NEURODEVELOPMENTAL DISORDERS

Getting with the reprogram

Timothy syndrome is a rare monogenic neurodevelopmental disorder that can be associated with autism. The underlying genetic defect in this syndrome is a point mutation in *CACNA1C*, which is the gene that encodes the α_1 subunit of the L-type voltage-gated calcium channel Cav1.2. This mutation is known to affect the inactivation properties of Cav1.2, but the cellular defects that arise from these altered properties remain unclear, as does how these defects cause neurological manifestations. Using induced pluripotent stem cells (iPSCs) derived from patients with Timothy syndrome, Dolmetsch and colleagues now show that this disorder is associated with impaired cortical neuron differentiation and abnormal catecholamine signalling.

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The authors isolated skin fibroblasts from two patients with Timothy syndrome and three control individuals and used somatic cell reprogramming to turn these fibroblasts into iPSCs. In turn, these stem cells were differentiated into cortical neural progenitor cells (NPCs) and neurons.

Through use of single cell reverse transcriptase PCR to detect genetic markers of neurotransmitter type and cortical layer identity, the authors characterized the iPSC-derived cultures. Interestingly, cultures from both patients and healthy individuals exhibited similar numbers of neurons and showed no differences in the proliferation and migration of NPCs. Patch clamp recordings and calcium imaging, however, revealed differences between the cultures in the physiological properties of neurons. Notably, Timothy syndrome neurons exhibited wider action potentials than did control neurons and showed a considerable rise in the sustained intracellular calcium level following depolarization.

The authors assessed the effects of the Timothy syndrome mutation on activity-dependent gene expression in the iPSC-derived cultures, as Cav1.2 has a crucial role in this process. They found that after depolarization, 135 genes were upregulated and 88 were downregulated in Timothy syndrome neurons compared with control neurons. Of note, several of these differentially regulated genes were linked to catecholamine signalling.

Dolmetsch and colleagues examined whether the genetic defect underlying Timothy syndrome affected neuronal differentiation. Compared with control cultures, Timothy syndrome cultures had a decreased number of neurons expressing lower cortical-layer markers but an increased number of neurons expressing higher cortical-layer markers, suggestive of deficits in neuronal differentiation. They also found that Timothy syndrome cultures were associated with a specific deficit in callosal projection neurons.

The authors went on to show that the proportion of neurons expressing tyrosine hydroxylase — a marker of catecholamine synthesis — was greater in the Timothy syndrome cultures than it was in the control cultures and that this increased expression was associated with higher levels of noradrenaline production. Interestingly, analysis of the Timothy syndrome cultures revealed that the increase in tyrosine hydroxylase expression was not associated with a specific neuron class, suggesting that the mutation affecting Cav1.2 does not drive cell fate towards a catecholamine neuronal type.

Finally, the authors assessed whether the increase in tyrosine hydroxylase expression could be reversed by targeting L-type calcium channel activity. Strikingly, roscovitine, which promotes the inactivation of such channels, was able to reduce the number of tyrosine hydroxylase-positive neurons in Timothy syndrome cultures by 68%, confirming that a lack of Cav1.2 inactivation underlies the rise in tyrosine hydroxylase expression.

Here, the authors identified a number of neuronal cellular deficits that are associated with Timothy syndrome. Interestingly, abnormal catecholamine signalling and cortical neuron differentiation have been implicated in other models of autism spectrum disorders, suggesting that they underlie the symptoms of autism that can be observed in patients with this syndrome. More broadly, this study reveals the potential of stem cell reprogramming approaches for elucidating mechanisms of disease.

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ORIGINAL RESEARCH PAPER Paşca, S. P. *et al.* Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nature Med.* 27 Nov 2011 (doi:10.1038/nm.2576)