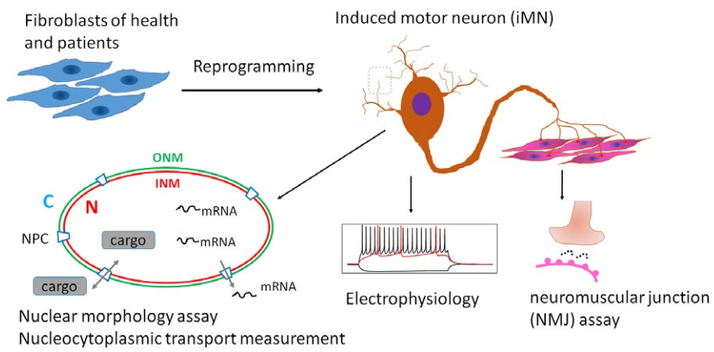
 Baojin Ding

Patient-specific neurons key in new dystonia discoveries

Dystonia, a movement disorder, currently has limited treatment and no cure. The current disease models of dystonia have limitations. Although animal models are available, they do not give a full understanding of cellular disruptions, and patient brain tissues are inaccessible to study. Dr Baojin Ding and his team at Louisiana State University Health Sciences Center, Shreveport, have developed a new disease model using reprogrammed patient-specific neurons from dystonia patients' skin cells. Exciting studies using these models have discovered a new potential target for treatment in the form of the nuclear protein, LMNB1.

Dystonia is a neurological movement disorder that induces involuntary muscle contractions, causing the body to twist into uncomfortable positions that result in cramps or spasms. The disorder has varying levels of severity between patients: it can be localised or it can affect the whole body, and the symptoms can be continuous or intermittent. Dystonia is painful and affects the daily life of patients, making normal activities difficult. Although treatments are available to alleviate symptoms for some patients, currently there is no cure for the condition.

There are many different causes of dystonia, such as brain trauma or a stroke, which can lead to the disorder occurring later in life. However, it can also be hereditary. The most common inherited form is DYT1 dystonia (also known as childhood-onset Torsin dystonia). DYT1 dystonia is caused by a deletion mutation in the *TOR1A* gene, which leads to dysregulation of the Torsin A protein that this gene codes for.



Baojin Ding and his team investigate the complexity of the cellular pathology of dystonia.

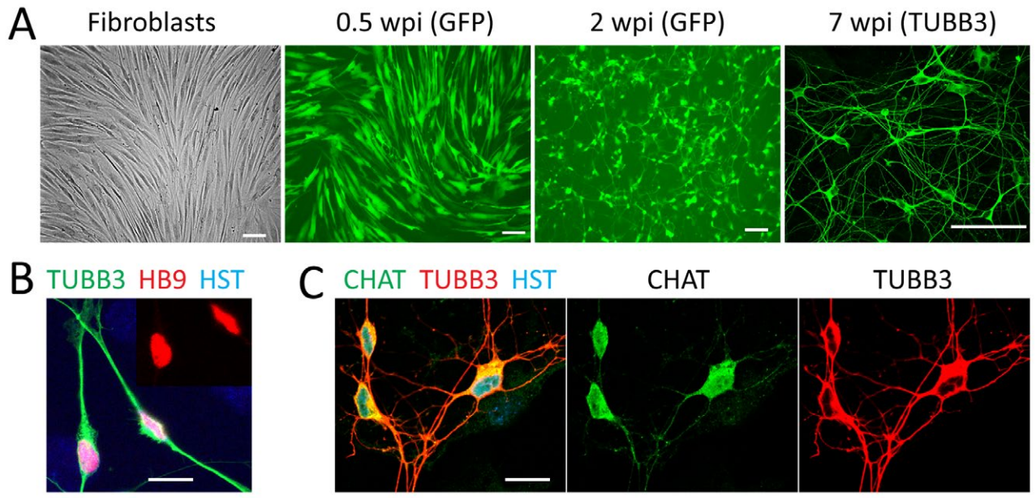
THE ROLE OF TORSIN A

Torsin A is an enzyme (an 'ATPase') involved in many essential cellular functions throughout the central nervous system. To carry out its roles effectively, Torsin A must interact and bind with various other proteins. The deletion mutation to the gene seen in DYT1 patients blocks this binding, resulting in the disruption of many cellular processes. Torsin A is located in the endoplasmic reticulum (where proteins are made within the cell) and in the nuclear envelope (the structure that separates the nucleus from the rest of the cell), where it is involved in maintaining the structure of the nuclear envelope. In addition, Torsin A plays a role in arranging nuclear pore subunits, which are vital for transporting molecules into and out of the nucleus.

