2015

Immature Interneurons Create a Lasting Impression

Janice R. Naegele
Wesleyan University, jnaegele@wesleyan.edu

Laura Grabel
Wesleyan University, lgrabel@wesleyan.edu

Gloster B. Aaron
Wesleyan University, gaaron@wesleyan.edu

EPILEPSY CURRENTS

Current Literature
In Basic Science

Immature Interneurons Create a Lasting Impression

hPSC-Derived Maturing GABAergic Interneurons Ameliorate Seizures and Abnormal Behavior in Epileptic Mice.

Cunningham M, Cho J-H, Leung A, Savvidis G, Ahn S, Moon M, Lee PKJ, Han JJ, Azimi N, Kim K-S, Bolshakov VY, Chung S.
Cell Stem Cell 2014:15:559–573.

Seizure disorders debilitate more than 65,000,000 people worldwide, with temporal lobe epilepsy (TLE) being the most common form. Previous studies have shown that transplantation of GABA-releasing cells results in suppression of seizures in epileptic mice. Derivation of interneurons from human pluripotent stem cells (hPSCs) has been reported, pointing to clinical translation of quality-controlled human cell sources that can enhance inhibitory drive and restore host circuitry. In this study, we demonstrate that hPSC-derived maturing GABAergic interneurons (mGINs) migrate extensively and integrate into dysfunctional circuitry of the epileptic mouse brain. Using optogenetic approaches, we find that grafted mGINs generate inhibitory postsynaptic responses in host hippocampal neurons. Importantly, even before acquiring full electrophysiological maturation, grafted neurons were capable of suppressing seizures and ameliorating behavioral abnormalities such as cognitive deficits, aggressiveness, and hyperactivity. These results provide support for the potential of hPSC-derived mGIN for restorative cell therapy for epilepsy.

Commentary

Noteworthy recent developments in stem cell biotechnology have opened up new possibilities for regenerating inhibitory synaptic connections within the damaged areas of the brain. In severe temporal lobe epilepsy (TLE), repeated seizures are thought to cause hippocampal sclerosis, often associated with loss or dysfunction of GABAergic interneurons. Several sources of GABAergic interneurons for cell replacement include neural progenitors from fetal brain tissue, embryonic stem cells (ESCs), and induced pluripotent stem cell (iPSCs). Progenitors harvested from the medial ganglionic eminence (MGE), a small region of the embryonic forebrain that produces specific subtypes of cortical and hippocampal GABAergic interneurons, differentiate into mature GABAergic interneurons and suppress seizures after engraftment into the adult rodent hippocampus (1, 2), providing proof-of-principle in the mouse pilocarpine model that GABAergic interneuron engraftment may be a promising treatment for severe TLE. These and many other studies have helped to define some of the parameters for fetal stem cell–based treatments for seizure disorders, one of the major goals in this field. However, an alternative stem cell source may be required moving into the clinic, as the ethical problems associated with obtaining large quantities of fetal human MGE cells make them unlikely cell sources for treating patients.

Human iPSCs are one alternative cell source; they can be generated from a patient's own fibroblasts and are thus immunologically compatible. Owing to fiscal and temporal constraints, however, it might not be feasible to develop a new cell line for each patient. Significant advances occurred from 2013 to 2014, when five separate laboratories published in vitro protocols for generating human GABAergic interneurons from human ESCs (3–7). The protocols recapitulated important signaling events for patterning the human MGE. Each uses growth factor–depleted conditions to establish the neural lineages and ventralizes the neural progenitors by treating them with sonic hedgehog (SHH). The Chung group (6) had previously documented that treating human ESCs (hPSCs) with secreted signaling molecules, coupled with a sonic hedgehog agonist and fibroblast growth factor 8 (FGF8), provided the necessary signals to differentiate human MGE-like progenitors in vitro. These hPSC-derived MGE cells were induced to mature further by adding the growth factors Brain Derived Neurotrophic Factor (BDNF) and Glia Derived Neurotrophic Factor (GDNF), and the Notch inhibitor, *N*-[(3,5-Difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine-1,1-dimethylethyl ester (DAPT).

