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REVIEW

Neural stem cells and epilepsy: functional roles and disease-in-a-dish models

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Abstract Epilepsy is a disorder of the central nervous system characterized by spontaneous recurrent seizures. Although current therapies exist to control the number and severity of clinical seizures, there are no pharmacological cures or disease-modifying treatments available. Use of transgenic mouse models has allowed an understanding of neural stem cells in their relation to epileptogenesis in mesial temporal lobe epilepsy. Further, with the significant discovery of factors necessary to reprogram adult somatic cell types into pluripotent stem cells, it has become possible to study monogenic epilepsy-in-a-dish using patient-derived neurons. This discovery along with some of the newest technological advances in recapitulating brain development in a dish has brought us closer than ever to a platform in which to study and understand the mechanisms of this disease. These technologies will be critical in understanding the mechanism of epileptogenesis and ultimately lead to improved therapies and precision medicine for patients with epilepsy.

Keywords Epilepsy · Seizure · Neural stem cells · Organoids · Adult neurogenesis

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> "People think that epilepsy is divine simply because they don't have any idea what causes epilepsy. But I believe that someday we will understand what causes epilepsy, and at that moment, we will cease to believe that it's divine."
> Hippocrates

Introduction

Epilepsy can be traced as far back as early 2nd millennia BC Mesopotamia. At that time, the disease was referred to as the "falling disease" (Engel 2013). Later, it would be called the "sacred disease" due to the overwhelming belief that seizures were a result of possession by gods or evil spirits (Engel 2013). Modern medicine and neuroscience has come quite a distance since then and we now know that epilepsy is a disorder of the central nervous system (CNS) characterized by spontaneous recurring seizures. Risk factors for epilepsy include traumatic brain injury (TBI), stroke, cancer, CNS infection and genetic factors affecting brain structure and development (Engel 2013). One of the most common forms of epilepsy in adults is mesial temporal lobe epilepsy (mTLE). Due to the strong clinical features, proclivity to affect the hippocampus, a region of the brain involved in adult neurogenesis and amenability to epilepsy surgery, models of mTLE have advanced our understanding of the pathophysiology of epilepsy. While anti-seizure medications (ASMs) may reduce seizure frequency, they do not modify the disease to prevent epilepsy occurrence (Engel 2013). However, regardless of the suspected cause, nearly 1/3 of patients do not respond to available therapy. In addition, ASMs have significant side effects and require prescribed daily medication regimens with noncompliance rates ranging from 25 to 75% (Conrad 1985).

Epilepsy is a complex disease that not only involves seizures but also causes a significant psychological, social and economic impact on the patients, families and communities that it affects (Jacobs and Jensen 2012).

In the following sections, we will review some of the histological and pathological findings in epilepsy. First, we will explore the role of neural stem cells and adult neurogenesis in rodent mTLE models. Next, we will discuss some of the newest technological advances to model genetic causes of epilepsy, and go into detail about how the use of human-induced pluripotent stem cells (iPSCs) has the potential to dissect mechanisms of epilepsy and screen therapeutics.

Aberrant neurogenesis in the epileptic brain

The generation of new neurons, or neurogenesis, is a process that occurs in two areas of the adult brain, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampus (Gage 2002; Gould et al. 1999; Gross 2000). Within these regions, multipotent neural stem cells can be found that undergo proliferation and give rise to newborn neurons throughout the life of mammals (Gage 2002; Gross 2000). However, in the case of prolonged seizures, the number of proliferating neural stem cells drastically increases in rodents (Parent et al. 1997; Scott et al. 1998, 2000). Many of these newborn cells will go on to migrate ectopically into the hilar region of the dentate where they form abnormal connections with resident neurons (Fig. 1). Additionally, a portion of these newborn neurons will develop aberrant properties such as hilar basal dendrites, mossy fiber sprouting, and increased dendritic arborization (Jessberger et al. 2007; Parent et al. 1997). Because the hippocampus is the main region of the brain implicated in mTLE, it has been hypothesized that the aberrant neurogenesis seen in this region after seizure may contribute to the development of recurrent seizures in patients with epilepsy (Parent 2002; Parent and Lowenstein 2002). Further, the hippocampus plays a significant role in learning and memory, mood regulation and resistance to depression. A dysfunctional hippocampus may lead to the associated behavioral comorbidities of mTLE, including depression, anxiety and memory deficits (Danzer 2012; Deng et al. 2010; Eisch and Petrik 2012; Zhao et al. 2008). In the next section, we will further discuss how acute seizures affect new, developing, and mature neurons and what effect each may have on the development of recurring seizures.

