Defects in GABA-mediated inhibition are associated with a large number of neurologic disorders, including epilepsy. Therefore, it is not surprising that cell replacement therapy is considered a potential option to replace lost or dysfunctional interneurons in the diseased brain. Previous studies demonstrated that interneuron progenitors derived from the embryonic medial ganglionic eminence (MGE) possess a unique ability to migrate long distances away from injection sites after transplantation into neonatal or adult brain (1). Following early postnatal transplantation, greater than 95% of these MGE-derived progenitors integrate as GABAergic interneurons that can selectively elevate GABAA receptor-mediated inhibition to principal neurons (2) and dramatically suppress spontaneous seizures in a mouse model of pediatric epilepsy (3). These studies provided proof-of-principle that interneuron transplantation could have disease-modifying effects in the epileptic brain and raised hope for an epilepsy cell therapy. Derivation of a transplantable mouse or human MGE cell line from which relatively pure and safe populations of interneurons can be produced in large quantities is a critical next step in this field.

Generation of an interneuron cell source first requires a thorough understanding of interneuron diversity and origin. Interneurons represent a broad class of GABAergic cells encompassing many subtypes, each with distinct morphologies, physiological properties, gene expression profiles, and connectivity patterns. Many of these subtypes have distinct spatial and temporal origins within the embryonic subpallium, and it is now well established that, at least in rodents, cortical interneurons are primarily generated in the medial or caudal ganglionic eminences (CGE) and preoptic area (4). Cell-type specification within these progenitor zones is tightly regulated by a combination of genes, transcription factor codes, and secreted molecules to produce distinct populations of interneurons. For example, most parvalbumin (PV), somatostatin (SST), and neuronal nitric oxide synthase (nNOS) subtypes arise from the MGE, and this region typically expresses the transcription factors Dlx1/2, Nkx2.1, and Lhx6. Whereas, vasoactive intestinal peptide (VIP), bipolar calretinin (CR), and reelin interneuron

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**Differentiation and Functional Incorporation of Embryonic Stem Cell-Derived GABAergic Interneurons in the Dentate Gyrus of Mice With Temporal Lobe Epilepsy.**


Cell therapies for neurological disorders require an extensive knowledge of disease-associated neuropathology and procedures for generating neurons for transplantation. In many patients with severe acquired temporal lobe epilepsy (TLE), the dentate gyrus exhibits sclerosis and GABAergic interneuron degeneration. Mounting evidence suggests that therapeutic benefits can be obtained by transplanting fetal GABAergic progenitors into the dentate gyrus in rodents with TLE, but the scarcity of human fetal cells limits applicability in patient populations. In contrast, virtually limitless quantities of neural progenitors can be obtained from embryonic stem (ES) cells. ES cell-based therapies for neurological repair in TLE require evidence that the transplanted neurons integrate functionally and replace cell types that degenerate. To address these issues, we transplanted mouse ES cell-derived neural progenitors (ESNPs) with ventral forebrain identities into the hilus of the dentate gyrus of mice with TLE and evaluated graft differentiation, mossy fiber sprouting, cellular morphology, and electrophysiological properties of the transplanted neurons. In addition, we compared electrophysiological properties of the transplanted neurons with endogenous hilar interneurons in mice without TLE. The majority of transplanted ESNPs differentiated into GABAergic interneuron subtypes expressing calcium-binding proteins parvalbumin, calbindin, or calretinin. Global suppression of mossy fiber sprouting was not observed; however, ESNP-derived neurons formed dense axonal arborizations in the inner molecular layer and throughout the hilus. Whole-cell hippocampal slice electrophysiological recordings and morphological analyses of the transplanted neurons identified five basic types; most with strong after-hyperpolarizations and smooth or sparsely spiny dendritic morphologies resembling endogenous hippocampal interneurons. Moreover, intracellular recordings of spontaneous EPSCs indicated that the new cells functionally integrate into epileptic hippocampal circuitry.
subtypes arise from the CGE and express the transcription factors Dlx1/2, CoupTfl1, or the ionotropic serotonin receptor, 5-HT3A. Knowledge of these transcription factors has fueled early efforts to generate MGE embryonic stem (ES) cell lines.

