Commentary

Neurons Derived From Patient-Specific Induced Pluripotent Stem Cells: A Promising Strategy Towards Developing Novel Pharmacotherapies for Autism Spectrum Disorders

Ryan Mokhtari, Herbert M. Lachman *

Albert Einstein College of Medicine 1300 Morris Park Ave. Bronx, New York 10461

One of the primary obstacles facing researchers interested in autism spectrum disorders (ASD) and other neuropsychiatric disorders is the lack of direct access to live brain tissue. Animal models can be used as a surrogate. However, data from the mouse and human ENCODE projects show substantial differences in regulatory domains, which suggests that some unique pathophysiological features may exist in humans, and that novel pharmacotherapies may not necessarily work in both species (Kavanagh et al., 2013; Yue et al., 2014). However, the introduction of induced pluripotent stem (iPS) cell technology has provided a promising platform for disease modeling and drug screening that addresses these obstacles (Brennand et al., 2011; Pedrosa et al., 2011; Zhao et al., 2012).

The challenge of developing effective pharmacotherapies for ASD is also limited by our incomplete understanding of its etiology, as well as genetic and clinical heterogeneity. However, despite the complex genetic underpinning of ASD, the clinical phenotype can be attributed to a distinct genetic abnormality in a significant proportion of patients (Persico and Napolioni, 2013). This subgroup of ASD individuals can be a helpful resource for developing iPS cell-derived neurons to identify dysfunctional underlying biological pathways, as well as for the development of personalized therapeutic interventions targeting specific genetic abnormalities.

One such gene is *SHANK3*, one of several ASD risk genes that code for synaptic proteins. SHANK3 is a scaffolding protein that helps form the post-synaptic density (PSD), which plays a critical role in regulating glutamatergic transmission. *De novo* loss-of-function mutations have been identified in some ASD individuals, and is believed to be a key gene involved in the behavioral phenotypes associated with 22q13 deletion syndrome (DS) (Durand et al., 2007; Harony-Nicolas et al., 2015). *SHANK3* haploinsufficient neurons derived from patients with 22q13DS display decreased glutamatergic transmission, which can be rescued by a *SHANK3* cDNA expression vector (Shcheglovitov et al., 2013). Similarly, autistic phenotypes in adult mice can be rescued after restoring *SHANK3* expression (Mei et al., 2016).

In light of the preceding findings, the study by Darville and colleagues (Darville et al., 2016) is timely. They explored the therapeutic potential of more than 200 pharmacological agents, using neurons differentiated from iPS cells derived from ASD individuals heterozygous for *SHANK3* null mutations. Darville et al. applied a comprehensive step-wise approach to identify potentially effective compounds. Using a qPCR-based high throughput screening (HTS) method, they quantified the relative expression of *SHANK3* after treating day-14 neurons with the test compounds, some of which were FDA-approved psychotropic drugs.

The authors found six compounds that increased *SHANK3* mRNA in a concentration-dependent manner, of which three were validated at the protein level: lithium, valproic acid (VPA), and fluoxetine. They performed a quantitative immunostaining analysis of synapses using pre-synaptic and post-synaptic neuronal markers. Only neurons treated with lithium and VPA showed evidence of staining for all the synaptic markers - indicative of increased *SHANK3* activity. Recording spontaneous calcium oscillations (SCO) in the neurons at a later stage revealed that only lithium and VPA increased their intensity and frequency. Transcriptome analysis demonstrated that, compared to VPA, lithium targets a narrower range of neuronal and synaptic genes, which perhaps could translate into a more specific and safer treatment.

To identify molecular pathways that lithium and VPA possibly share in their effect on *SHANK3* expression, the authors hypothesized that Glycogen Synthase Kinase 3 (GSK3) and/or class I Histone Deacetylase (HDAC) could be the possible common targets. Treating the iPS cell-derived neurons with two agents, CHIR99021 (a selective GSK3 inhibitor) and Trichostatin A (a selective HDAC inhibitor) revealed that only the latter agent increased *SHANK3* mRNA. Similarly, a pathway enrichment analysis on genes regulated by lithium and VPA showed that histone modifications, but not GSK3 *per se*, is the more likely mechanism shared by these two drugs in the upregulation of *SHANK3*.

Another important feature of this study was the use of a human embryonic stem cell (hESC) line as a control. When studying epigenetic phenomena in iPS cells, such as involvement of HDAC in a drug response, it is important to consider the fact that epigenetic differences may exist in iPS cell lines related to reprogramming and cell of origin, which can theoretically affect experimental findings. Thus, the observation by Darville et al. that the response to lithium and valproate was the same in the hESC line and iPS cells is significant.

Finally, the study is complemented by a trial of lithium administered to one of the ASD patients whose cells were used in the screening study. The authors reported some improvement in the clinical presentation after one year, based on several quantitative scales. However, worsening of some measures (*e.g.*, ADHD symptoms) was also observed. The authors appropriately caution that at this stage, the use of lithium to treat patients with *SHANK3* haploinsufficiency is premature. Nevertheless, this study is an interesting illustration of the potential use of neuronal cells derived from iPS cells for high-throughput drug screening, and the development of personalized medicine for complex neuropsychiatric disorders.

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