Neural stem cells or mature neurons: who poisoned the well?

Adult neurogenesis is a transient process by which neural stem cells are "born", differentiate and eventually integrate into the existing circuitry of the dentate gyrus. There is evidence to suggest a significant role for the newborn stem cells in the development of epilepsy. Animals in which neurogenesis is reduced or ablated in the kindling model of seizure induction using ionizing radiation targeted to the hippocampus have a lower seizure threshold, suggesting that the surviving neurons in this region are more hyperexcitable and contribute to the development of epilepsy (Althaus et al. 2015; Jenrow et al. 2001; Raedt et al. 2007). Further, cells born up to 1 week before the initial seizures can develop aberrant dendrites into the hilus when exposed to kainic acid, a potent neurotoxic glutamic acid analogue used to induce acute seizures in rodents (Jessberger et al. 2007). Many of the changes that occur to newborn neurons in the hippocampus after seizure—ectopic migration, hilar basal

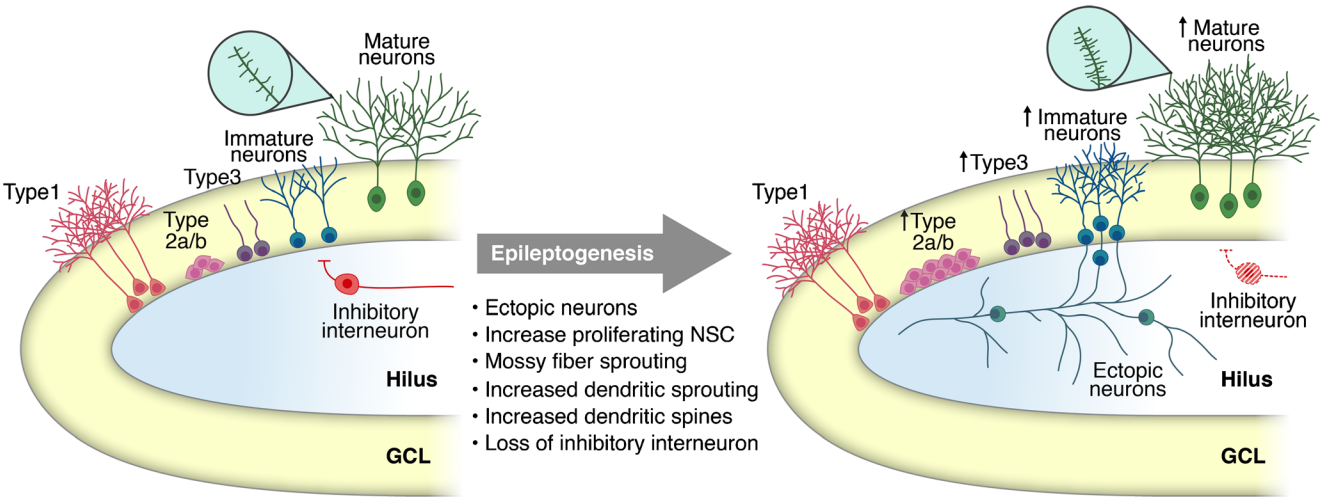

Fig. 1 Some of the key changes related to adult-born granule cells that occur in the epileptic dentate gyrus. *GCL* granule cell layer