Here, Maisano and collaborators examined the differentiation of mouse ES-derived progenitor cells in the epileptic brain, with the goal of replacing hilar SST interneurons lost or damaged after status epilepticus. Similar to the Lhx6-green fluorescent protein (GFP) GFP cell line strategy described by Anderson and colleagues (5), the authors used a Sox1-GFP mouse cell line to generate progenitor cells directed toward a ventral forebrain fate. When grafted into the dentate gyrus of adult mice 2 weeks after pilocarpine injection, and analyzed 2 to 3 months later, Sox1-GFP-derived ES cells formed a heterogeneous group of cells that expressed a mature neuronal marker and included a proportion of interneurons. Of interest, transplanted progenitor cells survived in the adult hippocampus of pilocarpine-treated mice and were shown to exhibit mature firing properties; in addition, some received functional excitatory inputs. Important next steps include determining whether these cells have disease-modifying activity in this model, and more complete characterization of the entire graft-derived cell population. In terms of the latter, anatomic and physiological findings were only reported for a small group of cells that appeared to differentiate into mature interneurons and not for the remaining population of Sox1-GFP derived cells. Of these cells, nearly all displayed firing properties consistent with regular spiking non-pyramidal neurons, but fewer than 10% of these cells were immuno-reactive for any of the standard interneuron neurochemical markers examined, and none were SST+. A low expression of the MGE-specific transcription factor Nkx2.1 during differentiation in vitro, the relative absence of SST and PV interneuron subtypes in vitro and in vivo, and very limited migration after transplantation suggest that the Sox1-GFP ES-cell differentiation protocol may not recapitulate the native embryonic MGE environment. This is not altogether surprising as Sox1 is a transcription factor restricted to neuroectoderm that is then expressed in probably all neural progenitors, and Sox1-GFP ES cell lines were previously shown to generate both neurons and glia (6, 7).

These studies, while an important step in stem cell biology, highlight a number of significant challenges that must be overcome to produce specific subsets of neurons for therapeutic purposes. These include inefficient and uncontrolled differentiation of ES cells into mixed cell populations, a relatively slow course of differentiation and development in vivo, and the tendency for ES cells to form tumors after transplantation. In this study, only one mouse developed an “apparent teratocarcinoma.” However, tumor formation following grafting of ES cells may be more prevalent than initially realized, as a follow-up manuscript from this group revealed that cell grafts using an identical mouse Sox1-GFP ES cell differentiation protocol resulted in tumors in nearly 90% of the host animals (8). Efforts to reduced tumorigenesis are ongoing (8, 9), and there are positive signs from human ES-cell protocols that enriched populations of GABAergic striatal projection neurons (10) or bipolar calretinin neurons (11) can be generated.

As the stem cell field advances, and additional cell lines emerge, the fundamental question remains whether transplantation of interneuron progenitors can suppress spontaneous seizures in an adult model of epilepsy. Temporal lobe epilepsy (TLE) with pharmacoresistant seizures is a common epileptic disorder and presents unique challenges for any cell-based therapy. In rodents, the most widely used TLE models have relatively high seizure frequencies, which is advantageous from an experimental perspective. However, significant mortality, reported variability in seizure onset locations, high sensitivity of some interneurons to repetitive seizures, and injury-induced homeostatic changes in circuit function might make it difficult to effectively “replenish” vulnerable interneuron subtypes in these animals. Whether acquired models of TLE in rodents represent the best system in which to test novel cell therapies is an open question. Nevertheless, it seems likely that a successful cell therapy for epilepsy will require generation of a highly pure population of transplantable GABAergic interneurons that migrate and integrate into the host circuitry. This study by Maisano and collaborators raises awareness for a more stringent characterization of ES-derived cells and provides an early step in the progression toward an interneuron-based cell therapy for epilepsy.

by Robert F. Hunt, PhD, and Scott C. Baraban, PhD

References


Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

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