerbury, University of Wollongong, has been awarded the Bill Gole Postdoctoral MND Fellowship for 2009 – 2011. He has written the following report on the scientific sessions he attended at the Symposium.

Symposium Report

Cell biology and Pathology

Previous work had discovered a mutation in the gene encoding angiogenin which was associated with MND in the Scottish/Irish population of MND patients. Angiogenin is a protein that aids in the formation of new blood vessels. Dr Vasanta Subramanian from the University of Bath, UK, presented work that suggested that the mutant version of this protein was indeed detrimental to neurones grown in the test tube. This implies that the mutation gives a harmful property that may cause MND in some cases.

Researchers from the University of Pittsburgh, USA, have been studying the role of retinoid signalling in sporadic MND patients. Retinoids are a class of compounds related to vitamin A. Dr Robert Bowser and his graduate student Christi Kolarcik found differences in the amount of retinoid binding proteins in post mortem lumbar spinal cord motor neurones in MND. This suggests that the retinoid signalling pathway which controls growth and differentiation of some cell types, may play a role in sporadic MND.

Mitochondria are the energy powerhouses of the cell and are usually very abundant in motor neurones. Dr Alexander Panov and team at the Carolinas Medical Centre, USA, have found that there are significant losses in mitochondria number in both the brain and spinal cord tissue of rats genetically modified with the human SOD1 gene that causes MND. They also found that the mitochondria found in the SOD1 rats produced higher amounts of free radicals.

Professor John Ravits and his team at the University of Washington have microdissected motor neurones from the spinal cord of G93A SOD1 mice in an attempt to understand the processes motor neurones switch on or off as motor neurone disease progresses. They found distinct molecular events switched on in the early phase of the disease (before symptom onset) compared to late disease stage, which mostly consisted of cell death programming. They also found differences in events taking place in the motor neurones when compared to surrounding cells. Although an understanding of these events and their roles in MND are not yet fully understood it demonstrates that events occurring late in MND are different to and are probably a result of early events. Therefore, understanding the early phase of the disease may identify targets suited for investigation into therapeutics.

Work coming out of the Motor Neurone Disease Research Group, Kings College, London suggests that mutations in the gene encoding VAPB (vesicle-associated membrane protein-associated protein B) may cause MND by disturbing transport of molecular machinery down the extraordinarily long motor neurone axons. Axons are the long projections from neurones that distribute the “electrical” signals. Dr Kurt De Vos reported that mitochondria, the cell’s powerhouse, were particularly affected with the number of mitochondria transported decreased significantly. This has implications for the energy hungry motor neurones which require mitochondria in all parts of the cell.

Translational Strategies (from laboratory to therapy)

Dr Thierry Bordet from the biopharmaceutical company *Trophos* in Marseilles, France, has screened a catalogue of 45,000 drugs on rat embryonic motor neurones under stress from nutrient deprivation. One of the stand out molecules, TRO19622, rescued axons and promoted axon growth upon nerve injury in rodents. It has also

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prolonged lifespan (by two weeks) of SOD1 mice. This drug has been through stage I clinical trials (to show safety) and is about to enter stage II (to see if it is effective). It is thought that this drug, if successful, would be used in combination with riluzole.

Previous work coming out of the Hannover Medical School, Germany, had suggested that levels of the protein Nrf2 were decreased in MND. Nrf2 is an activator of the cell's antioxidant response. Pamela Shaw's Laboratory at the University of Sheffield, UK, has been screening molecules already approved for use in humans for their ability to increase the levels of Nrf2. Dr Adrian Higginbottom presented his findings that showed two of these molecules increased Nrf2 in cells grown in the test tube. This finding has implications for sporadic MND where Nrf2 is low, however, these drugs have the potential to increase SOD1 in motor neurones so would not be useful for familial MND SOD1 families.

It is important to detect MND as early as possible since it is thought that any potential treatment would be more effective in the earlier stages of the disease. Thus detecting disease onset is a vital avenue of research. Dr Robert Bowser and co-workers from the University of Pittsburg are searching for biomarkers in cerebrospinal fluid and blood plasma that correlate to ALS disease progression. A small number of proteins, cystatin C, phosphor-neurofilament H and anti-thrombin III were found to differ in their levels in spinal fluid over the disease duration.