dendrite formation, mossy fiber sprouting and increased excitability—have been hypothesized to create a recurrent excitatory loop in the dentate leading to impaired gating function of information entering the hippocampal circuit from the cortex resulting in spontaneous seizures (Jessberger and Parent 2015). Earlier studies using non-specific approaches to ablate dividing cells prior to induction of status epilepticus in the dentate led to an attenuation in the number of seizures (Jung et al. 2004, 2006). More recently, using a transgenic mouse model, selective ablation of neurogenesis prior to induction of acute seizures using the pilocarpine mouse model of chronic mTLE, led to a 40–50% reduction in the total number of seizures (Cho et al. 2015; Hosford et al. 2016). In this work, ablation of neurogenesis prior to acute seizures reduced the total number of aberrant and ectopically migrating new neurons, providing evidence that there is a pool of susceptible newborn cells at the time of the initial insult that go on to mature and drive epilepsy progression (Cho et al. 2015; Hosford et al. 2016).

The factors driving epileptogenesis are not completely accounted for by aberrant neurogenesis, as near-complete ablation prior to acute seizures does not prevent the development of spontaneous recurrent seizure activity. Retroviral labeling reveals significant mossy fiber plasticity amongst the mature neuron population in the hippocampus in the pilocarpine model of epilepsy (Althaus et al. 2016). In addition, many studies demonstrate a significant loss of mature GABAergic neurons from the dentate circuitry in various models of rodent epilepsy. Loss of inhibitory GABAergic neurons is hypothesized to make the hippocampus more permissible to excitatory input (Ekdahl et al. 2003; Houser 2014; Pollard et al. 1994). Further, increased innervation of the CA2 region of the hippocampus after seizure bypasses inhibitory neurons that help suppress excess activity flowing into the dentate. Together, these findings suggest an important role for not only newborn neurons of the hippocampus but also mature neurons.

Monogenetic epilepsy models

In 6 out of 10 cases of epilepsy, the cause is unknown, suggesting that genetics may play an underlying role in epileptogenesis. Epidemiological studies have long confirmed the heritable nature of epilepsy, specifically the idiopathic generalized epilepsies (Peljto et al. 2014). Since the completion of the human genome project in 2001 (Lander et al. 2001; Venter et al. 2001), there has been a rapid discovery of monogenic causes of epilepsy. Monogenic epilepsy now likely represents approximately 10–20% of medically refractory epilepsies in childhood and this will expand with the discovery of new genetic variants and the increasing availability and affordability of genetic sequencing (Noebels 2015; Wang et al. 2014). Indeed, our discovery of these monogenic causes of epilepsy has outpaced our scientific resources to study how these genetic mutations cause disease and our clinical capacity to provide specific therapeutics. Genetics is the new frontier in understanding mechanisms of epileptogenesis and developing precision medicine in epilepsy.

The most common monogenic epilepsies occur in children, often starting within the first year of life. These children are more likely to exhibit additional CNS problems such as neurodevelopmental delays, autism spectrum disorders, and intellectual disability. There are many challenges in treating children with monogenic epilepsy, as seizures in this group are commonly resistant to ASMs and, in some cases, treatment may have neurodevelopmental side effects and contribute to behavioral issues. However, in spite of a desperate need for improved therapies, as of 2015, less than half of all known genetic epilepsies have a validated in vivo or in vitro model (EpiPM Consortium 2015). One example of a monogenetic epilepsy is children with *KCNQ2* gene mutations, which codes for a voltage-gated potassium channel (Singh et al. 1998). Mutations in *KCNQ2* have been associated with epileptic encephalopathy with intractable neonatal seizures and Ohtahara syndrome (Weckhuysen et al. 2012). In vitro models of *KCNQ2* mutations using hippocampal pyramidal cells have shown increased cell firing frequency and hyperexcitability (Miceli et al. 2013). Retigabine is an ASM that targets the voltage-gated potassium channel Kv7.2/Kv7.3, encoded in part by *KCNQ2*. In rodent models, administration of retigabine decreased kainic acid-induced seizure activity in *Kcnq2* knock-in mice when compared to phenobarbital (Ihara et al. 2016). However, while this is promising pre-clinical data, translation to humans has been challenging. While Retigabine (also known as ezogabine in the U.S.) is approved for use in adults with focal seizures, there are limited clinical data on its use in children. Small open label trials have shown some benefit; however, larger blinded prospective studies are needed (Millichap et al. 2016). Retigabine will no longer be available clinically after June 30, 2017; however, related compounds are in early development. In addition, developing human-specific pre-clinical models may lead to better screening of compounds directed to the Kv7.2/Kv7.3 receptor, possibly advancing the path to precision medicine in patients affected by *KCNQ2* mutations.

