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Gene and stem cell therapies for treating epilepsy.

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

Xu Maisano
Wesleyan University, xliu@wesleyan.edu

33 Gene and Stem Cell Therapies for Treating Epilepsy

Janice R. Naegele and Xu Maisano

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INTRODUCTION

Epilepsy afflicts over 60 million people worldwide, putting it among the most prevalent of neurological disorders. The highest incidence occurs in children under the age of 5 and in the elderly (Theodore et al., 2006). A relatively large proportion of patients with temporal lobe epilepsy (TLE) are resistant to antiepileptic drugs (AEDs) or experience debilitating side effects from long-term treatment such as cognitive impairment, depression, or dementia. Surgery to remove the epileptic tissue may offer an improvement over AEDs, but it is only an option for patients with focal unilateral seizures in brain regions that can be safely removed without causing severe cognitive or sensory deficits. Novel approaches based on gene and stem cell therapy offer the potential for curing epilepsy, rather than treating the symptoms. Coupled with a better understanding of neurological changes caused by seizures, the goals of this emerging area of research are to modify the progression of epilepsy and cure the underlying defects.

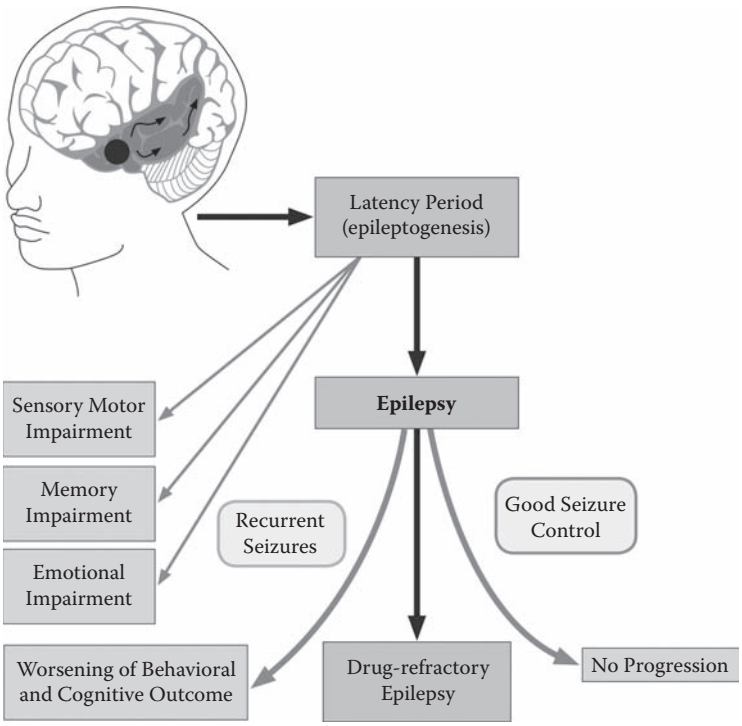

FIGURE 33.1 Scenario for patients developing temporal lobe epilepsy (TLE) after seizures, traumatic brain injury, high prolonged fevers, or strokes. Following an initial seizure or seizures, the patient may be seizure free for an indefinite latency period lasting weeks, months, or even years. Neuroplastic changes during this period may make temporal lobe circuits hyperexcitable and lead to the development of spontaneous seizures, a process referred to as *epileptogenesis*. Patients with TLE may be seizure free with antiepileptic drug (AED) medications, but in many individuals the TLE is resistant to AEDs or adverse side effects from these drugs, and seizures can produce cognitive and emotional impairments. (Adapted from Acharya, M.M. et al., *Prog. Neurobiol.*, 84(4), 363–404, 2008.)

This chapter discusses advances in the fields of gene transfer and stem cell therapy that are fueling new treatments for refractory epilepsy. We first discuss gene therapy for treating TLE and inherited forms of epilepsy and then stem-cell-based therapies for TLE. We highlight limitations and important hurdles that must be overcome before patients can be treated. We then describe some of the newest strategies for tailoring embryonic stem (ES) cell-based therapy for TLE, based on recent discoveries about embryonic origins and molecular codes that regulate GABAergic neuron fates.

BACKGROUND

Developmental disorders of neuronal migration, genetic mutations, and traumatic brain injury are three of the most common causes of epilepsy. Most cases of temporal lobe epilepsy are acquired after an initial episode of status epilepticus (SE) or severe head trauma (Figure 33.1). The risk is particularly high when limbic circuits become hyperexcitable following brain injury in early childhood that is caused by neurological insults such as prolonged febrile seizures, brain tumors, or spinal meningitis. How an initial precipitating injury alters limbic circuitry to promote epileptogenesis is not well understood. It is now thought that neural plasticity occurs mainly during the latent period (the period between the initial brain insult and the development of spontaneous seizures), which changes the balance between excitation and inhibition, and this imbalance is responsible for

