**Perspective**

**Novel insights into the pathogenesis of DYT1 dystonia from induced patient-derived neurons**

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**Dystonia** is a common movement disorder characterized by sustained or intermittent muscle contractions causing abnormal movements and/or postures (Keller Sarmiento and Mencacci, 2021). The dystonic syndromes are classified as primary dystonia (dystonia is the only motor feature without tremor) and the secondary dystonia (dystonia is combined with other movement disorders, such as Parkinsonism).

Based on the age of onset, dystonias are also dichotomously classified as childhood onset or adulthood onset. The distribution of affected body parts may change over time and progressively spread of dystonia to previously uninvolved sites. Because the clinical characteristics and underlying causes of dystonia are very heterogeneous, the pathological mechanisms of dystonia remain largely unknown. The diagnosis and etiological definition of this disorder remain challenges. The current therapies, such as anticholinergics, intramuscular botulinum toxin injection and deep brain stimulation, are largely symptom-based and only partially satisfactory (Balint et al., 2018).

The childhood-onset torsin dystonia, also called DYT1 dystonia, represents the most frequent and severe form of hereditary primary dystonia, providing an excellent example to understand the pathogenesis of this disease (Gonzalez-Alegre, 2019; Keller Sarmiento and Mencacci, 2021).

Most cases of typical DYT1 dystonia are caused by a heterozygous 3-bp deletion in exon 5 of the TOR1A gene, resulting in the loss of one of the two adjacent glutamate residues (E302 or E303) in the carboxy-terminal region of TOR1A protein (ΔE) (Keller Sarmiento and Mencacci, 2021). Protein TOR1A is a member of conserved AAA++ (ATPases Associated with diverse cellular Activities) ATPase, which typically use the energy of ATP hydrolysis to disassemble protein complexes in diverse biological processes, such as membrane trafficking, cytoskeleton dynamics, vesicle fusion, response to stress, and the biogenesis of nuclear pore complex (Laudermilch and Schlieker, 2016; Rampello et al., 2020).

In humans, TOR1A is widely distributed throughout the central nervous system with high expression in cerebral cortex, striatum, substantia nigra pars compacta, hippocampus, cerebellum and spinal cord, underscoring its critical roles in neural development and functions. At the cellular level, most TOR1A proteins are embedded in the lumen of the endoplasmic reticulum and the endomembrane space of nuclear envelope. The activity of TOR1A depends on the association with one of two transmembrane co-factors, LAP1 (lamina associated polypeptide 1, also named TOR1AIP1) and LULL1 (laminal domain like LAP1, also named TOR1AIP2), suggesting that TOR1A works in a membrane-spanning machinery (Laudermilch and Schlieker, 2016). Although ΔE has been shown to be a loss-of-function mutation, the actual functions of the TOR1A protein in DYT1 dystonia and how it leads to changes in the nervous system to cause dystonia remain poorly understood.

While rodent models provide insights into disease mechanisms, significant species-dependent differences exist because animals with the identical heterozygous mutation fail to show pathology (Gonzalez-Alegre, 2019). However, inaccessibility to patient neurons greatly impedes our understanding of the pathological mechanisms for dystonia.

It is fascinating that reprogramming of human neurons from adult fibroblasts provides an unprecedented approach to deciphering the molecular pathogenesis underlying neurological diseases. Recently, we modeled DYT1 by using human patient-specific cholinergic motor neurons (MNs) that are generated through either direct conversion of patients’ skin fibroblasts (Ding et al., 2020) or differentiation of induced pluripotent stem cells (iPSCs) (Sepehrimanesh and Ding, 2020; Ding, 2021). These human MNs naturally localize in the cytoplasm (Figure 1H). In DYT1 MNs, upregulated LMNB1 causes thickening of nuclear lamina, thereby leading to abnormal nuclear morphology and impaired NCT, resulting in nuclear accumulation of mRNAs and mislocalized proteins (Figure 1H). Progressive LMNB1 accumulation and subcellular mislocalization are critical pathological features of DYT1 MNs, providing a novel molecular target for therapeutic intervention.

To further understand the dysregulation of LMNB1 underlying the pathogenesis of DYT1 dystonia, the following questions need to be addressed in future studies (Figure 1H). First, to examine the neuronal subtype specificity of LMNB1 dysregulation in DYT1 cells. Dysregulation of LMNB1 has been recapitated by either ectopic expression of the mutant TOR1A gene or shRNA-mediated downregulation of endogenous TOR1A in healthy control MNs, suggesting that LMNB1 mislocalization are caused by TOR1A hypoactivity (Ding et al., 2021). In non-MNs that were generated by spontaneous differentiation of DYT1 neuronal progenitors and differentiation of human SH-SYSY neuroblasts with ectopic and the spinal cord, leading to movement problems (Kohler et al., 2018). Similarly, I think the increased LMNB1 also contributes to these cellular abnormalities in DYT1 neurons. Consistently, overexpression of LMNB1 indeed disrupts nuclear morphology (Figure 1F). Downregulation of LMNB1 can largely ameliorate all the cellular deficits in DYT1 MNs, including neurodevelopment, nuclear morphology, NCT activities (Figure 1G). These results indicate that dysregulation of LMNB1 may constitute a major molecular mechanism underlying DYT1 pathology. Importantly, this study describes the first cellular model system for human Dystonia using techniques of direct conversation and iPSC system, revealing the great value of disease modeling with human patient-derived neurons.

