Modeling epilepsy with pluripotent human cells

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1. Introduction

Epilepsy is one of the most common neurological diseases, characterized mainly by atypical neuronal network electric activity leading to a series of spontaneous and transient seizure episodes [1,2]. Such erratic brain activity can eventually interfere with normal behavior and perception, producing, among others, a variety of sensory, motor and even cognitive manifestations. The cause(s) of epilepsy remains largely elusive. Imbalance between inhibitory and excitatory neurotransmitters, in favor of the latter, is usually considered to contribute [3]. Inherited mutations, mostly in ion channel genes, have been linked to several forms of epilepsy [4]. Structural brain abnormalities resulting from injuries, infection, or malformations can also induce epilepsy. In that sense, epilepsy is not a single disorder but a cluster of syndromes with a large spectrum of symptoms, all involving episodic abnormal brain electrical activity. Temporal lobe epilepsy (TLE) is the most common (around 40% of affected patients) and often intractable form of TLE that adult neurogenesis is affected, altering the number, maturation, and integration of newborn neurons in the dentate gyrus [8–10]. TLE-associated seizures may also have secondary generalization, spreading from the seizure foci and damaging other brain regions.

Unfortunately, a substantial number of patients with TLE have chronic seizures that are resistant to antiepileptic drugs designed to attenuate excessive excitation of neuronal networks. As a consequence, patients with pharmacoresistant TLE have poor quality of life with multiple cognitive deficits, including learning and memory impairments, as well as depression [11–14]. Furthermore, antiepileptic drugs can provide only symptomatic treatment and have only little efficacy in controlling disease progression [15]. Alternatively, surgery for removal of the epileptic focus represents a therapeutic option. Clinical application of stem cell-based therapies for TLE, by grafting and/or activating endogenous neural stem cells (NSCs) in the hippocampus, has previously been considered [16–18]. However, despite promising results in animal models, it is of fundamental importance to determine if TLE in rodents adequately models the human condition. In many circumstances, data generated from animal models do not seem to exactly reflect the human condition [19]. For instance, the granular cell dispersion accompanied by enhanced neurogenesis and followed by seizures in the adult rodent hippocampus is not clearly observed in samples of patients with TLE [20–22]. Moreover, the hippocampus structure per se might be different. In contrast to rodents, several human and other primates' granular cells extend dendritic processes toward the hilus, indicating that such cells might be a normal feature of the primate dentate gyrus [23–25]. Thus, "humanized" models are urgently needed if we are to understand the fundamental biology of TLE and to develop alternative therapeutic approaches that suppress disease progression.

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ABSTRACT

Pluripotency is generally defined by the ability to differentiate into cell types representing all three germ layers: ectoderm, mesoderm, and endoderm. Human pluripotent stem cells hold great promise in regenerative medicine and in cell replacement therapies because of their ability to self-renew and their developmental potential to become all cell types in the body. Moreover, pluripotent cells represent a unique system in which to study the normal development of the human nervous system and the several instances where the process may fail. Here, I propose several strategies for how pluripotent stem cells, both human embryonic stem cells and induced pluripotent stem cells, can potentially be used to gain insights into the biology of temporal lobe epilepsy.
2. Pluripotent human cells

Pluripotent human embryonic stem (hES) cells have been successfully generated from early-stage human embryos and can be induced to differentiate in vitro and in vivo into various cell types [26]. However, to develop cellular models of human disease, it will be necessary to generate new cell lines with genomes that are prone or can lead to diseases. Such new cell lines, representing diverse genetic backgrounds, are important to our understanding of the variation in responses to therapeutics among different patients. The generation of disease-specific and/or genotypically diverse hES cell lines will have great value in disease modeling, drug discovery, and toxicological testing. Somatic cell nuclear transfer has been achieved in nonhuman primates, but has not yet been successfully reproduced in humans [27]. Recently, reprogramming of somatic cells to a pluripotent state by overexpression of specific genes (induced pluripotent stem (iPS) cells) has been accomplished using mouse and human cells [28–30]. Resultant iPS cells are isogenic to the donor individual; that is, they carry the identical genetic background. Isogenic pluripotent cells are attractive not only for a potential therapeutic purpose with lower risk of immune rejection, but also to understand complex diseases with inheritable and sporadic conditions. Because iPS cells can be generated from live patients, the information gained studying their differentiation potential may be applied back to the patient during his or her lifetime. Noticeably, there are still several pitfalls before iPS cells might be considered for cellular therapy, such as the use of oncogenes and retroviruses that may induce malignant cell transformation [31]. It is expected that these technical challenges will be solved in the near future.

