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REPROGRAMMING PATIENT-DERIVED CELLS TO STUDY THE EPILEPSIES

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Abstract

The epilepsies and related disorders of brain circuitry present significant challenges for using human cells to study disease mechanisms and develop new therapies. Some of these obstacles are being overcome with the use of induced pluripotent stem cell techniques to obtain patient-derived neural cells for *in vitro* studies and as a source of cell based treatments. The field is evolving rapidly with the addition of genome editing approaches and expanding protocols for generating different neural cell types and three-dimensional tissues, but the application to neurological disorders and particularly to the epilepsies is in its infancy. We discuss the progress made to date, the unique advantages and limitations of using patient-derived cells to study or treat epilepsy, and critical future directions for the field.

The derivation of induced pluripotent stem cells (iPSCs) through somatic cell reprogramming by Shinya Yamanaka in 2006¹ has led to a revolution in translational research using patient-derived cells for disease modeling and potential regenerative therapies. This discovery in mouse was rapidly followed by the generation of human iPSCs^{2,3} and then patient iPSC-derived models by differentiating the iPSCs into tissues relevant for investigating specific diseases^{4,5}. Disorders of the central nervous system (CNS) are typically not amenable to acquiring diseased human tissue for *in vitro* study during life. Thus, the iPSC method offers a unique opportunity to investigate CNS disorders using patient-derived neural tissue. Because of excellent progress in neural differentiation of human pluripotent stem cells over the past decade (reviewed in⁶), many groups have taken advantage of the iPSC strategy to model CNS diseases (see^{6–9} for reviews). Moreover, iPSCs have obvious appeal for stem cell-based transplantation therapies for neurological disorders and are being actively studied in this regard^{10–13}. In this review, we describe iPSC methodology and specific applications of iPSC technology to epilepsy disease modeling and cell-based therapy.

iPSCs are generated by the forced expression of specific transcription factors in somatic cells that reprogram the cells to a pluripotent state. The initial factors used by Yamanaka -

Oct4, Klf4, Sox2 and c-Myc (OKSM) - convert a fraction of the starting cells (about 0.1–1%), most commonly fibroblasts, to a pluripotent state resembling human embryonic stem cells (hESCs)². Although human iPSC and hESC lines exhibit differences in gene expression, epigenetic profiles and differentiation capacity¹⁴, many of these differences likely reflect line-to-line variability; in fact, some iPSC lines share more similarities with hESC lines than with other iPSC lines, and vice versa¹⁵. As noted by Sandoe and Eggan⁹, a more important issue for disease modeling is the variability between different iPSC lines derived from any given patient or control. Methodological factors involved in this potential variability include the specific type of somatic cell starting material, the reprogramming method and efficacy, passage number, culture conditions and differentiation protocols. These issues have been reviewed in detail^{6,9,16,17} and we will only highlight key points here. First, most studies involve reprogramming of skin-biopsy derived fibroblasts due to the ease with which they are acquired, cultured and reprogrammed. However, the field is moving toward the use of hematopoietic cells as starting material for reprogramming^{18–20} given that a blood draw is less invasive and easier to acquire. Yamanaka and colleagues initially reprogrammed using retroviral gene transfer¹, but approaches such as this with integrating vectors introduce potential oncogenic and other unwanted effects of genomic integration. To avoid these effects, most protocols currently involve reprogramming with non-integrating episomal or Sendai virus vectors^{21,22}. Once cells are reprogrammed, the best strategy for systematically characterizing the lines is less straightforward but evolving towards more standardized approaches^{23,24}. Another critical issue that needs to be addressed involves the lack of uniformity in application of iPSC protocols across different laboratories. The absence of uniformity makes it difficult to compare data between different groups.

Uses of iPSCs

The iPSC method applied to human cells offers the potential to advance understanding of basic developmental biology and disease mechanisms, and to develop regenerative therapies. Human iPSCs have been used to understand the molecular mechanisms controlling stem cell pluripotency²⁵. They also provide a model to study the earliest stages of human embryonic development, stages for which samples are otherwise limited due to accessibility and ethical issues.