Traditionally, dystonia has been considered a disorder of the basal ganglia, a subcortical nuclei involved in diverse functions including motor control and motor learning. Later studies suggest that dystonia is a network disorder, with involvement of a basal ganglia–cerebellum-thalamo-cortical circuit (Balint et al., 2018). Interestingly, recent reports also indicated that the cerebellum and the spinal cord could be other major sites of dysfunction in Tor1a mutant mice (Zhang et al., 2017). This finding that deficits of presynaptic inhibition caused by impaired GABApep neurons in the spinal cord suggest that the abnormalities of proprioceptive and/or muscle spindle feedback contribute to the DYT1 dystonia (Zhang et al., 2017). Using patient-derived MNs, our study further provides evidence that lower MNs are implicated in the pathogenesis of dystonia (Ding et al., 2021). In healthy MNs, nuclear morphology, neurite outgrowth and NCT activities are normal. The majority of mRNAs localize in the cytoplasm (Figure 1H). In DYT1 MNs, upregulated LMNB1 causes thickening of nuclear lamina, thereby leading to abnormal nuclear morphology and impaired NCT, resulting in nuclear accumulation of mRNAs and mislocalized proteins (Figure 1H). Progressive LMNB1 accumulation and subcellular mislocalization are critical pathological features of DYT1 MNs, providing a novel molecular target for therapeutic intervention.

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expression of ΔE, no mislocalized LMNB1 can be noticed (Ding et al., 2021), suggesting that LMNB1 mislocalization could be MN-specific. A recent report that nuclear increase in LMNB1 also contributes to nuclear dysfunction in Huntington’s disease (Alcala-Vida et al., 2021). Other DYT1 neural subtypes should be examined to test whether TOR1A hypacoactivity causes LMNB1 mislocalization in a MN-specific manner. Second, to verify these abnormalities in clinical samples, if these abnormalities identified using in vitro cellular system also present in clinical samples, these findings will lead to potential therapeutic strategies for DYT1 dystonia. Third, to identify nuclear accumulated transcripts in DYT1 neurons. Impaired nuclear export activity in DYT1 neurons causes the nuclear accumulation of mRNAs (Figure 1H). Are these mRNAs proportional to all transcripts or do they represent some particular subset that are involved in certain neural functions? Do the retained mRNAs share common sequences or secondary structures? These questions could be addressed by biochemical approaches of RNA isolation together with the next-generation sequencing to identify mislocalized transcripts. Fourth, to decipher the molecular mechanisms of how TOR1A ΔE causes dysregulation of LMNB1 and how mislocalized LMNB1 contributes to DYT1 dystonia. Protein co-immunoprecipitation coupled with mass spectrometry analysis could be used to identify ΔE-disrupted factors and mislocalized LMNB1-interacting proteins in DYT1 MNs. The abnormal protein-protein interactions and/or disrupted signaling pathways could provide novel insights into the dysfunctions of DYT1 neurons. No matter RNAseq assay or co-immunoprecipitation coupled with mass spectrometry analysis, generation of patient-specific MNs with high purity and yield is absolutely required. Excitingly, we have developed a protocol by which highly pure patient-specific MNs could be generated from iPSCs (Sepehrimanesh and Ding, 2020; Ding, 2021), providing a solid foundation to address these questions using biochemical approaches.

This work was supported by National Institute of Neurological Diseases and Stroke, No. NIH/ NINDS NS112910 (to BD) and Department of Defense (DoD) Peer Reviewed Medical Research Program (PRMRP) Discovery Award, No. W81XWH2010186 (to BD).

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Date of submission: March 21, 2021

Date of review: April 23, 2021
Date of acceptance: May 26, 2021
Date of web publication: July 30, 2021

https://doi.org/10.4103/1673-5374.320978

How to cite this article: Ding B (2021) Novel insights into the pathogenesis of DYT1 dystonia from induced patient-derived neurons. Neural Regen Res 17(3):561-562.

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Open peer reviewer: Carlo Colosimo, Santa Maria Hospital of Terni, Italy.

Additional file: Open peer review report 1.

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P-Reviewer: Colosimo C; C-Editors: Zhao M, Liu WJ, Li JY; T-Editor: Jia Y