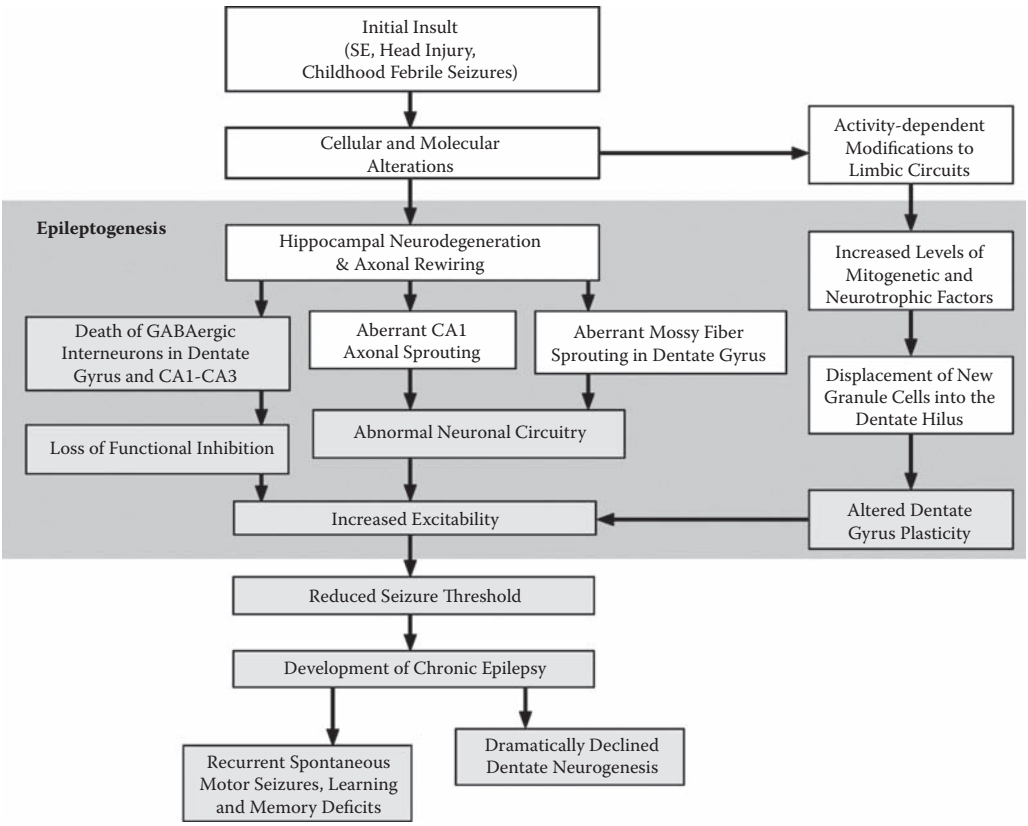

FIGURE 33.2 Events in the hippocampus after initial brain injuries that lead to the development of recurrent seizures and temporal lobe epilepsy. Initial damage to the temporal lobes can result from a variety of neurological traumas, including head injury or prolonged seizures such as status epilepticus. Experimental animal models of SE indicate that a large number of cellular and molecular alterations resulting from the initial insult lead to neuroplastic changes and rewiring of hippocampal circuitry. These changes, indicated in the box with gray shading, include changes to inhibitory neurotransmission, axonal sprouting in CA1 and the dentate gyrus, and alterations in adult neurogenesis in the dentate gyrus subventricular zone. Which of these plastic changes critically determine the development of recurrent seizures is not well understood.

epileptogenesis. The latent period, during which seizures are not evident, can extend for months or years after the initial insult. In childhood-onset TLE, the hippocampus shows progressive damage and sclerosis, whereas adult-onset TLE is not always associated with distinctive neurological signs that are visible with magnetic resonance imaging (Figure 33.2).

Studies of mesial TLE (MTLE) in animal models have extended our understanding of how the limbic lobe is altered by seizure experience, particularly the cellular and molecular changes that take place during epileptogenesis (Pitkanen et al., 2006, 2007). The central structure implicated in MTLE is the hippocampus, an archicortical region of the temporal lobe with three layers and extensive connections to other cortical and subcortical structures. Glutamatergic projections from neocortical pyramidal neurons form the perforant pathway; they become hyperexcitable in MTLE, synapse with granule neurons of the dentate gyrus, and form the first stage in a three-synapse loop. The second connection in the trisynaptic loop is made by mossy fibers from the granule neurons, which form giant synapses on the dendrites of CA3 pyramidal neurons and CA3 interneurons. Schaffer collaterals from the CA3 pyramidal neurons terminating on CA1 pyramidal neurons and interneurons form the third stage of the trisynaptic loop.

The hippocampal granule cells and pyramidal neurons are the principal cells. The pyramidal neurons, located in CA1 to CA3, comprise a relatively homogeneous, excitatory population that releases the neurotransmitter glutamate. Contrasting with the principal neurons in the hippocampus, the nonprincipal cells are morphologically, neurochemically, and electrophysiologically diverse. These GABAergic interneurons are distributed throughout the dentate gyrus and other hippocampal subfields and form short-range connections. The hilus of the dentate gyrus is enriched in interneuron subtypes that appear to be especially important for controlling seizures. Two of the nonprincipal interneuron types in the hilus of the dentate synthesize and corelease somatostatin (SOM) or neuropeptide Y (NPY), generally evoking inhibition of postsynaptic neurons; an additional subset contains the calcium-binding protein parvalbumin. These cells are highly vulnerable to traumatic brain injury and seizures and degenerate in some patients with severe MTLE. As discussed later, GABAergic interneurons play a central role in maintaining inhibitory tone, and their demise following injury is thought to be a critical event leading to MTLE. For this reason, a number of gene and stem cell therapy approaches have focused on the GABAergic system.

In addition to GABAergic interneuron cell death, additional changes include aberrant neurogenesis and displacement of new granule cells in the dentate gyrus, altered expression of neuropeptides and neuromodulators, gliogenesis, axonal injury, mossy fiber sprouting, and immune modulation (Acharya et al., 2008). It is not known which of these changes is responsible for the development of spontaneous seizures; however, recent studies with gene and fetal stem cell therapy in rodent models of epilepsy show that it is possible to inhibit the development of epilepsy or modify epileptogenesis by strategies that increase inhibitory tone (Loscher et al., 2008). Cell transplantation has also demonstrated that genetically engineered cells releasing inhibitory neurotransmitters or neuropeptides can suppress seizures and restore the balance between excitation and inhibition. This work provides "proof of concept" that reducing excitatory neurotransmission in the hippocampus can be beneficial for preventing or controlling epilepsy.

Most studies evaluating gene and stem cell therapies for treating epilepsy have been conducted in a small number of experimental rodent models. These include the electrical kindling model of TLE, in which repetitive electrical stimuli are delivered at subthreshold levels into limbic brain regions until the animals are kindled and show generalized seizures; acute chemoconvulsant seizures induced by systemic or focal brain injections of kainic acid (KA); recurring spontaneous seizures that develop weeks after KA- or pilocarpine-induced status epilepticus; and a few genetic models of inherited epilepsies. The experimental models that test therapeutic efficacy after SE develops are regarded as being more comparable to human TLE.