Human iPSCs differentiated to tissue-specific cells are used in translational studies to test drug toxicity in cells, such as cardiac myocytes, hepatic cells and neurons, that are difficult to obtain in large numbers from humans. Similarly, the iPSC method may be used to derive these and other relevant cell types from patients to study disease mechanisms and screen drugs to develop new treatments. Patient-derived cells generated in large numbers via iPSCs also provide a source for regenerative therapy to restore or modify diseased tissues, potentially obviating the need for immunosuppression after autografting²⁶. The remainder of this review will describe these translational applications of patient-derived iPSCs to neurological disorders with a focus on the epilepsies.

iPSC Studies of CNS Disorders

While advances in imaging and electrophysiological techniques have led to amazing progress in understanding systems-level aspects of human CNS development and function, discovering the molecular causes of nervous system disorders requires methods that can evaluate functional molecular interactions within distinctly identifiable types of neural cells. Directing iPSCs into disease-relevant neural cell types is an invaluable approach for conducting biopsy-like experiments on living tissue from individuals with CNS disorders, with the added capacity to study the initial development and progression of pathology. In general, applying the iPSC approach for modeling brain and spinal cord diseases has proven relatively straightforward given the propensity of pluripotent stem cells to form neural tissue (particularly forebrain-like cells) and the development of straightforward neural differentiation protocols devised using knowledge of neurodevelopmental mechanisms (reviewed in⁶). However, many reports do not sufficiently characterize the types of neurons generated, and work remains to be done to develop protocols for generating many specific neuronal and glial subtypes.

Initial reports of the generation of iPSC-derived neurons from patients with neurological disorders^{4,5} quickly led to the use of patient iPSC-derived models showing potential disease-related phenotypes as well as response to therapeutic manipulations *in vitro* (reviewed in²⁷). To date, patient-derived iPSC models for over 20 different neurodevelopmental or neurodegenerative disorders have been reported⁶, and this number is rapidly increasing. Those models relevant to epilepsy will be described below, but others include adrenoleukodystrophy, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), ataxia telangiectasia, Down syndrome, Friedreich's ataxia, Huntington's disease, Machado-Joseph disease, Niemann-Pick type C disease, Parkinson's disease, primary microcephaly, schizophrenia, spinal muscular atrophy and others (see^{6,28,29} and references therein).

Two examples of particular relevance to the epilepsy field deserve special focus. Wainger and colleagues generated motor neurons from ALS patient iPSCs harboring disease causing mutations in three different genes and found hyperexcitability due to reduced delayed rectifier potassium currents³⁰. They corrected the hyperexcitability phenotype and reduced motor neuron death with the potassium channel opener retigabine/ezogabine, a medication approved in the U.S. and other countries to treat epilepsy, and this work has led to initiation of a Phase 1 clinical trial to test the drug in ALS. Another group elegantly showed that human iPSCs may be grown as cerebral organoids, three-dimensional cultures that yield semi-organized brain tissue *in vitro*³¹. Remarkably, the cultures preserved dorsal forebrain-like progenitor zone and primitive cortical laminar patterning, as well as regional marker (albeit not structural) specification of hippocampus and ventral forebrain. The authors also derived cerebral organoid cultures from a patient with autosomal recessive primary microcephaly, a disorder often associated with epilepsy or epileptic encephalopathy, due to compound heterozygote cyclin-dependent kinase 5 regulatory subunit-associated protein 2 (*CDK5RAP2*) mutations. The cultures showed defects in neuroepithelial progenitor size and cell divisions, with altered spindle orientations and premature neurogenic divisions. The defects were partially rescued by overexpressing wild type *CDK5RAP2* in patient organoids, and a similar phenotype was seen after RNAi knockdown of *CDK5RAP2* in control

organoids. Together, these examples illustrate the utility of patient-derived iPSCs for elucidating disease mechanisms for both neurodegenerative and complex neurodevelopmental disorders, and for identifying novel potential pharmacotherapies.

iPSC Studies of the Epilepsies

The epilepsies are a constellation of syndromes that share the defining feature of spontaneous recurrent seizures. They are typically divided into two main groups: focal epilepsies, with seizures starting in a limited area of the brain, or generalized epilepsies consisting of seizures beginning simultaneously in both hemispheres. The epilepsies are also grouped based on the cause being acquired or genetic (either identified or presumptive genetic etiology). Animal models, typically involving rodents, exist for many of the epilepsies. Nonetheless, many decades of study have failed to yield conclusive insight into the mechanisms for any of the epilepsy syndromes, including monogenic epilepsies. Not surprisingly, therapy is directed solely at the main symptom of epilepsy - spontaneous recurrent seizures. About 30–40% of patients cannot be adequately controlled on anti-seizure medications³². Although surgery controls epilepsy in some, only a fraction of medically refractory patients are candidates, and even fewer are evaluated for resective brain surgery. Importantly, no therapies exist for preventing epilepsy after an acquired brain insult or in the setting of a predisposing genetic cause or brain malformation.