Due to its involvement in many processes, the mutation in the *TOR1A* gene and its subsequent effect on the Torsin A protein causes chaos in cells and leads to dystonia. However, it is unclear what exactly drives the disorder and symptoms. Shedding some light on which processes are affected is vital for finding targets that could be used for treatment.

STUDYING DYSTONIA

Previously, DYT1 dystonia has been studied using mouse animal models



Skin cells (fibroblasts) are gradually converted into neurons during the reprogramming process.

that mimic the deletion seen in the *TOR1A* gene. These mouse models have shown that many parts of the brain are affected by this genetic change, as expected due to the important role of Torsin A. They have also revealed abnormal nuclear morphology (characterised by 'blebbing' or 'bulging'), with the nuclear envelope being affected in various parts of the central nervous system in these mice. However, while these models give us a clearer understanding of molecular level disruptions, they do not present the usual symptoms associated with the disorder in humans. Notably, the mice do not exhibit any muscular contractions seen in patients, suggesting that there are species-dependent factors at play and we are not getting the full picture from these animal models. This presents a problem, particularly since patient neurons are also inaccessible compared to many other cell types, making them very challenging to study.

NEW HUMAN CELL MODELS

It is here that Dr Baojin Ding and his team at Louisiana State University Health Sciences Center, Shreveport (LSU Health Shreveport) have provided some vital answers to elucidate the complexity of the cellular pathology of dystonia. Instead of directly taking motor neurons from patients, they generated them by reprogramming cells using two different methods. The first was a direct conversion of patients' skin cells (called fibroblasts) into motor neurons. The second method used induced pluripotent stem cells (iPSCs) which were then differentiated to motor neurons.

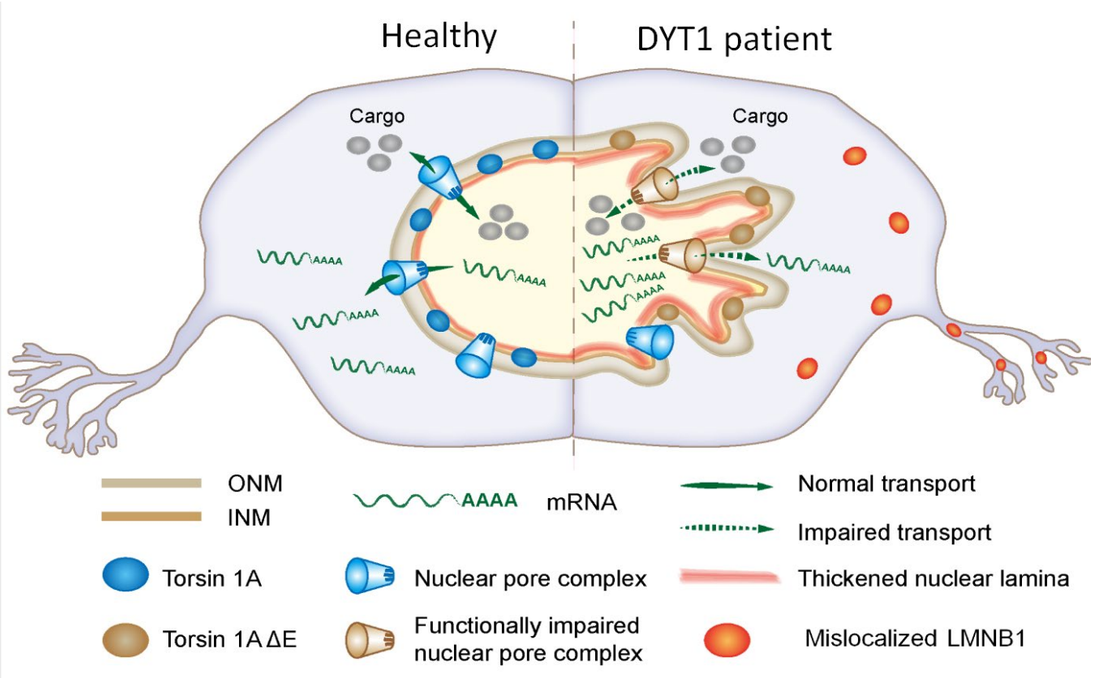
A series of experiments demonstrated that both of these methods for reprogramming human neurons were successful. The physical forms of the cells had changed shape to become more neuron-like and they also expressed specific neuronal proteins and markers, confirming their neuronal identity. Excitingly, these new patient-specific motor neurons could now be used to answer questions left unclear by previous animal models.

To overcome the limitations of inaccessible brain tissues, the researchers generated patient-specific neurons from skin cells.

