note, these modified clones are not strictly isogenic relative to their parental lines because they also appear to have acquired other genetic variation during clonal expansion, which may account for many of the SNVs and indels discovered. Additional studies in which more cell clones modified by nucleases targeted to different sites will be needed to further assess the generality of these findings.

Importantly, the full genome-wide spectrum of off-target mutations induced by engineered nucleases remains as yet undefined by these studies. WGS is unlikely to fully address this important issue for at least two reasons. First, as observed by Veres et al. and Suzuki et al., systematic sequencing artifacts can make it difficult to discern nuclease-induced alterations, even with high fold-coverage sequencing, and bioinformatic filtering strategies can also remove some bona fide mutations as well (Suzuki et al., 2014). Second, WGS is currently impractical for identifying lower frequency off-target mutations. For example, there is only a 95% probability of identifying off-target sites that are mutated with a frequency of 40% when sequencing three diploid single-cell clones (as was done by Veres et al.), and off-target mutations that occur with more modest frequencies,

such as 10%, would routinely be missed in such experiments. Indeed, to have a 95% chance of finding off-target mutations that occur with frequencies of 10%, 1%, or 0.1% at least once, one would need to sequence 15, 150, or 1,500 diploid single-cell clones, respectively. Furthermore, the actual number of genomes that would need to be sequenced is higher because convincingly distinguishing bona fide nuclease-induced mutations from those acquired by routine culture of cells requires identifying indels at a given site more than once among a population of genomes.

Clearly, an unbiased, genome-wide method that is also sensitive enough to identify even lower frequency off-target effects is required to globally define designer nuclease specificities. While the field awaits description of such an approach, the findings of Veres et al., Suzuki et al., Smith et al., and Kiskinis et al. take an important step forward in showing that it is possible to identify individual nuclease-modified cell lines that bear few, if any, unwanted nuclease-induced alterations. These findings will undoubtedly spur further research on the use of genome-edited human PSCs for disease modeling and of genome-edited single-cell clones for potential therapeutic applications.

REFERENCES

Joung, J.K., and Sander, J.D. (2013). Nat. Rev. Mol. Cell Biol. 14, 49–55.

Kiskinis, E., Sandoe, J., Williams, L.A., Boulting, G.L., Moccia, R., Wainger, B.J., Han, S., Peng, T., Thams, S., Mikkilineni, S., et al. (2014). Cell Stem Cell 14, 781–795.

Sander, J.D., and Joung, J.K. (2014). Nat. Biotechnol. 32, 347–355.

Smith, C., Gore, A., Yan, W., Abalde-Atristain, L., Li, Z., He, C., Wang, Y., Brodsky, R.A., Zhang, K., Cheng, L., et al. (2014). Cell Stem Cell 15, this issue, 12–13.

Suzuki, K., Chang, Y., Qu, J., Li, M., Yao, X., Yuan, T., Goebl, A., Tang, S., Ren, R., Aizawa, E., et al. (2014). Cell Stem Cell 15, this issue, 31–36.

Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S., and Gregory, P.D. (2010). Nat. Rev. Genet. 11, 636–646.

Veres, A., Gosis, B.S., Ding, Q., Collins, R., Ragavendran, A., Brand, H., Erdin, S., Talkowski, M.E., and Musunuru, K. (2014). Cell Stem Cell 15, this issue, 27–30.

Human Induced Pluripotent Stem Cells: Now Open to Discovery

Omer Durak[1] and Li-Huei Tsai[1,*]
[1]Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: lhtsai@mit.edu
http://dx.doi.org/10.1016/j.stem.2014.06.014

Human induced pluripotent stem cells represent a promising tool for investigating the underlying causes of disease; however, this potential currently remains unfulfilled. In this issue of Cell Stem Cell, Yoon et al. (2014) used iPSCs derived from patients harboring common genetic risk variants as the starting point to discover novel insights into disease pathology.