Why study epilepsy (or other neurological disorders) with patient-derived iPSCs instead of animal models? The saying that “mice are not small humans” rings true for a number of systems, including the developing brain. In this vein, the pros and cons of using patient-derived iPSCs versus *in vivo* rodent preparations as model systems to study human brain disorders deserve mention. For complex network disorders, such as epilepsies and psychiatric disturbances, iPSCs have the same limitations inherent to all reductionist *in vitro* approaches regarding the failure to recapitulate complex, three-dimensional neural circuitry. In addition, iPSCs have the added challenge of being difficult to fully differentiate into mature cell types and especially into “aged” terminally differentiated cells *in vitro*, a particularly troublesome limitation for studying age-related brain disorders. However, creative approaches to overcome this obstacle and prematurely age the cells *in vitro* are being tested³³. These problems also may be obviated in part by grafting iPSC-derived neural progenitor cells (NPCs) into embryonic rodent brain (or large animal models) to allow them to integrate into developing networks and mature *in vivo*. Another concern involves modeling X-linked brain disorders with iPSCs because of variability in X-chromosome reactivation/inactivation and gene dosing effects^{34,35}, requiring careful monitoring of X-chromosome silencing in female-derived iPSCs.

Despite these issues, human iPSC models of CNS diseases have distinct advantages over rodent models. First, human and mouse brain development are very different. Perhaps 20% of CNS genes show distinct cortical expression patterns between human and rodent³⁶. The germinal zone of the developing cerebral mantle is also larger in humans than rodents, especially the human outer subventricular zone (SVZ) which contains many outer radial glia (oRG) that are rare in mice^{37,38}. The expanded outer SVZ and oRG seen in the human embryonic dorsal forebrain appear to be present in human iPSCs cultured as organoids³¹.

Moreover, iPSCs recapitulate the prolonged embryonic development of neurons followed by glia in the human brain. Brain development is more abbreviated in rodents, implying that some abnormalities originating in specific developmental windows in humans will be absent or limited to developmental ages that are difficult to predict in the rodent. Additionally, genetic background often affects disease severity in human epilepsies and other CNS disorders. The genetic background of a specific patient cannot be mimicked using inbred mouse strains but is inherent and retained in patient-derived iPSC neurons, and these neurons can be compared to controls with different backgrounds as well as isogenic controls obtained by genome editing.

Other disorders, such as deletion syndromes, are difficult to model by genetically modifying mice and highlight the power of the iPSC approach. For instance, iPSC-derived neurons from patients with Phelan-McDermid Syndrome (PMDS), an autism spectrum disorder (ASD) often accompanied by epilepsy, display defects in excitatory synaptogenesis caused by loss of *SHANK3*³⁹. Multiple mouse models failed to fully recapitulate this phenotype, likely due to the complex transcriptional regulation of the gene^{39,40}. In another example, Yoon and colleagues used patient iPSC-derived NPCs to show that 15q11.2 microdeletions, associated with an increased risk of schizophrenia, autism and epilepsy, disrupt adherens junctions and apical polarity⁴¹. These defects result from haploinsufficiency of one of the genes contained in 15q11.2, cytoplasmic FMR1-interacting protein 1 (*CYFIP1*), leading to altered cytoskeletal dynamics⁴¹. The iPSC findings then led the authors to reduce *Cyfip1* expression in developing mouse neocortex by in utero electroporation, revealing similar alterations in NPC polarity and cortical lamination defects. This elegant work together with the studies described above suggest that using both patient-derived cell models and animal models inform each other in important ways and provide complementary mechanistic and potentially therapeutic information.