Although gene- and cell-based therapies are still in preclinical stages and most require extensive validation in models of chronic epilepsy, safer viral delivery systems for gene therapy have been developed, and clinical trials are now being contemplated. Transplants of ES cell-derived neurons raise the prospect for repairing damaged circuits, eliminating a seizure focus, or correcting abnormal wiring that propagates seizures. As discussed below, however, both safety and ethical issues must be surmounted before ES cell-based therapy becomes possible in patient populations.

GENE THERAPY FOR TEMPORAL LOBE EPILEPSY

Gene therapy targets endogenous cells to modify gene expression, counteracting the alterations in these cells caused by genetic mutations or seizures. Adeno-associated virus (AAV) is one of the most promising vectors used to convey foreign genes because it can transduce postmitotic neurons (neurotropism), it can promote persistent and long-term expression of transgenes, and it has low toxicity (Figure 33.3) (McCown, 2005; Riban et al., 2008).

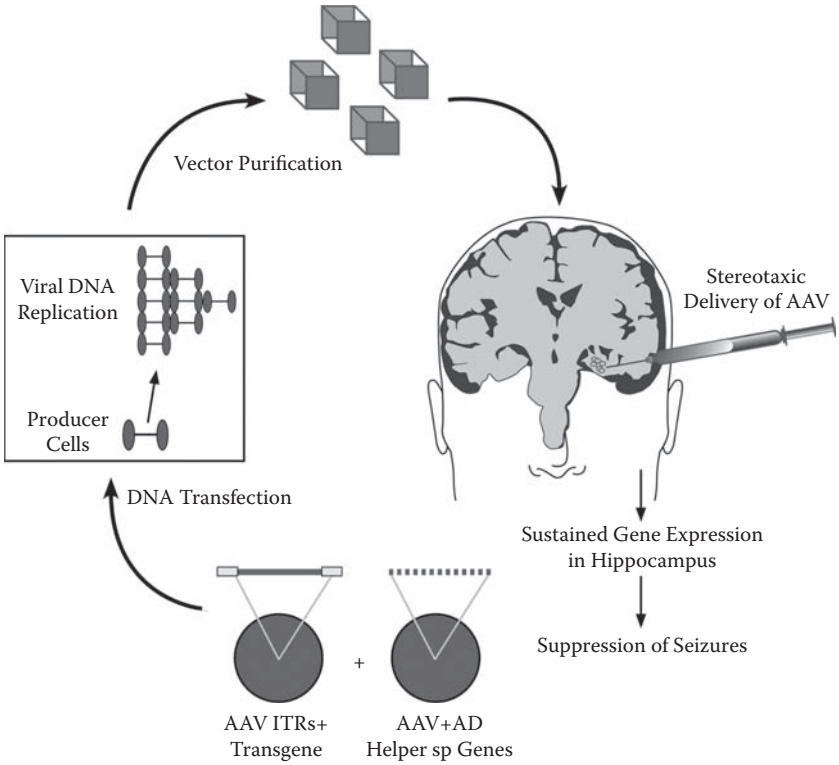

FIGURE 33.3 (See color insert following page xxx.) General approach for adenovirus-associated gene therapy. Adeno-associated virus (AAV) is a small virus that infects humans and primate tissues. It is not known to cause disease and does not elicit a strong immune response, making it ideal for human gene therapy. Recombinant AAV (rAAV) is coinfected with a helper virus (AD) and a second AAV vector containing inverted terminal repeats (ITRs), which are required for efficient amplification of the AAV genome and for integration of the AAV DNA into the host cell genome. For human gene therapy, ITRs are required *in cis* next to the therapeutic gene. The AAV ITR transgene and AAV+AD genes are cotransfected into a producer cell line for viral DNA replication. The rAAV is purified and delivered by stereotaxic injection into the nervous system to achieve sustained gene expression.

$GABA_A$ Receptors and GABA

Experimental analyses of the processes by which recurrent seizures develop in MTLE (epileptogenesis) have not yet explained why the latent period may be decades long after an initial brain injury in human patients. Studies examining the genes altered during the latent period suggest that alterations in the expression of $GABA_A$ receptors are an important determinant in patients and experimental animals (Brooks-Kayal et al., 1999; Hu et al., 2008; Raol et al., 2005, 2006; Roberts et al., 2005). These findings are supported by studies demonstrating that gene therapy for γ-aminobutyric acid type A ($GABA_A$) receptors can suppress seizures in rodents. When the dentate gyrus of rats subjected to pilocarpine-induced status epilepticus was infused with an AAV containing an activity-dependent promoter driving the expression of the $GABA_A$ receptor α_1 subunit, the incidence of developing chronic seizures was significantly lower in these animals. Moreover, seizure-free periods were three times longer in rats that did go on to develop chronic epilepsy (Raol et al., 2006).

Neuropeptide Y

One leading hypothesis to account for hyperexcitability in MTLE is that following the death of mossy cells and hilar GABAergic neurons, granule neurons are disinhibited and become hyperexcitable. Further support of this hypothesis has come from studies showing reduced numbers of GABAergic hippocampal neurons expressing NPY, calbindin, or SOM in mice with experimentally induced epilepsy or genetic mutations associated with a seizure phenotype (Gant et al., 2008; Tuunanen et al., 1997). Building on these findings, gene therapies have been designed to restore the expression of neuropeptides, including NPY, galanin, dynorphin, and SOM, to block epileptogenesis and suppress spontaneous seizures (Wasterlain et al., 2002). NPY is significantly upregulated by seizures (Mazarati et al., 2000) and is an endogenous anticonvulsant (Baraban et al., 1997; El Bahh et al., 2005; Richichi et al., 2004; Vezzani et al., 1999). NPY is released by GABAergic interneurons and arrests the progression of seizures through its inhibitory effect on presynaptic glutamate release by activating NPY-Y2 receptors located on glutamatergic axonal terminals (Vezzani et al., 2000). A strong anticonvulsant effect of a recombinant AAV (rAAV) vector expressing NPY (rAAV–NPY) has been demonstrated in acute models of epilepsy (Haberman et al., 2003; Lin et al., 2003; McCown, 2006; Noe et al., 2007; Richichi et al., 2004). Although these findings were promising, the studies did not test the efficacy of NPY gene therapy in a chronic seizure model in which neuronal death and synaptic reorganization had occurred before gene therapy. To test AAV–NPY gene therapy in neuropathological conditions that resemble human TLE with hippocampal sclerosis, an rAAV–NPY vector expressing the human NPY gene was injected into the brain 3 months after inducing status epilepticus by electrical stimulation of the CA3 subfield of the hippocampus. A majority of these rats developed spontaneous recurrent seizures, and NPY overexpression reduced seizure progression (Noe et al., 2008). Furthermore, compared with rats receiving control rAAV injections, rAAV–NPY injections significantly reduced spontaneous seizure frequency by 40%.