Awarded the 2012 Nobel Prize, the discovery that somatic cells can be reprogrammed into iPSCs has profoundly altered the landscape of human specific models for studying disease (Takahashi and Yamanaka 2006) (Fig. 2). It is important to emphasize, however, that iPSC science is still a very young field, with many unanswered

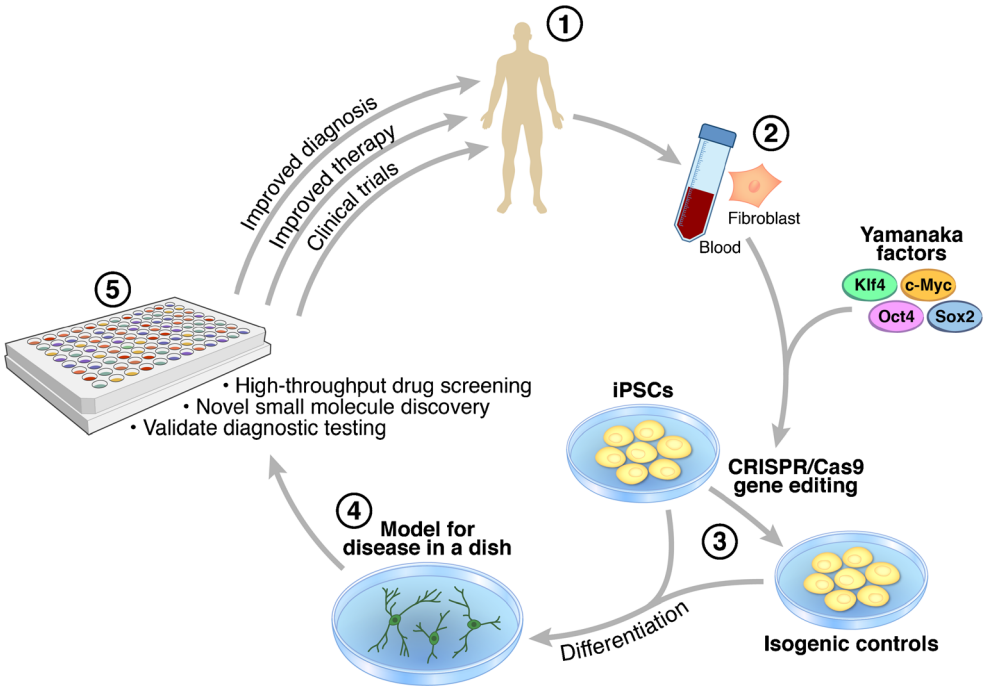

Fig. 2 Schematic illustrating utility of human induced pluripotent stem cells (*iPSCs*) in driving precision medicine for epilepsy: (*1*) patient recruitment, (*2*) reprogramming of human iPSCs from patient blood or fibroblasts, (*3*) CRISPR/Cas9 gene editing to obtain isogenic controls, (*4*) in vitro culture of human iPSC-derived neurons or organoids and (*5*) drug screening or diagnostic testing using epilepsy-in-a-dish model