As mentioned above, disorders of complex neural networks like the epilepsies are challenging to model *in vitro* using dissociated cells. However, several groups have successfully recreated and investigated disease-associated phenotypes using iPSCs. The epilepsies chosen for iPSC modeling have invariably been genetic epilepsies to take advantage of the suitability of the method for studying gene mutation effects using subjects' own cells that carry their specific genetic background. For acquired epilepsies, such as those caused by head trauma, stroke or other insults, the iPSC approach is less advantageous than animal studies for exploring disease mechanisms. Here we divide epilepsy patient-derived iPSC disease modeling studies into two groups: 1) brain disorders in which epilepsy is a variable symptom of a widespread disease involving intellectual disability, autism or other neuropsychiatric disturbances; and 2) diseases in which epilepsy is a defining or predominant manifestation. In both categories, the diseases studied thus far have largely been genetic disorders with early childhood onset. These features comprise the "low hanging fruit" for iPSC disease modeling given the higher likelihood that genetic (rather than acquired) epilepsies will show a phenotype *in vitro* and the relative ease of modeling early development (as opposed to diseases of aging) with cultured iPSC-derived neural cells. Moreover, protocols for generating different iPSC-derived neural cell types of relevance to epilepsy studies have been developed and facilitate epilepsy modeling with patient-specific

cells (Fig. 1). Although glial dysfunction is also implicated in epileptogenesis, the use of human iPSC-derived glia have been limited in epilepsy studies to date, in part because of the extended time required for glial differentiation.

The use of patient-derived iPSC modeling for the neurodevelopmental disorders primary microcephaly, PMDS, and 15q11.2 deletion syndrome, in which epilepsy is a variable phenotype, have been described above. Rett Syndrome, Fragile X syndrome (FXS) and Timothy Syndrome are additional ASD/intellectual disability disorders often associated with epilepsy that have been modeled using iPSCs. Rett syndrome, an X-linked disorder typically caused by mutations in the methyl CpG binding protein 2 (*MeCP2*) gene, shows the highest epilepsy penetrance of these disorders with seizures in 50–90% of cases⁴². Rett patient iPSC-derived neural cells have been studied by several groups (reviewed in⁴³). Neurons generated from Rett patient iPSCs demonstrate reduced soma size^{44,45}, dendritic spine density⁴⁴, neuronal size or differentiation^{46,47}, and decreases in excitatory synapse numbers, activity-dependent calcium transients, and spontaneous excitatory post-synaptic current (sEPSC) amplitude and frequency⁴⁴. Interestingly, treatment with insulin-like growth factor 1 (IGF1) rescues the decrease in excitatory synapses in Rett iPSC-derived neurons⁴⁴, and IGF1 also reverses excitatory synaptic defects in iPSC-derived neurons from another ASD associated with seizures, PMDS³⁹. Still, the link between the cellular phenotypes of Rett iPSC-derived neurons, particularly excitatory synaptic deficits, and seizures remains unclear. More recently, Williams and colleagues⁴⁸ showed that iPSC-derived astrocytes from Rett subjects inhibit the development of mouse hippocampal neurons and Rett patient iPSC-derived interneurons. They also found cell autonomous defects in Rett interneuron development, and showed that IGF1 or a small molecule mimetic partially attenuated both the Rett astrocyte-mediated and intrinsic interneuron defects. Thus, these studies demonstrated that multiple neural cell types are affected in Rett Syndrome and confirmed the utility of using iPSCs to dissect epilepsy mechanisms and screen for novel therapies.

FXS is the most common genetic cause of intellectual disability and is associated with epilepsy in 10–20% of cases⁴⁹. FXS usually results from a CGG trinucleotide repeat expansion in the FMR1 gene which leads to gene silencing and absence of fragile X mental retardation protein (FMRP)⁵⁰. FXS has been studied with both hESCs generated from affected embryos and patient-derived iPSCs. FXS hESCs show active FMR1 in pluripotent cells that is silenced upon differentiation⁵¹. FXS iPSCs unfortunately retain transcriptional silencing of FMR1 and have variability in repeat length, making the disease more challenging to study with iPSCs^{52,53}. Nonetheless, both hESC and iPSC FXS models display defects in neurogenesis, as well as intrinsic and synaptic electrophysiological alterations consistent with neuronal immaturity^{53–55}.

