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Stem cells as a potential therapy for epilepsy

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Abstract

Neural stem cells and neural progenitors (NSC/NPs) hold great promise in neuro-restorative therapy due to their remarkable capacity for self-renewal, plasticity, and ability to integrate into host brain circuitry. Some types of epilepsy would appear to be excellent targets for this type of therapy due to known alterations in local circuitry based on loss or malfunction of specific types of neurons in specific brain structures. Potential sources for NSC/NPs include the embryonic blastocyst, the fetal brain, and adult brain and non-neural tissues. Each of these cell types has potential strengths and weaknesses as candidates for clinical therapeutic agents. This article reviews some of the major types of NSC/NPs and how they have been studied with regard to synaptic integration into host brain circuits. It also reviews how these transplanted cells develop and interact with host brain cells in animal models of epilepsy. The field is still wide open with a number of very promising results but there are also some major challenges that will need to be addressed prior to considering clinical applications for epilepsy.

Keywords

Neural stem cells; Epilepsy; Transplant; Local circuits

Introduction

The discovery of neural stem and progenitor cells has opened the doorway for new cellular therapies for a variety of human neurological disorders. The true potential of these various cell types for neuro-restorative therapy is only beginning to be realized. Conversely, research into pre-clinical applications has made apparent the many challenges that must be overcome in order to make this promise a reality. Much of the therapeutic work with neural stem and progenitor cells has focused on Parkinson's disease, stroke and spinal cord injury. However, it would seem that epilepsy would be a promising target for cellular restorative therapy because many types of severe epilepsy are focal in nature and it is likely that many of these types of epilepsy result from a derangement of the neuronal circuitry in the region where seizures begin. In some cases there is a loss of specific types of neurons, such as inhibitory interneurons, in the epileptic focus. Therefore, selective, regional replacement or augmentation of specific neuronal subtypes would likely abolish the seizure-generating capacity of these areas. This is especially appealing since restoration of local circuitry may

not require establishment of long-range axonal projections and synaptic connections with distant brain sites, a goal that may be difficult to achieve in a mature host brain. In addition, the cellular alterations that result in seizures can also result in impairment of normal function of certain brain structures, such as the hippocampus. These cognitive co-morbidities can produce a burden on affected patients that can be as great as the seizures themselves. With a goal of restoring normal neural circuitry, cellular therapy offers the potential to reverse these cognitive deficits as well. Stem cells offer several potential advantages over current therapies for epilepsy. Anti-epileptic drugs have no regional specificity and are known to be ineffective at controlling seizures in 30–40% of people with epilepsy (Kwan and Brodie, 2000). Stem cells can be targeted to focal areas of epileptogenesis and tailored to affect only the dysfunctional constituents of the epileptic circuit. Surgical resection is quite effective for some focal epilepsies, but this treatment is limited by involvement of eloquent cortex and a poorly defined boundary of the region of epileptogenesis in some cases. Stem cells could theoretically be used in areas of eloquent cortex and could be more widely inserted into a region of epileptogenesis based on clinical response. This paper will review different cell sources and strategies for using neuronal stem and progenitor cells to treat epilepsy by establishing new neurons that incorporate into host brain circuits.

Overview of stem cell terminology

The wide variety of cells that can be expanded and ultimately differentiate into neurons has produced a lexicon that can sometimes be confusing. Stem cells are immortal, self-replicating cells that can produce a variety of different cell types, usually of different organ systems (Lindvall et al., 2004). These cells can be obtained from embryonic sources (embryonic stem cells, or ESCs) but may also be obtained from fetal or adult tissue. Under proper growth conditions, ESCs can be induced to produce specific cell lineages like neurons and glia (Brustle et al., 1997). These more differentiated cells are often called embryonic stem cell-derived neural precursors (ESNPs). Neural stem cells (NSCs) are self-replicating cells that can produce three-dimensional structures called neurospheres under particular in vitro growth conditions and can produce both neurons and glia (Reynolds et al., 1992). This term often refers to cells in the adult sub-ventricular zone of the lateral ventricles and the sub-granular zone of the dentate gyrus. Neuronal progenitor cells (NPs) are usually not immortal and are more restricted in their cell generating capacity (Higginbotham et al., 2010). They can produce neurons, sometimes only certain types of neurons such as inhibitory interneurons, and glia. NPs generally do not demonstrate the capacity to generate neurospheres. The term, progenitor cell, often refers to cells in the fetal ganglionic eminence and to certain types of cells in mature brain. Although there are general areas of agreement on what defines a stem cell vs a progenitor cell, it may be that these terms represent two points on a biological continuum and the differences are more often determined by functional properties rather than morphology. This means that, for a given study, it may not always be possible or feasible to determine the degree of “stemness” of a given donor cell. Therefore, we have relied on the term NSC/NP to acknowledge this ambiguity. There is also lack of clarity in the literature with respect to sources of the different cell types used for transplantation studies. For instance, it is not always clear when

an embryo becomes a fetus and even fetal stages of development are often defined by their embryonic (i.e., post-conception) age measured in days (e.g., E13 is 13 days after successful mating). For the purposes of this review, we will reserve the term “embryonic” to describe cells acquired from a blastocyst, “fetal” for cells derived from pre-natal, immature brain structures such as the ganglionic eminence, and “adult” for cells from the mature brain or other somatic tissues.