TDP-43

This year's recipient of the Forbes Norris Award for contributions to MND/ALS research Professor Michael Strong from the University of Western Ontario, USA, has been investigating the role of TDP-43 in MND pathology. He presented data that showed that TDP-43 is found associated with messenger RNA transport granules in normal motor neurones while it is found in stress or degradative granules in ALS patients. This means that transport for messenger molecules for important structural proteins such as neurofilaments will not be transported to where they are needed. This has implications for motor neurones since their axons are extremely long and consequently rely heavily on energy dependant transport of molecules. Other work coming from Professor Strong's laboratory was presented by Dr Katie Moisse whose work suggests that TDP-43 itself may not be toxic but rather a part of the cells response to injury. Dr Moisse studied the levels and locations of TDP-43 in response to neuronal injury and found that TDP-43 levels were increased transiently but decreased again once the injury was repaired. These data suggest that TDP-43 is a normal part of the stress response and may not itself be detrimental to motor neurones.

It had been known that deposits containing TDP-43 could be found in sporadic cases of MND. Dr Elizabeth Tudor from Kings College in London, UK, reported on data collected from mice genetically engineered to produce the human version of mutant VAPB. VAPB mutations are associated with some familial cases of MND. Dr Tudor found that the VAPB mice had deposits in their motor neurones that contained TDP-43. This is the first time TDP-43 has been found associated with pathological deposits in a MND mouse model.

Professor John Ravits presented work identifying the location of deposits in sporadic MND patients that contained TDP-43 and/or ubiquitin. Ubiquitin is a tag that cells use to label a old or damaged protein for destruction. TDP-43 was found located in motor neurones with ubiquitin. It was thought that by studying regions of heavy motor neurone loss and comparing this to areas in which motor neurones loss was not as great the role of TDP-43 may have become clear. However, it still remains uncertain if the TDP-43 deposited in motor neurones is a cause or result of cell death.

The role of TDP-43 in MND was cemented by the finding of cases of familial MND with mutations in the gene encoding TDP-43. There have been 14 mutations reported so far. Dr Jemeen Sreedharan from Kings College London, UK, reported that these mutations appear to affect one region of the protein suggesting that a specific function may be impaired. It is unclear as yet exactly what that may be. Dr Sreedharan predicted that the mutations may increase the appearance of an added phosphate on the protein that could in turn affect its function.

In addition, mutations may increase the likelihood of the protein being fragmented, or it may even change the way that it is distributed in the cell. More research is needed to clarify the role of TDP-43 in familial MND.

Use of the G93A SOD1 mouse in therapeutic testing

There has been a growing concern in the field of MND study that the G93A mouse model may not accurately represent what occurs in human MND. Drugs such as minocycline that appeared to prolong the life of G93A SOD1 mice have had little effect on humans. Sean Scott and co-workers from the ALS Therapy development Institute, USA, has also published work that demonstrates that most of the positive effects seen to date in the G93A SOD1 mice could be explained by “noise” in the model. This conference session heard from Professor Linda Greensmith from Kings College London, Dr Steven Perrin from the ALS Therapy Development Institute, USA, and Dr Richard Mead from the University of Sheffield, UK, all of whom presented work on ways to reduce the “noise” so as to collect data that represents a “real” result. It is clear that the international MND research community believes that this is the best possible model currently available and have shown that there are ways of ensuring valid results for future studies.

Role of non-neuronal cells

Previous studies have already indicated that motor neurones may not be the only cell types involved in MND pathology. Other cell types such as astrocytes, the neuronal “support” cells, and microglial cells, the immune modulators of the nervous system, may play a role in MND. Associate Professor Nicholas Maragakis and team from Johns Hopkins University, USA, has transplanted stem cell-like glial precursor cells into the spine of SOD1 mice. They found that the transplants resulted in the production of mature astrocytes and that the presence of mature astrocytes was associated with preservation of diaphragm function. This also prolonged the survival of the transplanted mice. This study opens the way for other studies involving non-neuronal cell stem cell implantation.

Researchers from the University of Milan, Italy, have conducted work that showed morphological changes in astrocytes before that of motor neurones. Dr Daniela Rossi also reported that the changes in astrocytes became more significant during onset of neuronal degeneration and appearance of symptoms. Work done in test tubes showed that astrocytes producing mutant SOD1 were more vulnerable to mild glutamate induced toxicity compared to normal astrocytes.

Professor John Weiss and co-workers at the University of California Irvine, USA, theorised that in addition to astrocytes causing motor neurone dysfunction increased free radical production in motor neurones may damage astrocytes in a feed forward cycle of cellular damage. Data presented showed that free radicals produced in motor neurones could in fact damage glutamate receptors on the surface of nearby astrocytes. This increase in free radicals was found to be due to the unusual ability of motor neurones to take up large amounts of calcium. The researchers propose that this interplay between motor neurones and astrocytes may explain the progressive nature of MND.