Timothy Syndrome is a rare cause of ASD due to a missense mutation in *CACNA1C* that also leads to multiorgan dysfunction including long Q-T syndrome, sudden death and seizures in about 20% of cases⁵⁶. The *CACNA1C* gene encodes the L-type Ca_v1.2 calcium channel. To study how mutant Ca_v1.2 causes arrhythmias and CNS dysfunction, Ricardo Dolmetsch's group generated Timothy Syndrome patient iPSC-derived cardiac myocytes (CMs) and neurons^{57–59}. They found altered CM contraction, calcium influx and excitability, and abnormalities in neuronal calcium signaling, gene expression and

differentiation^{57,58}. Both the CM and neuronal abnormalities were reversed by the calcium channel blocker roscovitine. A subsequent study of patient iPSC-derived and rodent neurons expressing Timothy Syndrome mutant $\text{Ca}_v1.2$ channels also revealed activity-dependent dendritic retraction that was independent of calcium influx⁵⁹. These findings should provide critical directions for determining potential arrhythmia, intellectual disability and seizure mechanisms.

Several disorders in which epilepsy is a defining or major manifestation, including Dravet Syndrome (DS), Angelman Syndrome (AS) and cyclin-dependent kinase-like 5 (*CDKL5*) gene mutations, have been modeled with patient-derived iPSCs. DS is a severe childhood epileptic encephalopathy typically caused by *de novo* heterozygous mutations in the *SCN1A* gene encoding the $\text{Na}_v1.1$ voltage-gated sodium channel⁶⁰. DS subjects develop epilepsy associated with regression in cognitive development and persistent intellectual disability⁶¹. Mouse models suggested specific loss of sodium current and excitability in inhibitory interneurons as a key mechanism underlying epilepsy in DS^{62,63}. Consistent with these observations, Higurashi and colleagues differentiated predominantly GABAergic neurons from one DS patient and found impaired electrical stimulation-induced action potential generation⁶⁴. In contrast, two other groups found hyperexcitability of DS patient-derived neurons. Jiao et al. generated glutamatergic neurons via iPSCs or directed reprogramming of fibroblasts to neurons in a DS subject, and showed increased evoked and spontaneous neuronal activity along with persistent sodium channel activation or delayed inactivation⁶⁵. Liu and colleagues generated a mix of GABAergic and glutamatergic neurons, predominantly GABAergic, from two DS subject and three control iPSC lines⁶⁶. They found increased sodium current density in putative inhibitory and excitatory DS neurons in association with increased evoked action potentials and spontaneous bursting consistent with an epileptic-like phenotype. The apparently paradoxical increase in sodium currents and excitatory neuron hyperexcitability was subsequently confirmed in a DS mouse model at a specific developmental time point that was not studied in initial investigations⁶⁷. These findings underscore the difficulty in *a priori* prediction of the appropriate mouse age to study when modeling childhood epileptic encephalopathies. Together, the observations from Dravet Syndrome patient iPSC-derived neurons and experimental mice suggest that reduced sodium currents from *SCN1A/Scn1a* haploinsufficiency lead to overcompensation via increased activity from another Na_v alpha subunit, perhaps $\text{Na}_v1.6$. Thus, the iPSC approach has suggested a novel epilepsy mechanism and should provide a future platform for drug testing that can be used in combination with animal models.

AS is a severe neurodevelopmental disorder with epilepsy as a manifestation in over 90% of cases⁶⁸. It is caused by loss of brain expression of the paternally imprinted ubiquitin-protein ligase E3A (*UBE3A*) gene most commonly due to maternal deletions of the 15q11.2-q13 chromosome region (paternal deletions cause Prader-Willi Syndrome [PWS] in which epilepsy is a less frequent and severe manifestation)⁶⁹. Chamberlain and colleagues generated iPSCs from two AS subjects and one PWS patient and found that they all maintained appropriate methylation imprints during reprogramming. The paternal *UBE3A* gene was repressed during neurogenesis of AS iPSCs leading to reduced protein levels, but no difference in the generation of functional neurons was evident. Thus, more studies will be

needed to determine how loss of UBE3A causes abnormalities of neural development or function. Several groups have generated patient iPSC-derived neurons to study how mutations in the *CDKL5* cause disease. *CDKL5* mutations lead to an early onset seizure variant of Rett Syndrome in females, and to a frank epileptic encephalopathy in males. Ricciardi et al used mouse and patient iPSC-derived neurons (comparing clones that inactivated only the mutant or only the wild type X chromosome) to show that loss of *CDKL5* leads to altered dendritic spine structure, and decreased excitatory synapse numbers and mini EPSC frequency and amplitude⁷⁰. Another group found altered expression of the glutamate D1 receptor in iPSCs and iPSC-derived neurons from patients with either *CDKL5* or *MECP2* mutations⁷¹. Similar to the Rett Syndrome studies described above, it is not clear how these changes cause seizures and further work is needed to decipher relevant epilepsy mechanisms and assay for potential therapeutics.

