The More, the Better: Modeling Dravet Syndrome With Induced Pluripotent Stem Cell-Derived Neurons

Dravet Syndrome Patient-Derived Neurons Suggest a Novel Epilepsy Mechanism.

Liu Y, Lopez-Santiago LF, Yuan Y, Jones JM, Zhang H, O'Malley HA, Patino GA, O'Brien JE, Rusconi R, Gupta A, Thompson RC, Natowicz MR, Meisler MH, Isom LL, Parent JM. *Ann Neurol* 2013;74:128–139.

OBJECTIVE: Neuronal channelopathies cause brain disorders, including epilepsy, migraine, and ataxia. Despite the development of mouse models, pathophysiological mechanisms for these disorders remain uncertain. One particularly devastating channelopathy is Dravet syndrome (DS), a severe childhood epilepsy typically caused by de novo dominant mutations in the *SCN1A* gene encoding the voltage-gated sodium channel $Na_v1.1$. Heterologous expression of mutant channels suggests loss of function, raising the quandary of how loss of sodium channels underlying action potentials produces hyperexcitability. Mouse model studies suggest that decreased $Na_v1.1$ function in interneurons causes disinhibition. We aim to determine how mutant *SCN1A* affects human neurons using the induced pluripotent stem cell (iPSC) method to generate patient-specific neurons. METHODS: Here we derive forebrain-like pyramidal- and bipolar-shaped neurons from 2 DS subjects and 3 human controls by iPSC reprogramming of fibroblasts. DS and control iPSC-derived neurons are compared using whole-cell patch clamp recordings. Sodium current density and intrinsic neuronal excitability are examined. RESULTS: Neural progenitors from DS and human control iPSCs display a forebrain identity and differentiate into bipolar- and pyramidal-shaped neurons. DS patient-derived neurons show increased sodium currents in both bipolar- and pyramidal-shaped neurons. Consistent with increased sodium currents, both types of patient-derived neurons show spontaneous bursting and other evidence of hyperexcitability. Sodium channel transcripts are not elevated, consistent with a post-translational mechanism. INTERPRETATION: These data demonstrate that epilepsy patient–specific iPSC-derived neurons are useful for modeling epileptic-like hyperactivity. Our findings reveal a previously unrecognized cell-autonomous epilepsy mechanism potentially underlying DS, and offer a platform for screening new antiepileptic therapies.

Commentary

Advances in cellular reprogramming have made it possible to generate virtually any cell type from pluripotent stem cells. Initially, embryonic stem cells were the only source of truly pluripotent cells. However, in 2007, it was reported that induced pluripotent stem cells (iPSCs) could be generated from human somatic cells (1, 2). This discovery enabled iPSCs generated from patients to be used as an in vitro model for studying disease mechanisms and testing therapeutics. Patient cells obtained from skin biopsy can be reprogrammed to pluripotency by addition of the four factors: Oct3/4, Sox2, Klf4, and cMYC (1). These iPSCs have infinite capacity for self-renewal and are pluripotent, making them an unlimited resource for differentiating any cell type for experimental studies. iPSCs provide a particularly attractive model for neurologic disease, where access to live human tissue suitable for culture is extremely limited.

Epilepsy Currents, Vol. 14, No. 1 (January/February) 2014 pp. 33–34
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In this study, Liu and colleagues generated iPSC-derived neurons to model Dravet syndrome, a catastrophic, infant-onset epileptic encephalopathy with pharmacoresistant seizures, developmental regression, and increased mortality (3). In over 80% of patients, Dravet syndrome is caused by heterozygous mutation of *SCN1A*, which encodes the voltage-gated sodium channel Nav1.1 (4). Most Dravet syndrome mutations result in loss of Nav1.1 function, suggesting that *SCN1A* is haploinsufficient. Initially, it was puzzling that *loss* of a voltage-gated sodium channel, which underlies action potentials, could lead to hyperexcitability. However, results from mice with targeted deletion of *Scn1a* or from mice engineered with a human nonsense mutation suggested that loss of Nav1.1 predominantly affected GABAergic inhibitory neurons (5, 6). These observations led to the hypothesis that Dravet syndrome is an "interneuronopathy," with hyperexcitability and seizures resulting from loss of inhibitory input onto excitatory principal neurons (pyramidal cells).