Galanin

Galanin is another powerful antiepileptic peptide that is produced by multiple neuronal cell types, including noradrenergic neurons in the locus coeruleus and serotonergic neurons in the dorsal raphe nuclei. It is involved in a wide range of behaviors, including feeding, cognition, mood, pain, opiate withdrawal, and seizures. It inhibits neurotransmitter release from neurons expressing norepinephrine, serotonin, or dopamine in the forebrain and inhibits neuronal firing in many classes of neurons (Holmes and Picciotto, 2006; Mazarati et al., 1998, 2000). In the hippocampus, galanin is expressed in cholinergic projections from the septum-basal forebrain and noradrenergic axons originating in the locus coeruleus and is thought to act through subtypes 1 and 2 G-protein-coupled galanin receptors (Lu et al., 2005).

Galanin knockout mice are more susceptible to seizures, and overexpression of galanin results in a seizure-resistant phenotype in mice (Mazarati et al., 2000). In addition, ectopic galanin overexpression in dentate granule cells, as well as hippocampal and cortical pyramidal neurons in transgenic mice, delays seizure development in the kindling model of human temporal lobe epilepsy (Kokaia et al., 2001).

Galanin has powerful anticonvulsant effects after viral gene delivery to the brain (Haberman et al., 2003; Lin et al., 2003; Loscher et al., 2008; McCown, 2006). Hippocampal infusion of galanin-containing AAV leads to a dramatic increase in galanin expression in the dentate molecular layer, hilus region, and mossy fiber tract (Lin et al., 2003). When kainic acid is infused several months later, the experimental mice have fewer seizure events compared with controls, demonstrating that galanin overexpression can have strong anticonvulsant effects.

Overexpression of a secreted form of galanin using an AAV vector with the strong cytomegalovirus promoter and a fibronectin secretion signal suppressed seizures and prevented hilar cell loss (Haberman et al., 2003; Mullen et al., 1992). The neuroprotective effects may be due to the ability

of galanin to reduce endogenous excitatory amino acid release in the rat hippocampus (Zini et al., 1993). In a second approach, galanin overexpression was induced in hippocampal mossy fiber–CA3 synapses in an activity-dependent manner with a rAAV vector encoding for galanin under the neuron specific enolase promoter. This vector delayed the initiation of convulsions but did not alter kindling development (Kanter-Schlifke et al., 2007b). These studies underscore the functional effects of different promoters in driving gene expression levels and the site-specific responses that can be achieved by focal gene delivery.

Glial Cell-Derived Neurotrophic Factor

Glial cell-derived neurotrophic factor (GDNF), a member of the transforming growth factor-β family of growth factors, promotes neuronal survival by activating the mitogen activated kinase pathway. GDNF has been utilized in gene therapy for temporal lobe epilepsy. In the kindling model of TLE, seizures upregulated GDNF, and delivery of recombinant GDNF suppressed seizures and reduced mossy fiber sprouting but did not alter epileptogenesis (Humpel et al., 1994; Kanter-Schlifke et al., 2007a; Loscher et al., 2008; Martin et al., 1995; Schmidt-Kastner et al., 1994). When rAAV–GDNF was delivered to the hippocampus of adult rats one week before kainic acid induced SE to increase the levels of GDNF protein, tonic–clonic motor seizures were suppressed and apoptosis inhibitor Bcl-2 was increased. This treatment prevented excitotoxic death of GABAergic neurons in CA3 (Yoo et al., 2006); however, when rAAV–GDNF was delivered ectopically to an existing seizure focus, it also increased seizure threshold, suggesting that increasing levels of GDNF in the hippocampus may have anticonvulsant and neuroprotective effects (Kanter-Schlifke et al., 2007a). These studies underscore the functional effects of different promoters in driving gene expression levels and the site-specific responses that can be achieved by focal gene delivery.

Gene Therapy for Neuroprotection

Several strategies employing viral vectors to enhance neuroprotection have been tested in animal models of epilepsy (Lawrence et al., 1995; McLaughlin et al., 2000; Yenari et al., 1998). Sapolsky and his colleagues induced ectopic expression of the glucose transporter-1 or Bcl-2 after seizures with a herpes simplex virus-1 system (McLaughlin et al., 2000). Herpes simplex virus vectors containing the rat brain glucose transporter under the control of strong promoters were shown to increase metabolism and reduce excitotoxic damage in the kainic acid model, even after seizures had occurred.

Prospects for Treating Genetic Forms of Epilepsy with Gene Therapy

Although many forms of epilepsy are heterogeneous disorders involving complex changes in the interactions between genes and environmental factors, another category of disorders are those caused by single gene mutations or well-defined microdeletions that cause epilepsy syndromes. Many of these are channelopathies caused by mutations in genes encoding individual subunits of ion channels that regulate potassium, sodium or calcium flux. Because the expression of ion channels is heterogeneous in different brain regions and different cell types, epilepsy channelopathies may respond to focal gene therapy. Toward this goal, viral-mediated gene transfer has been used to compensate for loss of the gene *kcna,* encoding the Kv1.1 potassium channel α-subunit in *kcna* knockout mice (Wenzel et al., 2007). This study provided evidence that focal gene therapy can be used for treating specific hippocampal subfields or neuronal populations to reduce seizure activity.