iPSC-Based Cell Therapy for Epilepsies

Beyond their capacity to model molecular and cellular pathology leading to epilepsy, human iPSCs hold tremendous therapeutic promise as source material for cell based therapies for epilepsy^{72,73}. Almost two decades ago studies in rodents demonstrated that cortical GABAergic interneurons originate subcortically in the forebrain basal ganglia anlagen, migrating extensive distances into the overlying cortex by a non-radial glia guided process^{74,75}. While the topic has been controversial^{76,77}, two recent studies provide strong evidence for a similar process in humans^{78,79}. The discovery of the non-radial glial guided migration of embryonic cortical interneurons led Alvarez-Buylla and colleagues to first demonstrate that they also have a distinctive capacity for migratory dispersion following engraftment into adult brain⁸⁰. These results suggested that interneuron precursors may be especially suited for use in cell-based therapies for conditions characterized by abnormal neuronal network excitability. Indeed, multiple rodent studies demonstrated that interneuron precursor transplants into postnatal cortex can give rise to mature GABAergic interneurons that integrate and enhance local synaptic inhibition. Such transplants also ameliorate electrophysiological and behavioral abnormalities in rodent models of epilepsy and related disorders of neural circuit function (for recent reviews, see^{72,73}).

Making patient-derived interneurons from iPSCs

The success of interneuron transplantation studies in rodent epilepsy models has generated tremendous interest in deriving interneurons from iPSCs. As mentioned earlier, using iPSCs as starting material is an ideal choice given the ability to generate large number of cells and the potential for autografts. The ability to generate cortical interneurons from mouse ESCs^{81–84}, together with the apparent conservation of genetic and cellular aspects of initial interneuron development in mice and humans^{78,79,85}, has led to the recent derivation of cortical interneuron-like cells from human pluripotent stem cells^{86–89}. These interneuron precursors display non-radial migration from medial ganglionic eminence (MGE) to cortex in an embryonic mouse forebrain slice preparation^{86,89}, and also migrate extensively into the surrounding cortical parenchyma following transplantation into neonatal mouse neocortex^{86,87}. Although human ESC-derived interneurons show afferent and efferent synaptic integration and neurochemical interneuron subtype expression when cultured for

one month with mouse neocortical cells⁸⁶, they unfortunately mature very slowly after transplantation into neonatal mouse neocortex^{87,88}. These observations suggest that human interneuron progenitors in xenographic transplants maintain the protracted developmental program characteristic of human NPCs. This slow maturation rate raises potential obstacles for human clinical trials unless the process can be accelerated.

Additional concerns are the lack of a protocol capable of efficiently generating pure populations of cortical interneurons from human iPSCs, the lack of ability to generate or isolate interneuron subpopulations, such as “fast-spiking” interneurons that may be particularly efficacious in blocking seizures, and the fact that undifferentiated cells present in the grafts may potentially give rise to teratomas or abnormal NPC growths. Overcoming these challenges will likely require a combination of positive and negative selection. Despite the remaining obstacles, exciting and rapid progress is being made with human pluripotent stem cell derived interneuron transplants. In fact, a very recent paper has demonstrated the capacity of human embryonic stem cell-derived cortical interneuron-like cells to synaptically integrate into the cortex of immune-suppressed mice, to mediate GABAergic synaptic inhibition in host pyramidal neurons when depolarized via optogenetic activation, and to reduce seizures induced by the pilocarpine model of epilepsy⁹⁰. It remains to be determined whether transplanted human PSC-derived interneurons can significantly enhance local inhibition through their activation from endogenous inputs, as demonstrated for MGE transplants^{91,92}. On the whole, however, this approach offers great potential for eventual therapy of medically refractory epilepsy.

