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## Spotlight on TDP-43

The human body is a miraculous organised system that requires balanced regulation of every cell within it. The integrity of the genes that encode the body's instructions for growth and maintenance forms the basis of the body's wellbeing. Equally important is the process of translating these genes into proteins that carry out these instructions. But in certain cell types, genes and proteins are particularly vulnerable to losing their normal control, leading to disease. An exceptionally high maintenance cell type is the motor neurone (MN), and MND is now linked to a growing list of affected genes and proteins. TDP-43 is one such protein that pops up conspicuously often in MND.

Mutations in the gene encoding TDP-43 cause some familial forms of MND (fMND), but abnormally clumped (aggregated) TDP-43 proteins are found in the MNs of a significant proportion of people with MND, including those who carry mutations in other genes. As we will see in this report, studies of TDP-43 are revealing that its levels need to be kept in tight control in MNs in order for disease to be avoided. We will also go through the latest revelations concerning disease mechanisms in different variants of MND, plus the discovery of a new potential therapeutic.

## TDP-43 and FUS: Independent leaders in the RNA world

Two proteins that are known to be involved in MND, TDP-43 and FUS, are both normally located in the nucleus of cells and share similar functions involving their binding to the RNA molecules that are copied from genes. They also both regulate the production of proteins from these RNA molecules. Because of their similarities, an intriguing question has been whether their contribution to MND is due to shared mechanisms.

Claudia Colombrita and a group of researchers in Italy and France used a cell model of MND to assess and compare the effects of the loss of TDP-43 and FUS on production of specific RNA molecules and their encoded proteins.

Claudia and her team revealed that the RNA targets regulated by these two RNA-binding proteins (RBPs) are mainly distinct from each other. However they do share a few common target genes that are involved specifically in the development of neurones and in the organisation of the cell's "skeleton", the cytoskeleton. Furthermore they both affected genes that are also involved in regulating RNA molecules, revealing that TDP-43 and FUS are at the top tier of what is essentially the RBP hierarchy. This group went further to test the effect of mutated forms of TDP-43 on production of its RNA targets, and found that mutant TDP-43 proteins were still able to maintain their RNA-regulating activity. What this indicates is that the levels of TDP-43 and FUS in cells, particularly in MNs, need to be well regulated in order for them to carry out their functions correctly. Loss of either protein causes changes that are particularly detrimental to MNs because, despite sharing some very important biological activities, they seem to act independently in controlling different aspects of the development and structure of neurones.

## TDP-43: A question of balance

Although TDP-43 needs to be kept at high enough levels in cells to carry out its functions, too much of the protein is also dangerous for MNs. In degenerating MNs of around 95% of people with MND, this protein accumulates in the cytoplasm which is the area inside cells that lies outside the DNA-containing nucleus. TDP-43 usually spends most of its time in the nucleus, but it does have some functions in the cytoplasm.

So a key question that researchers want to find the answer to is *why* and *how* so much TDP-43 ends up accumulating in the cytoplasm, and how its balance can be restored.



## MND Research Shorts

- *Researchers in Canada have discovered that chronic lipopolysaccharide-induced brain inflammation contributes to aggregation of TDP-43. This tells us that reducing inflammation in the central nervous system is important in preventing and perhaps alleviating neurodegeneration associated with TDP-43.*
- *Fat, or adipose cells, can be turned into stem cells (ASCs) that produce a multitude of protective chemicals that promote neurone growth. Researchers in the US and China found that liquid solution conditioned by ASCs protected MNs from degeneration in a SOD1-MND mouse model.*
- *Scientists in the US have discovered that the major protein implicated in Alzheimer's disease, APP, is affected by the same impaired protein transport mechanism as TDP-43, FUS and SOD1 in MND; a mechanism requiring a cellular machine called the endoplasmic reticulum.*
- *The C9orf72 gene mutation associated with 1/3 of fMND cases causes changes in the levels of different forms of the encoded protein in the MNs of affected patients. However, studies into the specifics of these changes have been lacking. Researchers in the US addressed this knowledge gap by examining patients carrying the mutation. They discovered that one specific form of C9orf72 was associated with a survival advantage in patients who produced that form, while other abnormally shortened forms of C9orf72 contributed to disease mechanisms.*