Other categories of mutations associated with inherited epilepsies are loss-of-function mutations in genes controlling GABA levels and transmission. Of a group of 21 genes identified to influence inhibitory synaptic transmission (reviewed in Noebels, 2003), some of these mutations alter interneuron excitability, synaptic levels of GABA, presynaptic release, or the postsynaptic responses

of GABA receptors. These include point mutations or truncation mutations in the gamma subunit of GABA receptors that have been linked to childhood tonic–clonic epilepsy with febrile seizures or generalized absence epilepsy and febrile seizures. Other genes were shown to alter the normal balance of excitation and inhibition by interfering with interneuron patterning, fate specification, migration, survival, and distribution. This category includes some of the mutations that are associated with mental retardation, myoclonic epilepsy, lissencephaly, and cortical dysplasia. Studies of gene therapy for treating inherited forms of epilepsy are very limited, but the large number of knockout and transgenic mouse models that have become available will facilitate future work.

From this brief review of the literature, we conclude that gene therapy is a promising avenue for epilepsy treatment in drug-resistant human MTLE. This approach has received considerable validation in experimental animal models, but has not been well studied in monogenic forms of inherited epilepsy. Further work with animal models for human monogenic epilepsies will help to identify which disorders can be treated by focal gene therapy. For those that are associated with early-onset pediatric epilepsy, future research should determine which disorders, if detected during brain development, can be corrected by gene therapy, before widespread damage or compensatory reorganization of neural circuits occurs (Boison, 2007; Conti et al., 2006; Loscher et al., 2008). Further tests for the safety and feasibility of viral vectors are also needed before treatments in human patients. One approach is to test viral gene transfer in cultured human tissue surgically removed to control intractable seizures (Freese et al., 1997). However, uncertainties about the safety of viruses for human gene therapy continue to hamper their development. The potential for viral vectors to transform into malignant types and the impact of foreign gene expression on endogenous gene expression await careful investigation before clinical trials are feasible.

STEM CELL THERAPY FOR TEMPORAL LOBE EPILEPSY

Cell-based therapies offer several advantages over gene therapy and other more traditional approaches for treating epilepsy, but also pose significant technical challenges. Unlike other tissues of the body, the nervous system has a very limited capacity for self-repair because mature neurons cannot regenerate, and, despite the presence of neural stem cells in the adult brain, their ability to respond to injury is limited. Improving the efficacy of stem cell therapies for replacing neurons or glial cell types destroyed by damage or disease is an extremely active area of investigation. To be successful, grafts of stem cells and their differentiated derivatives in the epileptic brain must not only survive for long periods of time but must also migrate correctly to the appropriate sites, integrate, and establish the correct types of synaptic connections with the host brain. The importance of this last point is underscored by studies showing that seizures induce the genesis of ectopically positioned neurons from endogenous neural stem populations, and these ectopic neurons can contribute to increased excitability and epileptogenesis (Parent, 2007; Scharfman, 2004; Scharfman et al., 2002).

The GABA Hypothesis of TLE

The lateral spread of activity in neocortical circuits is normally restrained by strong surrounding inhibition (Prince and Wilder, 1967). *In vitro* analyses of epileptiform propagation show that seizures can spread through stepwise recruitment of pyramidal neurons (Trevelyan et al., 2006, 2007). Spreading excitation is vetoed by surrounding inhibition; progressive failure of inhibitory networks may account for the slow Jacksonian march of seizures observed in human cortical epilepsy. In the GABA hypothesis, recurrent seizures occur because hippocampal GABAergic interneurons become unable to restrain activity from spreading throughout the limbic circuit. Studies using experimental models of TLE in rodents have supported this hypothesis by showing that a decrease in the functional properties of the GABAergic interneuron networks in the hippocampus is one of the key events underlying temporal lobe epileptogenesis (Cossart et al., 2001).

How can TLE be prevented or reversed by stem cell therapy? The goals of this research are to: (1) modify the fates of embryonic neurons or ES-derived neural stem cells to generate inhibitory neuron precursors; (2) deliver these precursor cells to the appropriate sites of damage in host brain circuits and ensure proper integration; (3) increase their viability in chronic conditions such as TLE; and (4) restore the balance between excitation and inhibition to within the normal range. As discussed below, recent cell transplantation studies in rodent TLE models support this hypothesis by demonstrating that increasing GABAergic neurotransmission or reducing levels of excitation can raise seizure thresholds and reduce both seizure severity and frequency.

Studies of Fetal Neurons as a Source for Transplantation

Limited repair of the adult hippocampus has been shown with grafts of neural stems cells with more restricted neural or glial fates (Shetty et al., 2008). For example, in the kainic acid model of TLE in rats, comparisons of grafts containing fetal neural precursors obtained from CA1 or CA3 showed that CA3 cell types are more effective for increasing GABAergic function (Shetty et al., 2000). However, detrimental effects of grafts forming abnormal connections with the host brain were found when fetal hippocampal cells were transplanted into the adult hippocampus; these grafts tended to increase epileptic discharges and spontaneous seizures (Buzsaki et al., 1991). By contrast, intrahippocampal grafts of fetal cells from the striatum, when pretreated with growth factors to aid survival of the transplanted cells, successfully reduced seizure frequency and severity in adult seizure models (Buzsaki et al., 1988; Hattiangady et al., 2008).

Fetal stem cell transplants have also included noradrenergic precursors from the locus coeruleus (Bengzon et al., 1993; Buzsaki et al., 1988), the hippocampal CA3 region (Loscher et al., 1998; Shetty and Hattiangady, 2007; Shetty and Turner, 1997), GABAergic precursors from the fetal striatum (Loscher et al., 1998), and GABAergic neural precursors from the ventricular zone of the ganglionic eminence in the embryonic ventral telencephalon (Alvarez-Dolado et al., 2006). When intrahippocampal grafts of striatal neural precursors for GABAergic neurons were transplanted into the hippocampus or substantia nigra in the kindling model of TLE, the grafted cells reduced the incidence of abnormal electrical discharges in response to electrical stimulation of the perforant path and afterdischarges (Buzsaki et al., 1988; Loscher et al., 1998). Moreover, when GABAergic neural precursors from the embryonic medial ganglionic eminence were transplanted into forebrains of young mice, they showed widespread migration across the hippocampus and neocortex. The grafted cells differentiated into functionally distinct types of GABAergic interneurons, functionally integrated, and increased inhibitory tone in the host cortex and hippocampus (Alvarez-Dolado et al., 2006).

