Turning Skin Into Brain: Using Patient-Derived Cells to Model X-Linked Adrenoleukodystrophy

Until recently, investigators have been largely unable to explore the pathophysiology of neurological diseases by direct manipulation of live neural tissues derived from patients. With the advent of the induced pluripotent stem cell (iPSC) technique, however, patient-specific disease models have become a reality. The iPSC method involves the reprogramming of embryonic or adult somatic cells into pluripotent stem cells that behave very much like embryonic stem cells (ESCs; reviewed in Chamberlain and colleagues[1]). The reprogrammed stem cells are then differentiated into the tissue of choice, including neurons and glia, for further study. The iPSC technique, first developed by Shinyu Yamanaka in mouse, involves transient, retrovirally-mediated expression of 4 key developmental transcription factors, sex determining region Y (SRY)-box 2 (Sox2), POU class 5 homeobox 1 (Pou5f1/Oct4), Kruppel-like factor 4 (Klf4), and c-Myc, to successfully reprogram somatic cells into pluripotent stem cells.[2] This approach was subsequently applied to newborn or adult human somatic cells using dermal fibroblasts or bone marrow–derived mesenchymal cells.[3–5]

Characterization of iPSCs from mouse and human show that they are similar to ESCs in nearly every aspect examined, including the expression of pluripotency genes, methylation state, differentiation into all 3 germ layers, and formation of embryoid bodies in vitro and teratomas in vivo (reviewed in Juopperi and colleagues[6]), although some differences likely exist.[7–10] More recently, different combinations of transcription factors and delivery via nonintegrating viral vectors, messenger RNA (mRNA) or protein have been used in place of retroviruses for reprogramming.[11–17] These methods are better suited for regenerative therapy as they reduce the oncogenic risks of c-Myc and viral integration. With further advances, the iPSC method should allow for autologous cell-based restorative treatments for a wide range of disorders. In addition, this approach will be useful for drug screening, studying early human developmental mechanisms, and exploring disease pathophysiology.

Several problems hamper the iPSC technique. These difficulties include the variability of iPSC colonies within and between subjects due to partial reprogramming, and the risk of teratoma formation with grafting. Some of these concerns may be obviated by the development of direct transdifferentiation, for example from fibroblasts to neurons or neural progenitors,[18,19] although these methods have their own limitations.

To date, patient-derived iPSCs have been used to model a variety of neurological and psychiatric disorders, including amyotrophic lateral sclerosis,[20] Parkinson's disease,[5,21] familial dysautonomia,[22] schizophrenia,[23,24] spinal muscular atrophy,[25] Rett syndrome,[26] and others. Disease modeling with patient-specific iPSCs should be extremely useful particularly in genetic disorders and those with limited animal models. X-linked adrenoleukodystrophy (X-ALD) fits both of these criteria, leading to the elegant work by Jang and colleagues[27] reported in the current issue of *Annals of Neurology*, in which they used patient-derived iPSCs to study X-ALD disease pathophysiology.

X-ALD is an inherited demyelinating disorder that is progressive in nature. The 2 main forms include the more severe early onset childhood cerebral ALD (CCALD), and the later onset adrenomyeloneuropathy (AMN). The latter mainly affects the spinal cord and peripheral nerves (see Ferrer and colleagues[28] for review). Both disorders are caused by mutations in the adenosine triphosphate (ATP)-binding cassette transporter superfamily D1 member (*ABCD1*) gene located on chromosome Xq28,[29] whose protein product is necessary for beta-oxidation of very long chain fatty acids (VLCFA) in the peroxisome. The buildup of VLCFA in various tissues, especially plasma, has been useful for diagnosing X-ALD. However, the mechanism by which peroxisomal accumulation of VLCFA leads to demyelination and subsequent white matter inflammation, the pathological hallmarks of X-ALD,[28] remains unknown. Also unclear is why the identical mutation may cause either CCALD or the milder AMN within the same family.[30]

To study disease mechanisms and to compare CCALD and AMN using patient-specific neural cells, Jang and colleagues[27] generated iPSCs from the fibroblasts of subjects with CCALD and AMN. Subsequent

neural differentiated led to the generation of neurons and oligodendrocytes that were indistinguishable between patients and controls, indicating that X-ALD does not adversely impact differentiation. In contrast, abnormal accumulation of VLFCA, the hallmark of X-ALD, was observed only in patient-derived cells differentiated into oligodendrocytes (and not in patient iPSCs or control oligodendrocytes). This VLFCA increase was greater in CCALD oligodendrocytes than in those derived from AMN subjects. Moreover, the excess in X-ALD oligodendrocyte VLFCA was partially ameliorated by chemical treatment to upregulate expression of the closely related *ABCD1* family member, *ABCD2*, that may have compensated for the mutant gene defect.

These findings suggest that the iPSC technique provides a very useful source of patient-specific neurons and oligodendrocytes to model X-ALD. This in vitro model selectively recapitulates the biochemical abnormalities associated with the disease and should prove useful not only for studying disease mechanisms, but also for in vitro screening of novel therapies. Moreover, iPSCs may offer earlier and more accurate diagnosis of disease subtypes than currently available methods (eg, assaying plasma VLFCA levels or examining cultured fibroblasts), although these questions were not directly addressed in the present study.

Several issues complicate the use of iPSC-derived neural cells in this study and for disease modeling in general. For example, age-related cell phenotypes were not examined and are a limiting factor for in vitro studies. Some disease phenotypes may not be apparent until cells are sufficiently aged, and such aging might not be possible in culture. Moreover, the regional specificity of the neurons and oligodendrocytes was not determined, and the susceptibility of neural cells is likely to differ based upon whether the cells are central vs peripheral in phenotype, and between regions of the neuraxis. The iPSC model is also not ideal for studying the development of inflammation in X-ALD. Nonetheless, the finding that patient-specific cells replicate key biochemical features of X-ALD is very exciting. The iPSC approach, perhaps considered "science fiction" less than a decade ago, should provide important pathophysiologic, diagnostic, and potentially therapeutic insight into X-ALD and many other neurological disorders.

Potential Conflicts of Interest

Nothing to report.

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