These studies paved the way for an exciting new study reviewed here that tested the clinical potential of human neural progenitors to regenerate damaged hippocampal circuitry in a mouse model of TLE. MGE-like human neurons were first purified by fluorescence-activated cell sorting (FACS) using antibodies recognizing neural cell adhesion molecule in its highly polysialylated embryonic form (embryonic NCAM: eNCAM, or ENCAM). They referred to the enriched population as "maturing GABAergic interneurons" (mGINs). The study also addressed an important concern for ESC-based therapies—whether or not there were residual undifferentiated hPSCs that could form tumors. The pluripotency marker SSEA4 was not expressed in the mGIN populations, consistent with the fact

Epilepsy Currents, Vol. 15, No. 3 (May/June) 2015 pp. 140–142

that none of the mice in this study developed tumors from the transplanted cells.

To test whether the human mGINs would differentiate, integrate into the adult mouse hippocampus, and suppress spontaneous seizures, they were transplanted into adult mice with pilocarpine-induced TLE and spontaneous seizures. Typically, xenografts can elicit a strong immune response and graft rejection. To overcome this limitation, the authors developed a method for inducing TLE in NSG/SCID mice, an inbred strain that lacks T- and B-cell–mediated immunity (8). Following transplantation, they compared seizure incidence in TLE mice receiving mGIN transplants to control TLE mice, by making EEG recordings over short periods of 5 to 10 days per mouse. Strong seizure suppression was found, and while more protracted recording periods are likely necessary to capture the characteristic clusters of seizures typical in the pilocarpine model (2), these findings are very promising. Of interest, the authors also found that when compared with control TLE mice, the mice with mGIN transplants showed improvements in several behavioral tests of cognition, as well as reduced aggressiveness and hyperactivity.

The likely mechanism responsible for these remarkable effects on both the incidence of seizures and cognitive impairments was that the transplanted human cells had differentiated and integrated into the hippocampal neural circuits that had become compromised by epilepsy. To examine differentiation, the authors studied the phenotypes of the transplanted human neurons at multiple time points; after just 2 weeks, the mGINs, distinguished from the host tissue by expression of human nuclear antigen, were clustered around the injection sites and expressed cellular proteins typical for neural precursors. But by 4 months, the mGINs had migrated into the hilus of the dentate gyrus, where some showed expression of the more mature MGE marker Lhx6 and a marker of mature neurons, NeuN. In addition, over 20% of the mGIN derivatives expressed somatostatin or calbindin, with lower levels of parvalbumin- and calretinin-positive cells.

To further determine whether the transplanted hPSC-derived cells developed properties characteristic of GABAergic interneurons, the authors conducted impressive single-cell patch-clamp recordings in hippocampal slices. These studies confirmed an interesting finding that has been noted about primate interneurons, in that they maintain a relatively immature phenotype for several months both in culture and in vivo, even when transplanted into a host animal with faster maturation (5). This is unlike mouse ESC-derived forebrain progenitors, which can mature in a matter of weeks (9). Electrophysiologic markers of full maturation include hyperpolarized resting membrane potentials (more negative than −60 mV) and fast action potentials that can occur at high rates, neither of which could be demonstrated in the present study. And yet, the transplants appear to strongly reduce spontaneous seizures. This finding raises the possibility that transplanting interneurons with the ability to produce high rates of action potentials may not be necessary to alleviate seizures.