These examples generally support the hypothesis that increasing GABAergic neurons in epilepsy models is efficacious for controlling seizures. These studies also underscore the fact that GABAergic precursors and other stem cell types from different regions of the nervous system are not all equally effective and may be detrimental. Moreover, the requirement for harvesting embryonic neurons from aborted human fetal tissue poses a formidable hurdle in the United States. Furthermore, fetal neuronal precursors transplanted into the mature brain do not migrate well, nor do they show long-lasting survival in chronic seizure models. Another hurdle is to stimulate these precursor cells to generate the specific types of neurons or glial cells needed to replace lost or damaged cell types and this has not been possible to date. Moreover, immune incompatibility between the donor and host requires that patients receiving fetal stem cells also take immunosuppressive drugs to prevent rejection of fetal cell grafts (Bjorklund and Lindvall, 2000). Autologous stem cell grafts, in which the patient is also the stem cell donor, may help overcome the problem of graft rejection. Skin-derived precursors (SKPs) are one promising type of stem cell located in the hair follicles in adult dermis (Fernandes et al., 2004). SKPs have been derived from rodent and human skin. They can generate immature neurons *in vitro* and *in vivo*, and they survive upon

transplantation into normal or kainate-injured hippocampal slices (Fernandes et al., 2006). Work to date, however, indicates that after transplantation they retain immature characteristics and do not fully differentiate into mature neurons.

Another hurdle for stem cell treatments for intractable epilepsy is to discover how to obtain sufficient quantities of particular cell types. Generating neural or glial precursors from pluripotent embryonic stem cells is an alternative to harvesting limited numbers of cells from the embryo. Embryonic stem cell culture methods have the capacity to be adapted for large-scale production of neural precursors *in vitro*. ES cells have the potential to generate any neuronal or glial cell type, because they originate from the inner cell mass of the embryo at the blastocyst stage and give rise to the entire brain and body. It is also relatively straightforward to genetically modify these neural precursors to express recombinant proteins, fluorescent markers, or luciferase to monitor their survival and migration in the living brain. ES cells from mouse, human, and canine ES lines are available for study, and these cell lines can be genetically engineered to reduce graft rejection.

Embryonic stem cell therapy bypasses some of the ethical roadblocks associated with harvesting stem cells from aborted fetuses, but it has its own ethical problems, including the fact that most of the human ES lines that are being studied were derived from fertilized human embryos. Another major hurdle for ES cell-based therapy is that the risk of tumor formation is high because they are pluripotent and mitotically active. To address this problem, human ES cells have been engineered with suicide genes to allow elimination if the transplanted cells proliferate excessively or form tumors (Schuldiner et al., 2003). As we discuss below, additional strategies for enriching and purifying specific cell types are being developed to help overcome the problem of tumor formation.

Genetically Engineered Cell Lines as a Source for Transplantation

In addition to transplanting acutely dissected fetal cells, transplanted cells have also been used either as a drug-delivery method or to provide local increases in neuromodulators or the neurotransmitter GABA. Most studies have engineered GABA- or adenosine-releasing cells to test their for efficacy in controlling seizures (Behrstock et al., 2000; Castillo et al., 2006; Gernert et al., 2002; Li et al., 2007, 2008; Nolte et al., 2008; Ren et al., 2007; Thompson, 2005; Thompson and Suchomelova, 2004). The duration and severity of established seizures have been reduced by transplanting modified GABA-producing cells into the piriform cortex, dentate gyrus, or substantia nigra (Castillo et al., 2008; Gernert et al., 2002; Thompson, 2005). One limitation of these cell lines is that they are not neuronal and do not integrate within the host brain; therefore, the release of neurotransmitters cannot be regulated by neuronal activity. However, genetic modifications using the Tat-regulatable promoter system (or other comparable regulatable promoters) have been done, to enable controlled GABA release from the transplanted cells when experimental animals are given doxycycline (Behrstock et al., 2000; Thompson, 2005).

Neuromodulation with Adenosine

Adenosine is a well-established endogenous neuromodulator that has both neuroprotective and anticonvulsant effects. Adenosine is expressed primarily by astrocytes and modulates neurotransmission. Seizure-induced astrogliosis is thought to increase neuronal excitability by causing adenosine-kinase-mediated downregulation of adenosine (Boison, 2006, 2008; Fedele et al., 2005). Adenosine kinase reduces adenosine several hours after SE, and this event is thought to play a decisive role in epileptogenesis (Gouder et al., 2004). Evidence linking loss of adenosine to epileptogenesis suggests that increased expression of adenosine kinase is predictive for epileptogenesis (Li et al., 2008). To test this hypothesis, encapsulated rat fibroblasts or myoblasts, engineered to release adenosine, were transplanted into the lateral ventricles and shown to suppress seizures in kindled rats (Guttinger et al., 2005). These results raise the possibility that focal release of adenosine by

intraventricular cell grafts is a feasible option for long-term treatment of TLE. To be used in human patients, this approach would require xenografting or the use of human fibroblasts derived from the patient.

Alternatively, adult stem cells were tested in an autologous grafting protocol that used RNA interference (RNAi) to knock down adenosine kinase. Human mesenchymal stem cells were transduced with a lentivirus containing an anti-adenosine kinase microRNA and grafted into the hippocampal fissure of mice following kainic-acid-induced injury to the amygdala. In this paradigm, lentiviral anti-adenosine kinase RNAi reduced adenosine kinase in the stem cells, reduced hippocampal cell loss, and significantly reduced seizure activity (Ren et al., 2007). It has been shown that transplantation of adenosine-kinase-deficient neural precursors, derived from modified ES cells, eliminated spontaneous seizures induced by kainic acid (Li et al., 2008).