3. Epileptic neurons and glial cells in a culture dish

Perhaps the most fascinating questions in TLE biology are when and how excitability affects neuronal morphology and networks in the human brain. In past decades, several groups used cultured human neural cells in an attempt to learn about cellular physiology [32–35]. However, the inaccessibility of the human brain contributes to the slow progress in the field. A useful cellular model would take advantages of the behavior of human neurons in an environment that simulates the initial seizure focus, providing a first step for modeling epilepsy in a culture dish. Human pluripotent cells represent an infinite source of human neurons in culture. Moreover, iPS cells derived from patients with TLE can be induced to differentiate into neurons and other cell types of the nervous system and, then, compared with normal controls. It would be extremely important to know if heritable and sporadic TLE-derived human neurons respond in a similar fashion when challenged with excitation. Such information will allow us to discriminate between stochastic and deterministic (nonrandom, genetic) models. Morphological parameters, such as neuronal arborization and synapse formation, and electrophysiology, as well as neuronal maturational time order, can be measured in the presence or absence of excitability. A co-culture system, in which neurons are in close contact with glial cells, can also be envisioned. Mixing healthy neurons with astrocytes differentiated from TLE-derived iPS cells and vice versa will be valuable in understanding the contribution of glial cells to neuronal hyperexcitability (Fig. 1).

The major obstacle in this approach is to authentically differentiate the pluripotent cells into the neuronal types of the affected structure, in the case of TLE, pyramidal and granular cells of the hippocampus. Neuronal complexity is achieved by different molecular mechanisms and environmental cues during brain formation [36]. It is crucial to understand the fundamental biology of neuronal diversity to advance the field. In the future, this simple system will be developed into a fine three-dimensional microcircuitry that simulates hippocampal connectivity, with different neuronal types, such as granular cells from the dentate gyrus and CA3 pyramidal neurons. In such a model, it would be possible to study the effect of GABA-expressing inhibitory neuron transplantation on hyperexcitable granular cells as an attempt to counterbalance excess excitation [37]. Furthermore, the model would allow an efficient way to screen for drugs that reduce excitability, for example, compounds that enhance glutamate uptake by glial cells. The information gained with the manipulation of this system at the various levels of organization (molecular, synaptic, cellular, and network) can be incorporated into a dynamic computer simulation. Lessons from different genetic backgrounds and incorporation of detailed information on patient socioeconomic environment and lifestyle may help to elucidate how the neuronal network will behave with a specific genetic mutation. The combination of all this information may help us understand the situations that are likely to induce seizures, allowing clinical application of the results [38,39]. For example, one can envision that a tailor-designed algorithm would help to predict when seizures will occur in a specific patient. Clinicians can then alert patients to periods of high seizure probability, use rational pharmacotherapy, or implant seizure-attenuation devices for better quality of life.