DISRUPTIONS IN THE NUCLEUS

Using their new disease models, the LSU Health Shreveport researchers are starting to unravel the molecular factors involved in dystonia patient cells. As previous studies show that the Torsin A protein has a role in nuclear import and export, Torsin A deletion (seen in dystonia patients) could therefore negatively affect this nucleo–cytoplasmic transport. This was confirmed in studies carried out by Ding and his team, using both types of patient-specific neurons.

One set of experiments used fluorescently-labelled mRNAs (the RNA that makes proteins) to track these molecules in the induced neurons. In normal control cells, the mRNAs were mostly in the cytoplasm as expected. Conversely, in the cells derived from DYT1 patients, the mRNAs remained stuck in the nucleus. This transport of mRNAs was not the only cellular process affected. Similar experiments highlighted disruption to protein transport. The nuclear pore complex was studied more closely using electron microscopy to take a closer look at what was causing these changes. This revealed that there were far fewer of these complexes in patient cells, confirming the role of Torsin A in nuclear pore formation and highlighting its role in nuclear transport.



A working model elucidates the possible pathogenesis underlying DYT1 dystonia.

The studies also revealed that the nuclear envelope itself was affected, as well as transport across it. The nuclear morphology was vastly different in patient cells compared to controls. While the controls still had a smooth nucleus, patient-specific cells exhibited nuclei with sharp angles, folds, and a thicker nuclear lamina. This change to the shape of the nucleus was also seen in animal models (and therefore was not unexpected) but it presented itself in different ways. For example, the blebbing seen in mice did not appear in these cells. This once more confirms that there are many differences between the human and animal models for dystonia, highlighting the importance of the new models developed by Ding and colleagues.

PROTEIN MISLOCALISATION

Following on from their discovery that the nuclear morphology in DYT1 neurons is altered, further experiments were undertaken focusing on the nuclear envelope and nuclear lamina. Notably, these demonstrated that nuclear lamin B1 (LMNB1) was mostly found in the cytoplasm rather than the nucleus. It was also found at a higher concentration in the DYT1 cells. LMNB1 is a nuclear protein that contributes to the structural integrity of the nucleus, therefore this dysregulation in terms of location and concentration is key to the disruption to nuclear morphology seen in DYT1 dystonia.

To confirm that this effect was caused by the changes to the Torsin A protein in patients, the same *TOR1A* mutant was expressed in the control cells.

Discoveries from the Ding laboratory offer a hopeful outlook for the future of dystonia patients.

The control cells then showed the same increase of LMNB1 levels in the cytoplasm as the patient-specific cells.

In another set of experiments, LMNB1 was downregulated in the patient-specific neurons (meaning that it was no longer expressed at high levels). Interestingly, this counteracted the previously seen mislocalisation: LMNB1 was mainly found in the nucleus as would be expected in normal conditions. It also reversed the changes to nuclear morphology and mRNA nuclear transport. These factors clearly contribute to symptoms in patients, so being able to reverse them is incredibly promising.

A NEW TARGET

The discoveries from the Ding laboratory offer a hopeful outlook for the future of dystonia patients. As always, any clarity that can be gained into what is going on at a cellular level can point us to new paths to be explored. Here, the researchers have learnt that the key pathological changes in patient-specific models are in nuclear transport, nuclear morphology, and protein localisation. In addition, their studies offer a new target to focus on: LMNB1. Notably, the downregulation of LMNB1 leads to a reversal of the key pathologies seen in the disease models. If this could be targeted in a clinical setting it could change the lives of patients for the better.

Behind the Research

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Research Objectives

Understanding the molecular mechanisms in neurodevelopment and neurodegeneration, and modelling neurological diseases using patient-derived neurons.

Detail

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Bio
Dr Baojin Ding received his PhD in Biochemistry and Molecular Biology from Louisiana State University and his postdoctoral training in Neurosciences at the University of Massachusetts Medical School. He has worked as a research faculty for two years at UT Southwestern Medical Center. He built his independent laboratory in the University of Louisiana at Lafayette in 2018, and then moved to Louisiana State University Health Sciences Center-Shreveport in early 2022.

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NIH grant (NIH/NINDS NS112910 to BD) and Department of Defense (DoD) Peer Reviewed Medical Research Program (PRMRP) Discovery Award (W81XWH2010186 to BD).

Collaborators
Research colleagues and students who are co-authors of Dr Ding's research articles.

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Personal Response

What do you think the next steps are after this exciting LMNB1 discovery?

"In the next steps, two major research lines will be focused. One is to further validate these findings in clinical samples from dystonia patients. The other is to elucidate the molecular mechanisms underlying dysregulations in DYT1 neurons, including how torsin mutant causes LMNB1 mislocalisation and how dysregulated LMNB1 contributes to dystonic symptoms."