In summary, engineered cells expressing inhibitory neurotransmitters such as GABA or neuromodulators such as adenosine are providing new vistas for therapies aimed at seizure control and preventing the development of epilepsy. Unless the secretion of GABA or adenosine is activity dependent, however, release will not be compensatory during seizures and may be excessive during interictal periods.

ES Cell-Derived Neurons for Cell Transplantation Therapies

Studies are now underway investigating the use of ES cell-derived neural stem cells as a source of transplantable neurons for treating epilepsy. When mouse or human ES derived neural precursors were transplanted into the CA3 region of the hippocampus of mice one week following kainic-acid-induced seizures, they migrated into the dentate gyrus. Thereafter, they became integrated into the host brain, differentiated into neurons, received synaptic connections, and survived for prolonged periods of up to 2 months (Carpentino et al., 2007). ES cell-derived neural progenitor cells appear to be superior to fetal cells in terms of their ability to adapt to the host environment, as shown by broader migration and more robust effect in controlling seizures (Li et al., 2007). However, only a fraction of the cells survive after they are transplanted into the adult brain. Although very small numbers of surviving cells may be sufficient to produce therapeutic benefits in some neurological conditions, neuroprotective growth factors such as vascular endothelial growth factor or erythropoietin may be required for protecting immature grafted neurons in chronic conditions such as epilepsy (Ferriero, 2005). In addition, gliosis and concomitant inflammation are aspects of chronic epilepsy that may reduce graft survival and impair efforts for repairing the hippocampus.

Generating Specific Types of Neurons for Transplantation from ES Cells

Before ES cell-based therapies can be used for repairing neural circuits, it must be determined how to generate specific cell types (or their precursors), such as GABAergic neurons or principal neurons that release glutamate and form long-range axonal projections (Figure 33.4 and Figure 33.5). Both of these cell types are damaged or lost in temporal lobe epilepsy. Knowledge gained in the field of developmental neurobiology will greatly aid these efforts. In addition, most studies have been done with transplants made before the development of recurrent seizures. Validation of this approach requires studies of rodents with spontaneous seizures to determine whether the transplants are successful in chronic epilepsy. We now understand that most, if not all, of the functionally distinct subtypes of forebrain GABAergic neurons are derived from microdomains of the ventricular zone in the ventral telencephalon. The different types of interneurons form in the ganglionic eminences, transient bulges in the ventricles of the ventral forebrain, where combinatorial codes of transcription factors regulate their genesis and specify cell fates (Cobos et al., 2005a,b; Gonzalez et al., 2002; Lavdas et al., 1999; Marin et al., 2000; Parnavelas, 1992; Trinh et al., 2006; Wonders and Anderson, 2005a,b, 2006; Wonders et al., 2008).

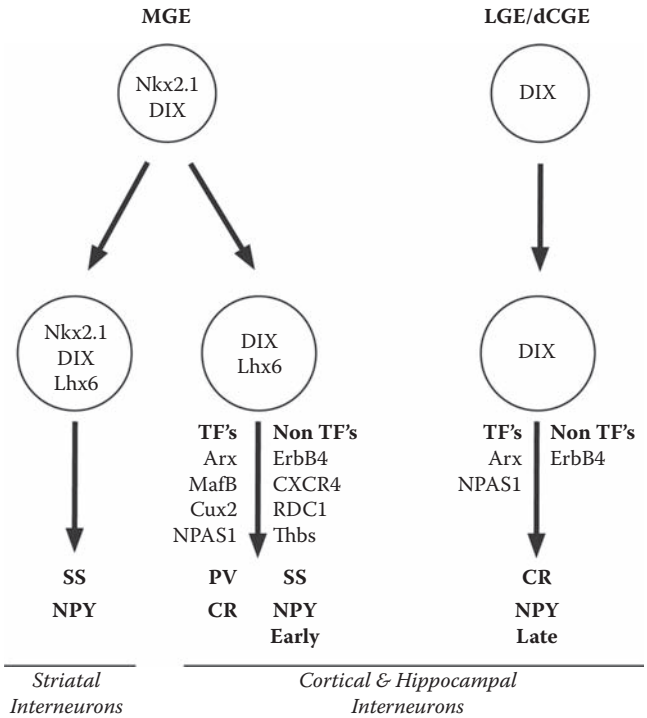

FIGURE 33.4 Model of transcriptional regulatory mechanisms controlling the fates and migrations of GABAergic interneurons derived from the ganglionic eminences (see, for example, Zhao et al., 2008).

Interneurons take multiple routes from the ventral telencephalon as they migrate to reach the striatum, cerebral cortex, or hippocampus, and a complex code of adhesive and repulsive cues guides this process (Alifragis et al., 2004; Anderson et al., 2001; Denaxa et al., 2001; Friocourt et al., 2008; Marin et al., 2003). Information about the molecular codes for regulating GABAergic interneuron identity and migration should now make it possible to direct ES cell-derived neural progenitor differentiation into particular fates by stable transfection with suitable expression vectors that drive expression of transcription factors specifying GABAergic interneurons in the embryonic brain (Anderson et al., 1997; Butt et al., 2007; Du et al., 2008; Wonders and Anderson, 2005a; Wonders et al., 2008; Xu et al., 2003, 2004, 2005).