The successful reprograming of somatic cells into induced pluripotent stem cells (iPSCs) by Takahashi and Yamanaka in 2006 was seen as a landmark event in disease research. This new technology promised to provide a powerful tool for modeling human pathology that could be used to understand the underlying causes of various human diseases. The following years saw a stream of new and improved approaches for converting somatic cells into more differentiated cell types such
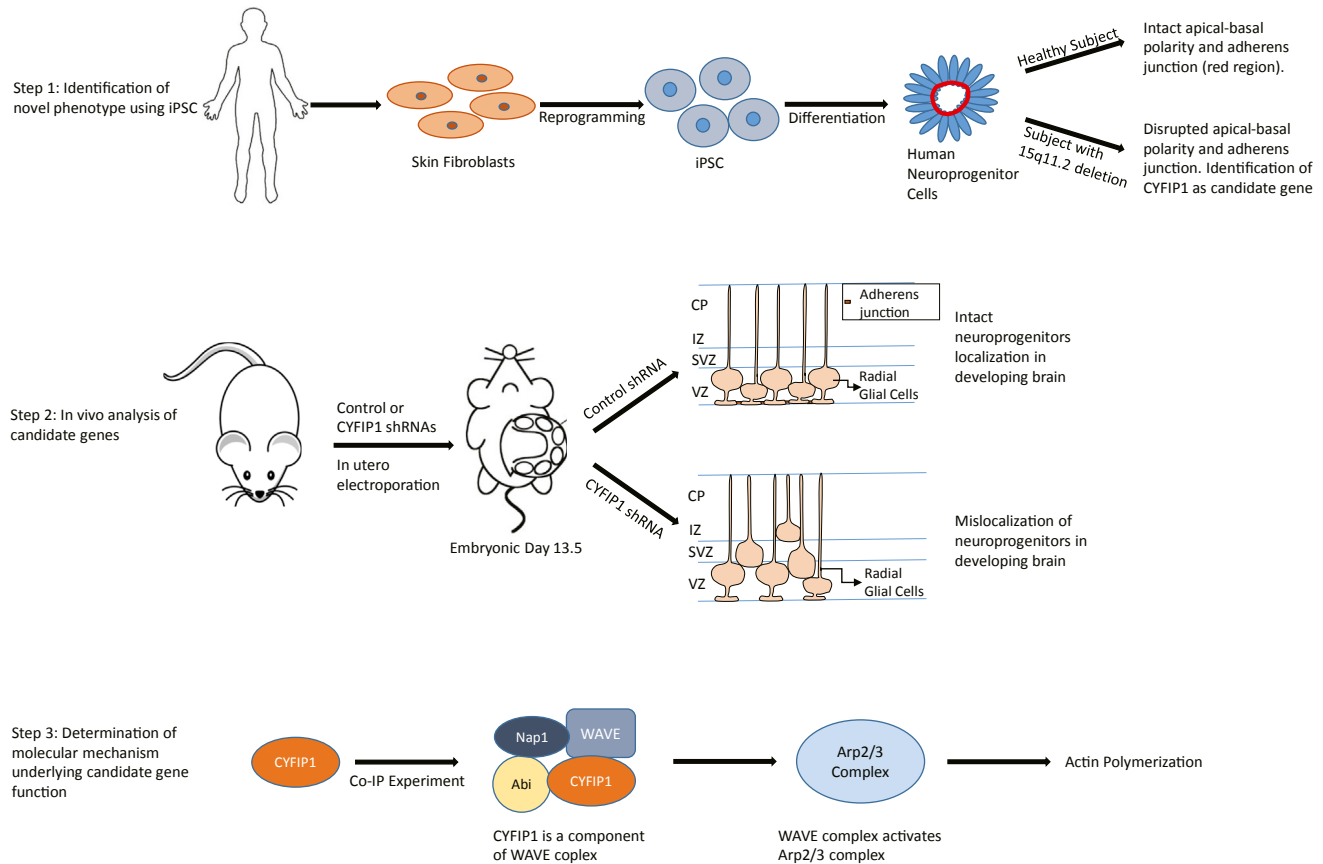
Road Map to Discovery with iPSCs (15q11.2)

Figure 1. Road Map to Discovery with iPSCs (15q11.2)
A multifaceted approach to understand disease pathology with human-derived iPSCs as the starting point to discover novel phenotypes and candidate genes is illustrated. In vivo function of the candidate genes can be studied using mouse brain development followed by determination of the molecular mechanism(s).

as neurons. However, until recently the iPSC technology was mostly used as a tool to confirm previously identified phenotypes thought to underlie disease etiology. In this issue of *Cell Stem Cell*, Song and colleagues (Yoon et al., 2014) make a significant advance in the application of iPSCs by using patient-derived cells as the entry point for discovering novel pathophysiological phenotypes associated with neurological disease (Figure 1). To achieve this they utilized iPSCs containing a DNA deletion at chromosome 15q11.2 (15q11.2del), a region known to be a prominent risk factor for schizophrenia and autism spectrum disorder (ASD) (Malhotra and Sebat, 2012).