Future Directions

The fields of iPSC disease modeling and regenerative therapy are new but evolving rapidly. Advances in several critical areas are necessary to meaningfully impact epilepsy research and therapy development using these approaches. For example, epilepsy patient iPSC-derived cells have the potential to reveal underlying mechanisms and identify potential treatments for the most devastating epilepsy complication, sudden unexpected death in epilepsy (SUDEP). iPSCs from subjects with epilepsies due to gene mutations affecting multiple cell types (e.g., ion channels), thereby conferring a high risk of SUDEP, may be used to study cellular functions in tissues implicated in this fatal disorder. Such tissues include heart, brainstem respiratory centers and autonomic nervous system (Fig. 2).

In comparison to direct reprogramming, in which forced gene expression is used to convert somatic cells of one type to a different type, such as fibroblasts to neurons⁹³, the use of iPSCs offers several advantages. Direct reprogramming requires the development of several separate and labor-intensive protocols for generating and selecting multiple different neural and non-neural cell types, whereas iPSCs are simply differentiated into various cell lineages under the appropriate conditions. iPSC-derived progenitors also provide more flexibility in generating cells with different regional identities in a given tissue and are more readily expandable for cell-based therapeutic approaches. However, direct reprogramming methods for generating neural progenitors at defined stages and of specific regional identities are rapidly improving⁹⁴ and this will be an important direction to pursue for epilepsy studies. In particular, the ability to generate MGE-like progenitors directly from fibroblasts for cell-

based epilepsy therapy would be attractive in avoiding the potential presence of pluripotent stem cells in the grafts, which is a risk when using interneuron progenitors derived from iPSCs.

Genome editing techniques, such as the clustered regularly interspaced short palindromic repeat (CRISPR) system, have quickly become the standard for correcting mutant iPSCs for isogenic controls or generating mutant iPSCs from wild type cells (reviewed in⁹⁵). Genomic background strongly affects the penetrance of epilepsy phenotypes, and the use of genome-editing technology preserves such relations in isogenic control lines, allowing for in-depth studies of relevant factors in comparison with unrelated human iPSC lines. In addition, introducing multiple mutations with genome editing techniques represents a powerful approach for studying epilepsy modifier genes. Lastly, methods for high-throughput drug screening in patient iPSC-derived NPCs are being developed. This approach will be challenging for epilepsy, but multi-well multielectrode array platforms and label-free technologies should greatly expand the capacity for high throughput screening in iPSC systems and the number of relevant phenotypes that can be explored using epilepsy patient-derived cells⁹⁶. Given the astonishing pace of advances in the iPSC field in the first eight years since its introduction, the future for iPSCs in understanding epilepsy mechanisms, identifying novel drugs and developing cell-based epilepsy therapies seems bright indeed.

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Figure 1.

iPSC differentiation into neural cell types to study epilepsy. Somatic cells, such as fibroblasts, are reprogrammed to iPSCs and then may be differentiated into various NPC types, including hippocampal-like progenitors (green, nestin; red, Pax6; blue, DAPI), medial ganglionic eminence-like progenitors (green, Nkx2.1; red, Foxg1), dorsal cortical-like progenitors (green, Pax6; red, nestin; blue, bisbenzimide) or glial progenitors (red, A2B5; blue, DAPI). The NPCs are then terminally differentiated into the relevant cell types for investigation, including dentate granule cell (DGC)-like neurons (green, MAP2; red, Prox1;

blue, DAPI), cortical-like inhibitory interneurons (green, Nkx2.1-GFP; red, GABA), excitatory cortical-like neurons (red, MAP2ab; blue, bisbenzimidazole) and astrocytes (green, GFAP; red, S100B). Hippocampal-like progenitors and DGC-like neurons shown with permission from⁹⁷, and glial progenitors and astrocytes with permission from⁹⁸.

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Figure 2.

Epilepsy patient-derived iPSCs may be differentiated into multiple cell types to study SUDEP mechanisms. iPSC differentiation protocols were used to generate: cardiac progenitors and then ventricular- and atrial-specific cardiac myocytes as demonstrated by immunostaining for myosin light chain 2v (MLC2v, red) and MLC2a (green), respectively; neural crest progenitors immunolabeled with P75 (red) and HNK1 (green) and then differentiated into peripheral neurons immunostained with TuJ1 (red) and peripherin (green) antibodies; and hindbrain neural progenitors which were then differentiated into serotonergic neurons immunolabeled for tryptophan hydroxylase (TPH, red) and MAP2 (green).