## When sharing is not caring: Prion-like spread of SOD1 in MND



Degeneration in MND characteristically begins in one localised area in the body and then progressively spreads. This process of spreading has been likened to the spread of prions in diseases such as Creutzfeldt-Jakob disease and “Mad Cow” disease. Prions are disease-causing forms of proteins that have

the ability to act as templates and convert other normal proteins into prions, thereby enabling disease to spread. Studies of MND have produced more and more evidence highlighting the centrality to disease of disruptions in the ways cells produce, manage and turnover proteins (proteostasis), and the involvement of protein aggregation. Protein aggregates are seen in the diseased MNs of all people suffering MND. Aggregates of SOD1 are found in a subset of these people, and cell-to-cell transmission of SOD1 aggregates has been shown in several studies, but the precise mechanism of this transmission has remained elusive. Addressing this issue, Rafaa Zeineddine of the University of Wollongong together with a team of researchers sought to define precisely how MNs take up SOD1 aggregates that have been released from neighbouring cells. They found that SOD1 aggregates interact with the surface of cells and stimulate a protein called Rac1 that induces the cell surface to ruffle and engulf substances surrounding the cell, a process called macropinocytosis. Via macropinocytosis, SOD1 aggregates are able to enter the cell where they convert normal forms of SOD1 into aggregates in much the same way as prions. Thus it appears that in addition to disrupted proteostasis in individual cells, disease is able to progress through the body as cells share their protein aggregates via macropinocytosis, a process that will now require attention in the development of an effective therapeutic.

### One more thing that changes as we get older

MND is an age-related disease; in most cases the age of onset is after 40 years of age. As we have already discovered, TDP-43 is found clumped together with other aggregated proteins in the cytoplasm of MNs in around 95% of MND patients. This means that less TDP-43 is in the nucleus and its role in regulating genes is impaired. However findings from a research team in Trieste, Italy, have revealed that this reduction in TDP-43 functionality isn't critical in young cells, but becomes a problem as cells age. Lucia Craganz and her group used a well-established fruit fly model of MND to examine the effects of TDP-43 reduction on the fly nervous system. They found that aggregated proteins “hijacked” TDP-43 molecules, clumping them into the aggregates, but this had no effect on the locomotion of the flies until they reached adulthood. Investigating this further, Lucia's team discovered that younger flies produced greater amounts of TDP-43 than older flies, thus were able to overcome the loss of TDP-43 into aggregates. This ability to compensate for TDP-43 loss declined with age as the flies produced less and less TDP-43.



Lucia gathered evidence showing that this reduced production of TDP-43 is an evolutionarily conserved process that likely also occurs in human cells as they age. What this ultimately suggests is that aggregated TDP-43 can exist in MNs of people at a young age without causing disease, but as we get older the lower of amounts of TDP-43 in the nucleus become critical and can contribute to MN dysfunction.

### RNA engineers re-establish control of C9orf72

Many people with fMND carry a mutation in the *C9orf72* gene in which a sequence of the DNA code, GGGGCC, is erroneously repeated hundreds to thousands of times. This repeat expansion causes the formation of structures made of RNA, RNA foci, that can capture proteins



in the cell. Expansions of the reverse sequence, CCCCGG, are also thought to be harmful. Researchers have thus had the aim of stopping the formation of RNA foci as a way to treat people carrying *C9orf72* mutations, a task that has proved challenging. Jiaxin Hu and others in Dallas, Texas, have devised a way of doing this by engineering duplex RNA molecules that can specifically bind to the GC-rich repeat expansion RNA. These duplexes are made of two linked strands of RNA that are designed to recruit an important mechanism called RNA interference (RNAi) that degrades unwanted RNA and stops the formation of their encoded proteins. This level of control over MND-linked genes and their encoded RNAs is anticipated to be a very important step in the treatment of MND.

### C9orf72 is no spelling bee

Many neurological diseases are associated with what we can think of as bad spelling in the language that is DNA, in which repetitive DNA



“letters” expand in the “words” that are genes. The GGGGCC repeat expansion in *C9orf72* is an example of this. This poor spelling causes DNA to form a variety of abnormal structures that can disrupt DNA replication, a process that is critical for our DNA to copy itself when our cells divide. By disrupting proper replication, repetitive DNA sequences can expand in the DNA and seriously interfere with the production of their encoded proteins. But how the repeat DNA sequences actually expand has remained a mystery. That is until Ryan Griffin Thys and Yuh-Hwa Wang decided to investigate it. These two researchers at the University of Virginia in the US investigated the effect of the length and structural orientation of the repeat DNA on the replication process. Using a human cell model of *C9orf72*-MND they discovered that the length of the repeat expansion determined how unstable the DNA molecule was. It also determined the efficiency of the replication process, and this was further affected by the orientation of the repeat sequence relative to the replication start site in the DNA. What is significant about these findings is that we now have an idea of how repetitive DNA sequences actually expand, so we can now work on targeting this mechanism to prevent expansion and its contribution to disease severity.