Song and colleagues first established iPSC lines using skin cells from three individuals carrying heterozygous 15q11.2del. These iPSC lines were then differentiated into cortical neural rosettes composed of human neural stem/progenitor cells (hNPCs) to understand how 15q11.2del could affect human brain development. Careful examination of the neural rosettes derived from patient cells, using atypical PKCλ and N-cadherin as markers, showed disruption of apical-basal polarity and adherens junctions of these hNPCs. During embryonic cortical development, correct organization of apical-basal polarity and maintenance of adherens junctions in neural progenitors ensures proper cell division in the ventricular zone (VZ) (Buchman and Tsai, 2007). This tightly controlled process is important for correct spatial development of the cortex and disruption of this organization has been implicated in the pathology of diseases such as schizophrenia and ASD (Durak et al., 2014; Ishizuka et al.,

2011). The 15q11.2 region contains four genes including CYFIP1, TUBGCP5 (also known as GPC5), NIPA2, and NIPA1; however, which of these could be responsible for the observed apical-basal polarity phenotype was unknown.

A likely candidate is CYFIP1, a protein that has previously been shown to regulate the WAVE complex, which is important for actin cytoskeleton organization (Silva et al., 2009). Song and colleagues have shown that CYFIP1 indeed binds to the WAVE complex proteins WAVE1, WAVE2, and NAP1 in hNPCs from normal/control patients. Interestingly, the authors found that WAVE2 protein, but not mRNA levels, was significantly reduced in hNPCs derived from 15q11.2del patients, indicating that CYFIP1 is important for stabilization of the complex.

The authors demonstrated that knockdown of CYFIP1 in control cell lines resulted in scattered expression of atypical PKCλ. Furthermore, restoration of normal levels of CYFIP1 in 15q11.2del cell lines was able to rescue the proper expression of atypical PKCλ at the luminal surface of neural rosettes. Importantly, it was shown that disruption of apical-basal polarity was specific to 15q11.2del cell lines as neural rosettes derived from cells carrying mutations in DISC1, another prominent psychiatric risk gene, did not exhibit similar phenotypes.

The authors then complimented these findings from patient-derived iPSCs with experiments to examine the role of CYFIP1 in mouse cortical development in vivo. This was important because the in vitro human iPSC system provides a simple monolayer cell culture tool lacking the tightly regulated in vivo transcriptional program that gives rise to the complex layered mammalian cortex. Knockdown of CYFIP1 in Radial glial cells (RGCs) using the in utero electroporation technique resulted in disorganized adherens junctions similar to the phenotype observed in 15q11.2del-derived iPSCs. Furthermore, they showed that depletion of CYFIP1 in RGCs resulted in aberrant localization of neural progenitors outside of VZ without affecting the proliferative properties of RGCs, suggesting that CYFIP1 is required for proper localization of RGCs, but not proliferation. Investigation of postnatal day 5 mouse cortices after knockdown of CYFIP1 in RGCs at embryonic day 13.5 revealed disorganization of cortical layer formation. Finally, it was demonstrated that CYFIP1 is a component of the WAVE complex in mouse NPCs and disruption of this complex resulted in aberrant placement of RGCs in the developing mouse cortex. These results suggest a conserved role for WAVE complex proteins in determining apical-basal polarity in both human and mouse NPCs.

A leading hypothesis for the etiologies of disorders such as schizophrenia and ASD is that defects during early brain development can lead to altered circuit formation, thus predisposing the brain to dysfunction during postnatal life (Fatemi and Folsom, 2009). Consistent with this notion, disruption of cortical structure and networks have been suggested to underlie schizophrenia pathology (Fatemi and Folsom, 2009). Furthermore, a recent study reported focal patches of abnormal cortical disorganization in children diagnosed with ASD (Stoner et al., 2014). The findings by Yoon et al. demonstrating altered mouse neocortical development after in utero knockdown of CYFIP1 are in support of this neurodevelopmental hypothesis for schizophrenia and ASD.

