RNA metabolism’s role in amyotrophic lateral sclerosis

Distinguished University Professor Michael Strong describes how he first became involved in amyotrophic lateral sclerosis research and the evolving concepts that are changing our understanding of the disease.

What first inspired you to research the pathobiology of amyotrophic lateral sclerosis (ALS)?

As a final year medical student studying at Queen’s University in Kingston, Ontario, I had the opportunity to spend three months in Denmark where I found myself on a neuromuscular service that was also the national referral centre for ALS. I was intrigued by the number of cases of a disease that I was taught was sufficiently rare that I might see only one or two in my lifetime. When I found out that Denmark has about the same incidence rates as are seen worldwide, I began reading more about the disease.

When I began my training as a neurologist at Western University, Canada, I studied with Dr Arthur Hudson who had written a great deal about the disease and established the second ALS clinic in North America. He really influenced my decision to do postdoctoral work on the topic.

In recent years, our understanding of ALS has changed to reflect the fact that it is a multisystem degenerative disorder, not a single-system one as once believed. Can you explain why developing our knowledge is important?

This needs to be viewed in two frameworks: at the level of cell biology and clinically. From the vantage of the cell, understanding that ALS can be at the intersection of a number of seemingly divergent biological processes going awry opens opportunities to develop individualised therapies. The analogy here is cancer biology in that multiple pathways can give rise to malignant transformation, so there are multiple ways as it currently stands, even if we only have alterations in RNA metabolism and impaired proteasome function as the two major pathways, we already have two seemingly unique pathways. Now, we need to understand how these can be identified.

Clinically, understanding that there is a significant percentage of the patient population who will have frontotemporal dysfunction immediately changes the game: is this an intersection of two disease processes or a single disease with the potential of multiple systems involvement?

How has your research contributed to improving the potential for novel ALS therapies?

If the basis of ALS is a disorder of RNA metabolism, and if this is due to alterations in the expression of specific RNA binding proteins, these proteins never exist in their native state. They are always going to be modified in a post-translational manner and this modification alters their function. So the question is: can we identify which state is the key interacting state and then identify drugs that will alter this state?

Could you discuss some of the evolving concepts in RNA metabolism with relevance to your work?

The concept that ALS may be a disorder of RNA metabolism for the majority of patients continues to evolve. We know that in response to injury or stress, the cell will divert its genesis of proteins towards those that are important to respond. It makes sense that the cell would ‘bundle’ together a number of these proteins and their related regulatory proteins into a single upregulation of gene expression at the level of the DNA. How this is regulated is less certain.

In what direction is research into RNA metabolism and ALS currently heading?

New questions are arising about how RNA granules are formed. DNA transcription leads to the production of the respective RNA species that are bundled together into granules within the nucleus and then transported into the cytosol. Depending on their composition, these granules may be loaded onto the transport mechanisms of the cell and sent where they are required (stress granules) or targeted for degradation (processing bodies). In being degraded, RNA transcripts are acted upon by microRNAs whose function is to regulate RNA degradation in a process known as RNA-mediated gene silencing.

The exciting concept is that these are formed as hydrogels – matrices of proteins that come together and disassociate ‘freely’ (nothing is free in the cell), so that by interchanging components, the function of the granule changes readily. There is recent work to suggest that the formation of the pathological RNA granules of ALS may in fact take their origin from these hydrogels. If so, this fundamentally changes how we think about the alterations in RNA metabolism that underlie this process.
In search of a cause

Thanks to groundbreaking research carried out in the Department of Clinical Neurological Sciences and the Robarts Research Institute at Western University in Canada the amyotrophic lateral sclerosis community has a clearer understanding of the disease’s pathogenesis than ever before.

IN 1923, A young man named Lou Gehrig spiced up the American baseball scene with his stamina and hitting prowess. A seven-time All-Star player and named the most valuable player on several teams that won the World Champion series, Gehrig was considered one of the best players of his day. It seemed like nothing would stop him.

In 1939, that all changed. Gehrig announced to his adoring public that he was leaving the game as he had been diagnosed with amyotrophic lateral sclerosis (ALS) – a disorder also known as Lou Gehrig’s disease.

WHAT IS ALS?
The ALS Association defines the disorder as a progressive neurodegenerative disease that affects nerve cells in the brain and the spinal cord. With time, these cells degrade and die, giving way to muscle weakness and atrophy throughout the body as the brain loses control. Eventually, ALS can lead to paralysis, respiratory failure and even death.

While an aggressive loss of motor neurons does indeed occur with ALS, more recent research has uncovered the fact that more than half of those affected with the disease also develop one or more syndromes of frontotemporal dysfunction. The involvement of non-neuronal cells has altered the classical single-system view, and ALS is now recognised as a multisystem degenerative disorder.

HALLMARKS OF DEGENERATION

The question for Strong, and the ALS community as a whole, is to find out what is happening in RNA metabolism that leads to the degeneration of motor neurons. Strong started this investigation by looking at hallmarks of motor neuron degeneration in ALS: neuronal cytoplasmic inclusions (NCIs). These inclusions contain neurofilament (NFL), a major structural component of the neuronal cytoskeleton that can be divided into three grades (light, medium and heavy) and fit together to form a final structural filament.