Looking only at the electrophysiology of the hPSC interneurons, one might even suspect that they are not inhibitory interneurons at all. Moreover, nonintegrating cells are a worry, as a prior study showed that transplants into the developing cortex of a high number of nonintegrating neural stem cells caused defects in cortical network function (10). These concerns are somewhat alleviated by single-cell reverse transcription-polymerase chain reaction (RT-PCR) experiments, demonstrating expression of genes indicative of GABAergic interneuron fates, as well as the synaptic physiology; fully mature or not, these transplants are well integrated into the host circuitry and can supply robust GABAergic inhibition to their neighbors, as demonstrated by an impressive number of intracellular recordings in the acute slice preparation in a variety of configurations. Similar to a previous study (2), they interrogated the mGIN-derived transplanted neurons optogenetically, while recording from nearby granule cells and showed that the transplanted neurons provided synaptic GABAergic inhibition to their neighbors, whereas excitation between host hippocampal neurons and the mGINs was shown by recording glutamatergic currents from the transplants. Synaptic integration into the circuit, which should enable the transplanted interneurons to provide on-demand inhibition in a most timely way, is the easiest way to understand at this point why, despite their apparent immaturity, these transplanted human interneurons might be so effective in seizure suppression.

by Janice R. Naegele, PhD, Laura B. Grabel, PhD, and Gloster Aaron, PhD

References

1. Hunt RF, Girskis KM, Rubenstein JL, Alvarez-Buylla A, Baraban SC. GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. *Nat Neurosci* 2013;16:692–697.
2. Henderson KW, Gupta J, Tagliatela S, Litvina E, Zheng X, Van Zandt MA, Woods N, Grund E, Lin D, Royston S, Yanagawa Y, Aaron GB, Naegele JR. Long-term seizure suppression and optogenetic analyses of synaptic connectivity in epileptic mice with hippocampal grafts of GABAergic interneurons. *J Neurosci* 2014;34:13492–13504.
3. Germain ND, Banda EC, Becker S, Naegele JR, Grabel LB. Derivation and isolation of NKX2.1-positive basal forebrain progenitors from human embryonic stem cells. *Stem Cells Dev* 2013;22:1477–1489.
4. Liu Y, Liu H, Sauvey C, Yao L, Zarnowska ED, Zhang SC. Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. *Nat Protoc* 2013;8:1670–1679.
5. Nicholas CR, Chen J, Tang Y, Southwell DG, Chalmers N, Vogt D, Arnold CM, Chen YJ, Stanley EG, Elefanty AG, Sasai Y, Alvarez-Buylla A, Rubenstein JL, Kriegstain AR. Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. *Cell Stem Cell* 2013;12:573–586.
6. Kim TG, Yao R, Monnell T, Cho JH, Vasudevan A, Koh A, Peeyush KT, Moon M, Datta D, Bolshakov VY, Kim KS, Chung S. Efficient specification of interneurons from human pluripotent stem cells by dorsoventral and rostrocaudal modulation. *Stem Cells* 2014;32:1789–1804.
7. Maroof AM, Keros S, Tyson JA, Ying SW, Ganat YM, Merkle FT, Liu B, Goulburn A, Stanley EG, Elefanty AG, Widmer HR, Eggan K, Goldstein PA, Anderson SA, Studer L. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. *Cell Stem Cell* 2013;12:559–572.
8. Leung A, Ahn S, Savvidis G, Kim Y, Iskandar D, Luna MJ, Kim KS, Cunningham M, Chung S. Optimization of pilocarpine-mediated seizure induction in immunodeficient NodScid mice. *Epilepsy Res* 2015;109:114–118.

9. Maisano X, Litvina E, Tagliatela S, Aaron GB, Grabel LB, Naegele JR. Differentiation and functional incorporation of embryonic stem cell-derived GABAergic interneurons in the dentate gyrus of mice with temporal lobe epilepsy. *J Neurosci* 2012;32:46–61.

10. Weerakkody TN, Patel TP, Yue C, Takano H, Anderson HC, Meaney DF, Coulter DA, Wolfe JH. Engraftment of nonintegrating neural stem cells differentially perturbs cortical activity in a dose-dependent manner. *Mol Ther* 2013;21:2258–2267.