Liu and colleagues sought to determine the effects of *SCN1A* mutations on human neuronal function using iPSC-derived neurons from Dravet syndrome patients. They generated iPSCs from three unaffected controls and two patients,

one with a nonsense mutation and another with a splice site mutation that results in a nonfunctional protein. The iPSCs were differentiated into forebrain neurons, resulting in neuronal cultures with 80 to 90% bipolar-shaped GABA-expressing neurons and 10% pyramidal-shaped neurons expressing the vesicular glutamate transporter. Using whole-cell patch clamp recording, they characterized sodium currents and evaluated excitability of the neurons. After 3 to 7 weeks of differentiation, bipolar- and pyramidal-shaped neurons derived from Dravet syndrome patients exhibited elevated sodium current densities compared with controls. Other parameters of sodium channel function, including voltage-dependence and kinetics of activation and inactivation, were not different between patient-derived and control neurons. To examine excitability, they recorded spontaneous and evoked activity from neurons 5 to 7 weeks after differentiation. Bipolar- and pyramidal-shaped neurons derived from Dravet syndrome patients had significantly reduced thresholds for action-potential generation and elevated repetitive firing compared with control neurons. Additionally, patient-derived neurons of both morphologies displayed spontaneous repetitive firing and bursting behavior that was absent in control neurons. These results show that *both* bipolar GABAergic neurons *and* pyramidal glutamatergic neurons derived from Dravet syndrome patients are hyperexcitable.

The discovery of hyperexcitable phenotypes in both inhibitory and excitatory neurons was unexpected given that results from mouse models showed decreased excitability of bipolar inhibitory neurons and no change in pyramidal excitatory neurons.

There are several plausible explanations for the discrepancies between mouse models and human iPSC–derived models. The most obvious difference is that one study used human-derived neurons and the others used mice. However, that alone probably does not fully explain the differences. Each model system has its own limitations that need to be considered. For a mouse model, one of the unavoidable and significant limitations is that it is not a human, which raises that possibility of species-specific specialized functions for Nav1.1 and differences in homeostatic responses to Nav1.1 loss. However, many aspects of the Dravet syndrome clinical phenotype are recapitulated in the mice, suggesting that at least some aspects of pathophysiology are shared (5–7). For a cell culture model of neurons derived from patient iPSCs, the most serious limitation is that it is a cell culture rather than fully networked, mature brain tissue. This raises the possibility that homeostatic mechanisms may be out of balance owing to the lack of appropriate network integration of neurons, altered neuron to glia ratios, and uncertain maturity level of the neurons (8).

The most important lesson from the discrepancies between the models is that we should be cautious in our extrapolation of model systems to human disease. Both models are likely to faithfully recapitulate some disease mechanisms, while other aspects may not be reproduced. Mechanisms shared between multiple model systems are the most likely to be salient features for disease pathophysiology. For example, patient-derived pyramidal neurons had elevated sodium current density and increased excitability compared with control neurons. Although initial studies showed no differences in pyramidal neurons from *Scn1a*$^{+/-}$ and wild-type mice at postnatal days 14 to 16 (5), subsequent studies at postnatal days 21 to 24 showed elevated sodium current density and increased excitability in *Scn1a*$^{+/-}$ pyramidal neurons (9). This shared feature of pyramidal-cell hyperexcitability in two model systems suggests that changes in pyramidal neurons may be important in Dravet syndrome pathophysiology and that the interneuron hypothesis should be reexamined.

This new iPSC-derived patient neuron model adds to the growing arsenal of Dravet syndrome models. Although each model system has intrinsic advantages and limitations, the availability of multiple complementary model systems is invariably advantageous. With models in mouse, zebrafish, and patient-derived neurons (5, 6, 9, 10), we are poised to make rapid progress in understanding the pathophysiology of Dravet syndrome and developing novel therapeutic strategies for improved treatment of patients.

by Jennifer Kearney, PhD

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