4. The chimeric brain

The purpose of using human pluripotent cells in a rodent chimeric brain is to explore the behavior of normal and TLE-specific neural cells in an in vivo setting. Such chimeras can be challenged with seizure-inducing drugs. Genetic manipulation of hES cells (by recombination or delivery of small interfering RNA) [40,41] or use of patient-specific iPS cells allows the generation of a large spectrum of human neurons that can then be assayed in a wild-type or mutant recipient brain, producing a rodent–human chimeric nervous system. Such a strategy may allow the direct examination of the effect of TLE-inducing drugs and potential therapeutic molecules on naïve (healthy) or patient-derived human neurons in a live and functioning nervous system, even in long-term paradigms. Several studies indicate that neurodevelopment may be recapitulated by differentiation of hES cells [42]. The central nervous system (CNS) originates from the neural tube, which is composed of neuroepithelial cells that proliferate, migrate, and differentiate into neurons, astrocytes, and glia [43]. The duration of neuronal and glial differentiation from hES cell-derived neural precursors in vitro seems to be similar to lineage allocation in vivo [42]. Many groups have established hES cell-derived neural progenitor cells that can differentiate into neural subtypes [44–49]. Recently, it was demonstrated that hES cells implanted in the brain ventricles of embryonic mice can differentiate into functional neural lineages and generate mature, active human neurons that successfully integrate into adult human forebrain [48]. Transplanted cells were identified in brain slices with enhanced green fluorescent protein (EGFP) (Fig. 2). A small fraction of the cells integrated into the host tissue, with morphometric dimensions similar to those of adjacent host cells, and adjusted to the preexisting cellular architecture. EGFP-positive cells co-localized with markers specific for mature neurons, such as NeuN. Interestingly, neurons in the hippocampus displayed the morphologies of granular cells. Evidence of synaptic inputs was apparent in the presence of arborized dendrites with spines, suggesting that glutamate-containing terminals contacted these dendrites. Ultrastructural analysis also confirmed that transplanted cells received synaptic input. Moreover, hES cell-derived neurons were electrophysiologically functional even a long time after transplantation. Recorded cells showed neuronal properties similar to those of neurons under comparable recording condi-
It is noteworthy to mention that neither teratomas nor immunological rejection was observed, indicating that the murine embryonic environment supports integration of human cells [48]. However, the integration in these experiments was not uniform in all animals. Some animals had no or few detectable cells. Such variation is likely for a variety of reasons, including cell number, cell passage, and manipulation or embryonic handling prior to transplantation. To reduce such experimental variation, the optimal conditions for in utero transplantation need to be established first. Alternatively, pluripotent cells can be induced to differentiate into neural precursors and then transplanted to differentiate into functional mature neurons in vivo. Such an option could improve the number of differentiated cells in the brain and make the method more reproducible and consistent.

Transplantation of these normal hES cells and TLE-derived iPSCs into mouse brains will help to elucidate the cell autonomous versus cell nonautonomous nature of the disease in a humanized context. Additionally, the strategy can be useful for the discovery of cellular and molecular causes of neuronal cell damage and death in TLE, and also to screen chemical libraries to find novel drugs that may eventually protect against neuronal loss, crossing the blood–brain barrier (Fig. 3).

5. Conclusions

To achieve a holistic view of the causes and consequences of epilepsy, I describe here the use of human pluripotent cell modeling as a first step toward this major goal. Two strategies were discussed, each one with advantages and disadvantages (Table 1). In the first one, an in vitro system using neurons derived from hES cells or derived from iPSCs from patients with TLE could allow several layers (molecular, cellular, synaptic, and network) of experimental manipulations. The morphological and behavioral differences between naïve healthy neurons and neurons derived from patients with TLE might be the key to understanding the role of seizure semiology and neuronal network dynamics in the context of epilepsy. Although it may sound unrealistic now, the incorporation of heritable and sporadic mutations plus environmental cues from patients may provide precious inputs for a computer simulation with potential clinical value in the future.

The second strategy takes advantage of cellular transplantation in the embryonic rodent brain. Mouse chimeric models have already produced insights into neurodegenerative diseases such as
amytrophic lateral sclerosis [50], and they are likely to reveal a nonautonomous component of a specific disease. The fact that human cells can survive and integrate into the neuronal network revealed the conservation and recognition of common signals for neural differentiation throughout mammalian evolution. The model allows for a unique opportunity to study human neural development in a live environment.

Human stem cells have a great potential for regenerative medicine. However, cell transplantation studies are in their infancy and there is a lot to learn about the behavior of these cells in vivo before therapeutics becomes a reality. Alternatively, the use of human pluripotent stem cells as a biology tool to understand complex disorders, such as epilepsy, may provide additional insights into the disease pathology and new compounds that ameliorate disease progression.

Conflict of Interest

The author has no conflict of interests to report that would influence the content of this paper.

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Table 1

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References