Role of Epigenetic Factors in Regulating the Production and Differentiation of ES Cell-Derived Neural Precursors for Transplantation

In addition to transcriptional codes that determine cell fates, secreted signaling molecules and epigenetic factors also appear to play a significant role in guiding neural stem cell differentiation. The production of neural stem cells for neuronal replacement therapies may therefore require additional steps that incorporate intercellular communication via cell surface receptors, secreted neurotransmitters, and other soluble factors. For example, the neurotransmitter GABA has long been known to potentiate the maturation of developing neurons (Antonopoulos et al., 1997). The secreted protein Sonic hedgehog (Shh), released from the ventral forebrain, plays a critical role in maintaining the expression of transcription factors in interneuron precursors (Xu et al., 2005). Sequential treatments of ES cells with retinoic acid and Shh can induce caudal and ventral spinal motor neuron

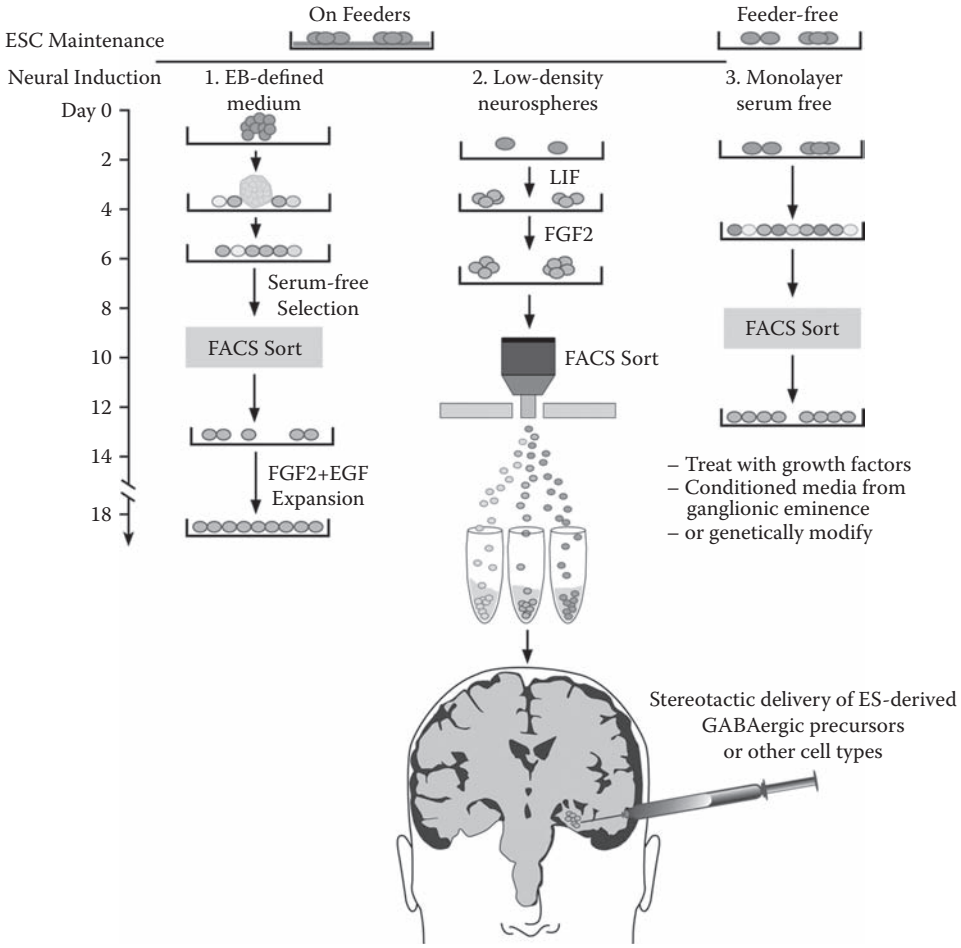

FIGURE 33.5 (See color insert following page xxx.) Approaches for producing and purifying embryonic stem (ES) cell derived neurons for stereotaxic delivery into limbic structures for treating temporal lobe epilepsy ES-based stem cell therapy, based on studies in many labs. *Abbreviations:* embryonic stem cell (ESC), embryoid body (EB), fluorescence activated cell sorting (FACS), leukemia inhibitory factor (LIF). Figure is based on work in our laboratory and others (see, for example, review by Cai and Grabel, 2007).

phenotypes (Li et al., 2005). Several recent studies described protocols for generating GABAergic and other neuronal cell types by adding a specific sequence of growth factors and diffusible molecules to ES cell cultures (Barberi et al., 2003). Serum treatment and coculturing ES cells with primary fetal cells are two additional approaches employed.

These studies suggest that inductive interactions and the factors secreted by more differentiated cells may influence the choices ES cells make toward specific lineages. In fact, the vast majority of ES cells at the undifferentiated stage are cultured on feeder layer cells such as fibroblast cells or stromal cell lines; therefore, the notion of deriving specific neuronal types from ES cells by coculturing with other cell types is not new. In most cases, however, the elements influencing ES cell differentiation in a coculture system are still undefined. Specific protocols, as well as detailed transcriptomes of interneurons, will soon be available and will ultimately make it possible to evaluate the relative strengths and limitations of different ES cell protocols that employ forced expression of transcriptional factors, defined media, or coculturing for producing specific neuron types.

One powerful tool that is under development is the construction of human and mouse ES cell lines with reporter genes that are driven by lineage specific promoters (Giudice and Trounson, 2008). In this approach, the green fluorescent protein (GFP) gene is turned on only transiently during the neural stem cell stage; the expression is then terminated upon differentiation into postmitotic neurons. Lineage-specific expression is advantageous because neural stem cells can be enriched by methods such as fluorescence-activated cell sorting (FACS), a state-of-the-art technique to pool cells based on their fluorescence expression profiles. FACS purification allows neural stems cells to be separated from undifferentiated and potentially tumorigenic stem cells. For example, transduction of ES cells with lentivirus carrying GFP genes under the phosphoglycerate kinase promoter turns on GFP during differentiation of the ES cells into hematopoietic lineages (Hamaguchi et al., 2000). One of the best characterized genes for this purpose is *Sox1*, the earliest marker currently known for neural stem cells (Ying et al., 2003). When ES cells expressing *Sox1*–GFP were FACS sorted prior to transplanting them, it was found that they produced well-contained grafts without forming tumors (Chung et al., 2006).

CONCLUSIONS

Only a few years ago, the potential to correct imbalances between excitation and inhibition or to make specific neuronal cell types for transplantation therapies to treat epilepsy seemed out of reach. The rapid progress in technology, including development of safer viral vectors for gene delivery, knowledge about the developmental programs for generating individual classes of neurons from ES cells, and strategies for preventing the death of endogenous neurons, is now opening the door for novel therapies to treat intractable epilepsy.

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