Looking at ALS cases, Strong and his team found that the expression ratio for the smallest NF (NFL) came up short. This scenario has been shown to cause motor neuron degeneration in transgenic mice. According to the researchers, there are two main possibilities for this deficiency in NFL: either it is a problem at the DNA level concerning reductions in gene expression, or it is down to the loss of RNA. “I really don’t like working with DNA so we did a ‘Hail Mary pass’ and designed an experiment that would test the RNA question,” recalls Strong.

Strong’s lab has led to profound shifts in the way humanity understands the biology of ALS.

The risk of bypassing the DNA angle paid off – it proved that the issue of RNA stability is indeed a major factor in explaining ALS pathogenesis. His team discovered that the NFL deficiency is caused by an instability in its RNA (NEFL RNA) that leads to the loss of the NFL transcript. Furthermore, it turns out that the fate of the messenger RNA that makes the NFL protein (NEFL mRNA) is targeted for degradation through RNA-mediated gene...
CLINICAL NEUROLOGICAL SCIENCES

OBJECTIVE
To understand the role of alterations in RNA metabolism as a fundamental disease process underlying the motor neuron degeneration of amyotrophic lateral sclerosis (ALS).

KEY COLLABORATORS
Dr Heather Durham, Montreal Neurological Institute, Canada
Dr Christine Vande Velde, University of Montreal, Canada
Dr Eran Hornstein, Weizmann Institute of Science, Israel
Dr Robert Bowser, Barrow Neurological Institute, Arizona

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CONTACT
Professor Michael Strong
Dean, Schulich School of Medicine & Dentistry, Distinguished University Professor
Western University
Don Rix Clinical Skill Building, 3rd Floor
London, Ontario, N6A 5C1
Canada
T +1 519 663 3874
E michael.strong@schulich.uwo.ca

MICHAEIL STRONG is Dean of the Schulich School of Medicine and Dentistry at Western University, a Distinguished University Professor and the Arthur J Hudson Chair in ALS Research. His basic research focuses on the study of alterations in neurofilament metabolism in degenerating motor neurons in ALS and how perturbations in RNA metabolism contribute to this process. His clinical research has been central to identifying the frontotemporal syndromes of ALS.

silencing. "Such alterations placed the motor neuron at an increased risk for both oxidative and glutamatergic injury," Strong notes.

BIOMARKERS TO SPOT THE DIFFERENCE
In cases of familial ALS, where there are mutations in superoxide dismutase 1 enzyme [SOD1] giving rise to the disease in some families, a protein is present that causes the loss of NEFL mRNA stability. Taken on its own it is difficult to draw a solid conclusion. However, upon learning at a conference that TAR DNA-binding protein 43 (TDP-43) aggregates could be found in ALS and that it is a dual DNA- and RNA-binding protein, Strong’s interest was piqued. “Within three months my lab showed that TDP-43 was an RNA-binding protein, that it interacted with NEFL mRNA and that it was a stability determinant," he states.

Strong and his team have shown that among the mRNA-binding proteins that contribute toward the stability of NEFL mRNAs is a collection of proteins – including mutated SOD1 and TDP-43 – which are now known to associate within the NCl s in ALS spinal motor neurons. Not only that, but these mRNA-binding proteins have been identified in association with specific variants of familial ALS. Based on their known roles in motor neuron degeneration, Strong employed these proteins as disease biomarkers in order to develop a novel means for discriminating between the individual variants of ALS [with or without genetic mutations]. Using a colorimetric immunohistochemistry [IHC] approach, this was the first study to try and exploit the distinctive immunoreactivity patterns of spinal motor neuron NCIs for such a purpose.

He found that the unique IHC signature of mutated SOD1 makes it possible to differentiate these cases from other ALS variants.

"Potentially, a routine IHC-based analysis of spinal motor neurons could be a huge help in identifying familial cases not previously thought to possess mutations of SOD1," he enthuses.

A PUZZLE OF A PROCESS
Strong’s groundbreaking studies have significantly impacted the way motor neuron degeneration in ALS is understood, bolstering his hypothesis that changes to RNA metabolism, while not the sole pathway, are major players in the aetiology of the disease. Indeed, it is the presence of NCIs that are considered to be ALS’s key neuropathological hallmark.

What is not certain, however, is the process leading to the formation of such pathological inclusions. Strong is looking at the cellular response to environmental stress – the formation of stress granules. "It’s the million dollar question. How do environmental conditions lead to these pathological inclusions?" he asks. By potentially altering post-translational modifications in RNA-binding proteins, environmental stress, along with RNA-binding proteins already prone to aggregation, could change the formation and function of stress granules. Strong thinks that these stress induced changes may in turn be responsible for producing protein aggregates that significantly impact RNA metabolism.

There are many questions that need to be answered before the pathogenesis of ALS can be fully revealed. If Strong’s hypotheses are correct, what then determines which RNA species are chosen and bundled into a granule? How is it decided which microRNA interact with which RNA species and in which order? Furthermore, how are these dysregulated in ALS? Answers to these questions may be slow to reveal themselves, but it is only through landmark research like Strong and his lab’s that these questions can be asked at all.