Genetics

A team of international researchers headed by Professor Robert Brown Jr from the Cecil B Day Neuromuscular Research Laboratory, USA, are investigating the possibility that there are genetic factors that modify survival in MND. In a set of 1829 sporadic ALS patients a small stretch of DNA was found to be associated with survival in these patients. The underlying mechanism of the extended survival is not yet understood. In contrast, Dr S Cronin and co-workers from Ireland and Poland were unable to find any genetic modifiers in the Irish or Polish ALS population.

In vivo models

Deficiency of a protein called SMN (for *survival of motor neurones*) causes Spinal muscular atrophy. Research presented by Dr Bradley Turner from the Howard Florey Institute, Australia, suggests that SMN levels may play a role in ALS. Dr Turner's results show that the presence of mutant SOD1 reduces the levels of SMN in the test tube and in SOD1 mice. This suggests that therapies relacing SMN or increasing the cellular levels of SMN may prove beneficial in ALS patients.

Dr Gareth Banks from the Institute of Neurology London presented data that showed that a SOD1 mice with a second mutation in the gene encoding glycine tRNA sythetase (whose job it is to add glycine into growing proteins) lived longer than those with the SOD1 mutation alone. How this mutation protects the mice is unknown.

Molecular chaperones are a large group of proteins that are a part of the cells defences against inappropriate protein deposits. One such chaperone is called HSJ1. Dr Wendy Mustill from University College London, UK, examined the role of HSJ1 by studying SOD1 mice with no HSJ1 or SOD1 mice that had high levels of HSJ1. The results showed that at 120 days of age the number of motor neurones depended on the levels of HSJ1. This evidence suggests that increasing the levels of molecular chaperones may be beneficial to ALS patients.

Results from Cynthia Soon from the University of Melbourne, Australia, showed a small increase in lifespan of SOD1 mice after administration of a copper complex known as Cu(ATSM). The mechanism by which this occurs is unknown at present.

Researchers from the University of Edinburgh, UK, have mutated the VAPB-like gene in fruit flies associated with MND in humans. Dr Guiseppa Penetta presented work showing that the fruit fly version of VAPB was important for synaptic remodelling and affected the number of contacts to muscle cells. The results suggest a role for VAPB in synaptic well being in general and also validate the use of evolutionarily distant species in MND research.

Dr Joan Coates from the University of Missouri, USA, detailed the result of spontaneous SOD1 mutations in dog species. In a similar fashion to humans, SOD1 mutations in canine species is characterised by progressive degeneration and limb paresis. This is the first know study to find a spontaneous SOD1 inherited motor neurone disease in a species other than humans and could possibly be used to evaluate therapeutic interventions.

SOD1 Pathogenesis

Research conducted at the Howard Florey Institute, Australia, implicates the endoplasmic reticulum in SOD1 pathogenicity. The endoplasmic reticulum is a compartment inside cells that is responsible for the production of mature proteins destined to be secreted from the cell. Dr Julie Atkin presented work demonstrating that endoplasmic reticulum stress is triggered by mutant SOD1. This stress is prior to inclusion formation. The mechanism of this effect on endoplasmic reticulum is not yet known. However, Adam Walker also from the Howard Florey Institute has been working on the protein PDI, which is an endoplasmic reticulum based molecular chaperone. When Dr Walker increased the levels of PDI in cells the SOD1 deposits were decreased. In fact, their research showed that a drug that mimics PDI decreases SOD1 aggregation and reduces endoplasmic reticulum stress.

Dr Victor Mulligan from the University of Toronto demonstrated, in the test tube, that SOD1 mutants upon stress lose their zinc atom more easily than the copper atom. The importance of the metals copper and zinc staying bound to the SOD1 enzyme is evidenced by the fact that zinc deficient SOD1 is more likely to aggregate and be toxic to cells.

Only a portion of the protein found in the ALS deposits is SOD1. Daniel Bergemalm from Umea University, Sweden, is attempting to address the question of what the other proteins in the MND protein deposits might be. Results have shown that only approximately 50% of the deposits consist of SOD1. Dr Bergemalm has found other proteins such as endoplasmic reticulum chaperones and intermediate filaments. The roles of these proteins in the pathology of MND are unknown.