questions regarding the biology and function of these special cells. Recent work with iPSCs has led to new in vitro models for many neurological diseases. There are investigations using human-derived iPSCs to model anorexia nervosa, Alzheimer's disease and neurodevelopmental diseases (Jones et al. 2017; Negraes et al. 2017; Tidball and Parent 2016). Experiments by Parent and colleagues successfully modeled Dravet syndrome using neurons in two-dimensional cell culture and postulated a novel mechanism of *SCN1A* mediated epilepsy (Tidball and Parent 2016). Dravet syndrome is most commonly associated with a loss of function mutation in *SCN1A*, encoding for the sodium channel, Nav1.1. The predominating hypothesis of *SCN1A* mutation pathogenesis is the "interneuron hypothesis" where epileptogenesis is due to decreased sodium current and loss of excitatory drive within GABAergic interneurons leading to generalized hyperexcitability (Escayg and Goldin 2010). In vitro iPSC models have challenged this hypothesis, showing that pyramidal neurons and bipolar interneurons are both unexpectedly hyperexcitable. These neurons show an increased sodium current and measureable repetitive spontaneous firing and bursting activity, indicating an epileptic phenotype in a dish (Liu et al. 2013). Another study, by Dolmetsch and colleagues, supported the interneuron hypothesis of Dravet syndrome. In this work, iPSCs were used to study Dravet syndrome and led to the discovery of deficits in sodium currents and action potential firing only in inhibitory neurons while excitatory neurons were functionally normal (Sun et al. 2016). Elucidating the mechanisms of childhood epilepsy will bring useful tools in analyzing epileptogenesis and may lead to precision medicine for genetic epilepsy syndromes (Du and Parent 2015). Understanding the fundamental changes perturbed by these monogenic epilepsies may in turn allow for the development of small molecule targets against epileptogenesis and other therapeutic or diagnostic possibilities.

Organoids and CRISPR/Cas9 to study genetic epilepsy syndromes

With approaches in human embryonic stem cells such as homologous recombination and gene-editing technologies such as TALENs and CRISPR/Cas9, it is now possible to investigate monogenetic human diseases with relative ease. Südhof and colleagues successfully studied neurodevelopmental diseases related to *SHANK3* haploinsufficiency using homologous recombination and CRISPR/Cas9 to mutagenize the *SHANK3* gene in human H1 embryonic stem cells (Yi et al. 2016). In these experiments, conditional knock-out neurons with matching controls from the same ES cell clones reduced subclone-to-subclone variability. In their study, combined electrophysiology and immunostaining experiments demonstrated that *SHANK3* haplo-insufficiency impairs intrinsic neuronal properties and HCN channel function, which is commonly associated with epilepsy and intellectual disability. Other studies using iPSCs from patients with amyotrophic lateral sclerosis and Huntington's disease have been conducted using isogenic controls through targeted CRISPR/Cas9-mediated gene correction (Wang et al. 2017; Xu et al.

2017). Further studies utilizing patient-derived iPSCs with isogenic controls created by CRISPR/Cas9 will allow for elucidation of the neurobiology of many monogenic diseases for which there is no validated experimental model. In addition, there are efforts to use CRISPR/Cas9 therapeutically in patients to correct causative gene defects; however, delivering this technology across the blood–brain barrier and directing it to specific target cell types are areas of ongoing research (Keener 2015).