15q11.2 deletion is a rare genetic mutation resulting in reduced expression of the genes contained in this region, and it is associated with an increased risk for schizophrenia. However, single nucleotide polymorphisms (SNPs) associated with genes located within the 15q11.2 region did not show genome-wide significance for association with schizophrenia. To account for this discrepancy, Song and colleagues hypothesized that SNPs within genes encoding other components of the WAVE signaling pathway might interact to increase the risk for schizophrenia. To test this, the authors conducted targeted pair-wise SNP-SNP interaction analyses for SNP variants associated with expression of genes in the WAVE signaling pathway. This analysis found that an interaction between an SNP located at ACTR2 (a component of the WAVE-interacting Arp2/3 complex) and another SNP affecting the expression of CYFIP1 and NIPA2 genes was significantly associated with an increased risk for schizophrenia. This result suggests an epistatic interaction of WAVE signaling pathway components affecting the risk of schizophrenia development.

CYFIP1 was recently implicated in the maintenance of dendritic complexity and stabilization of mature spines in hippocampal neurons. The findings by Song and colleagues provide insight into an earlier developmental role for CYFIP1, indicating multiple functions for this protein in brain development. Despite the clear importance of CYFIP1 in cortical development, it is worth noting that these findings do not preclude a role for the other 15q11.2del genes in neurological dysfunction. For instance, TUBGPC5 has previously been identified as a member of the γ-tubulin complex that is required for microtubule nucleation at the centrosome (Murphy et al., 2001). One could hypothesize that depletion of TUBGPC5 would affect cell proliferation. Therefore, it would be interesting to know if TUBGCP5 has a role in cortical development, especially in neural progenitor proliferation.

Finally, Yoon et al. demonstrated that iPSC technology could be successfully used not only to study monogenic disorders where disruption of a single gene causes the disease pathology, but also to understand diseases associated with disruption of multiple genes (i.e. polygenic diseases) such as 15q11.2del. A key challenge of studying polygenic disorders is the uncertainty in contribution of disrupted genes to disease etiology. As was demonstrated by Yoon et al., targeted candidate gene selection is possible using human iPSCs to characterize phenotypes associated with disrupted genes. Future studies taking advantage of the system to study diseases associated with deletion or duplication of multiple genes could give novel insights into pathophysiological phenotypes and mechanisms.

REFERENCES

Buchman, J.J., and Tsai, L.H. (2007). Nat. Rev. Neurosci. 8, 89–100.

Durak, O., de Anda, F.C., Singh, K.K., Leussis, M.P., Petryshen, T.L., Sklar, P., and Tsai, L.H. (2014). Mol. Psychiatry, in press. Published online May 13, 2014. http://dx.doi.org/10.1038/mp.2014.42.

Fatemi, S.H., and Folsom, T.D. (2009). Schizophr. Bull. 35, 528–548.

Ishizuka, K., Kamiya, A., Oh, E.C., Kanki, H., Seshadri, S., Robinson, J.F., Murdoch, H., Dunlop, A.J., Kubo, K., Furukori, K., et al. (2011). Nature 473, 92–96.

Malhotra, D., and Sebat, J. (2012). Cell 148, 1223–1241.

Murphy, S.M., Preble, A.M., Patel, U.K., O'Connell, K.L., Dias, D.P., Moritz, M., Agard, D., Stults, J.T., and Stearns, T. (2001). Mol. Biol. Cell 12, 3340–3352.

Silva, J.M., Ezhkova, E., Silva, J., Heart, S., Castillo, M., Campos, Y., Castro, V., Bonilla, F., Cordon-Cardo, C., Muthuswamy, S.K., et al. (2009). Cell 137, 1047–1061.

Stoner, R., Chow, M.L., Boyle, M.P., Sunkin, S.M., Mouton, P.R., Roy, S., Wynshaw-Boris, A., Colamarino, S.A., Lein, E.S., and Courchesne, E. (2014). N. Engl. J. Med. 370, 1209–1219.

Yoon, K.-J., Nguyen, H.N., Ursini, G., Zhang, F., Kim, N.-S., Wen, Z., Makri, G., Nauen, D., Shin, J.H., Park, Y., et al. (2014). Cell Stem Cell 15, this issue, 79–91.