Modeling of CNS disease is also possible in three-dimensional cultures called cerebral "organoids" (Fig. 3). Several groups have created cerebral organoids, which retain structural and cellular features reminiscent of cerebral regions, including the neocortex and hippocampus (Lancaster and Knoblich 2014; Lancaster et al. 2013). Previous work in the field has validated the use of organoids in studying normal human brain development, as well as structural defects like lissencephaly and the pathogenesis of congenital Zika syndrome (Bershteyn et al. 2017; Garcez et al. 2016; Matsui et al. 2017). Exciting new work from Paşca and colleagues shows that organoids can be used to evaluate the developmental characteristics of autism and other neurodevelopmental disorders such as epilepsy (Birey et al. 2017). In this study, using patient-derived iPSCs with Timothy syndrome, a rare monogenetic cause of autism, cortical spheroids (a specific type of cerebral organoid) enriched for glutamatergic and GABAergic neural progenitors and neurons were fused, revealing an in vitro phenotype of abnormal interneuron migration. Using advanced techniques, such as single cell RNA sequencing, live cell imaging and pharmacologic rescue, this phenotype was determined to be cell-autonomous to the cultured GABAergic neurons. Additionally, organoids are amenable to electrophysiological experiments by means of slice physiology seen in organotypic culture models or using multielectrode arrays (MEAs) to record network activity of the organoid. Looking to the future, it may one day be possible to screen drugs and other small molecules by utilizing patient cells grown in cerebral organoid culture. While in their relative infancy, these powerful new techniques hold great promise in the evaluation of human disease and will pave the way for precision therapy for epilepsy.

Future outlook

Use of in vivo rodent models in tandem with in vitro human-derived iPSC models represents the future of epilepsy research. Rodent models have elucidated many of the aspects of the environment of the epileptic brain. Due to the widespread availability of modified transgenic rodents, a variety of approaches exist to ask fundamental questions about the underlying pathophysiology of the epileptic brain. Rodent models have also been very useful in providing a better understanding of how various therapeutic interventions may affect behavioral co-morbidities associated with epilepsy, such as anxiety, depression and memory loss. Recently, with the development of several key technological advances, we are now also able to model epilepsy-in-a-dish. By directly harvesting human cells and using CRISPR/Cas9 technology to edit the genetic code, we are now entering a new era of personalized medicine where we are able to study the electrophysiological and histological changes that occur in patients with epilepsy without requiring human neural tissue, which is now only available from surgical specimens and autopsies. Looking to the future, both in vivo rodent models and in vitro patient-derived iPSC models will be critical to our understanding of different aspects of epilepsy and other neurodevelopmental disorders. Each of these models is likely to continue to contribute significantly to our understanding of epilepsy and will be complimentary in reaching our ultimate goal, a cure for epilepsy.

Conclusions

Epilepsy is a complex neurobiological condition characterized by recurrent seizures and significant co-morbidities. The role of neural stem cells in epilepsy is well described in mTLE models, but the exact contribution of aberrant neurogenesis to epileptogenesis is evolving. Moreover, there has been a dearth of translational therapies developed based on our current understandings of epilepsy. IPSC models of monogenic epilepsy provide a human-specific model, which has great potential to elucidate the biology of epilepsy and affect the

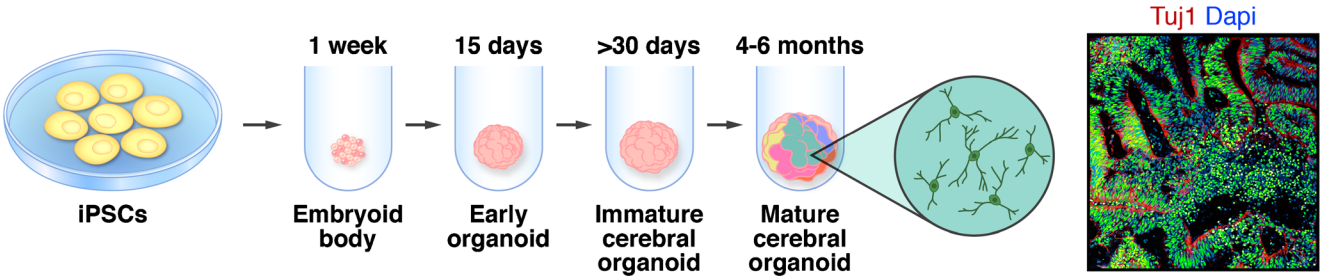

Fig. 3 Overview of human cerebral organoid culture and formation. *Far right* a representative section through a mature cerebral organoid stained with DAPI (*blue*) and Tuj1 (*red*), a marker of immature neurons

development of new therapies and strategies to impact the lives of patients with epilepsy.

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