Focal epilepsy as a disease of local circuitry

Epilepsy is a common and devastating health problem that affects 6.25/1000 in the United States (Engel, 1989). Although antiepileptic medications can control seizures in many patients, 30–40% of people with epilepsy are refractory to medical therapy (Kwan and Brodie, 2000). The majority of patients with intractable epilepsy have partial epilepsy with the temporal lobe (TLE) being the most common site of seizure onset. The most common pathological finding in TLE is hippocampal sclerosis (HS), a condition characterized by neuronal loss and synaptic reorganization in specific parts of the hippocampus (de Lanerolle et al., 1989). Hippocampal changes in TLE have been studied in human tissue and animal models of this disease. Many animal studies have examined the effects of convulsant-induced status epilepticus (SE) using pilocarpine or kainic acid (for review, see Coulter et al., 2002). These include loss of specific cells types in the dentate hilus including excitatory mossy cells and inhibitory somatostatin-containing interneurons (Buckmaster and Jongen-Relo, 1999; Obenaus et al., 1993). Another important change involves synaptic reorganization with sprouting of the dentate granule cells’ mossy fibers into the inner molecular layer of the dentate (Houser et al., 1990). After 30 years of intensive study, the relative contributions of these and many other changes to the clinical phenotype of epilepsy are still not settled and well beyond the scope of this review. However, HS does represent a clearly defined pathological state where regional loss of specific type of neurons and subsequent synaptic reorganization may lead to epilepsy.

Abnormalities of cortical development are a major pathological substrate of intractable epilepsy in childhood comprising about 40% of children treated with surgical resection (Farrell et al., 1992; Lerner et al., 2009). Cortical dysplasia (CD) is a general term used to describe a deviation from normal development of the cerebral cortex that results in abnormalities of lamination, spatial orientation of the neurons, neuronal morphology, and/or abnormalities of cell commitment. As with HS, a wide variety of abnormalities in neuronal circuitry have been described in human CD tissue as well as animal models of CD. But there is strong evidence that loss of inhibitory interneurons and reduced inhibition may play a role in some types of epilepsy associated with CD. Several investigators have reported a loss of inhibitory interneurons in surgical specimens from human focal CD (Ferrer et al., 1994; Spreafico et al., 2000). Calcagnotto et al. (2005) studied neocortical tissue from 7 patients with focal CD and 9 “control” patients with medial temporal lobe epilepsy. Recording from pyramidal cells, they found a significant reduction in the frequency of spontaneous and miniature inhibitory post-synaptic currents (IPSCs) in the CD specimens. In the in utero irradiation model of CD, numerous studies have shown reduced numbers of inhibitory interneurons (Roper et al., 1999), reduced frequency of IPSCs in pyramidal cells (Zhu and Roper, 2000), and reduced excitatory drive in the surviving interneurons in this model (Zhou

et al., 2009). These findings support the idea that loss of gamma-aminobutyric acid (GABA)-containing interneurons and reduced inhibition may be an important mechanism of epileptogenesis in some types of CD.

HS and CD are two examples where focal brain abnormalities are known to produce epilepsy and where there is experimental evidence to suggest that replacement of specific subtypes of neurons could be reasonably expected to decrease seizure susceptibility and treat epilepsy. The fact that loss of subtypes of GABAergic interneurons (rather than projection neurons) appears to be important in both conditions further supports the feasibility of cellular restorative therapy as discussed above.

Caveat: new-born cells as causes of epilepsy

Not all newly generated neurons in adult brain are the “good guys”. There is strong evidence that newborn dentate granule cells (DGCs) arising from neural progenitors in the subgranular zone of the dentate gyrus contribute to abnormal circuits that support seizure activity (for review, see Kuruba et al., 2009). In both the pilocarpine (Parent et al., 1997) and kindling (Bengzon et al., 1997) models of TLE, seizure activity dramatically increases neurogenesis in the sub-granular zone of the dentate gyrus. These newly generated DGCs produce the axons that result in mossy fiber sprouting, they produce basilar dendrites, and they also migrate ectopically into the hilus. Each of these changes has been shown to increase the excitability of the local circuitry. In addition, the developmental stage of the newborn DGCs at the time of the pilocarpine-induced status epilepticus (SE) determines which pathological features the DGCs will express as they mature (Kron et al., 2010). These studies provide a cautionary tale for therapeutic transplantation of exogenous neural stem cells in that pathological factors in the host brain may induce deleterious properties in the transplanted cells. However, it is useful to note that, in the pilocarpine model, DGCs born just before SE exhibited basilar dendrites and ectopic migration but only cells born after SE produced mossy fiber sprouting (Kron et al., 2010). This suggests that avoidance of potential pathological properties in the transplanted NSCs may be controllable by culture conditions and by the stage of development of those cells prior to transplantation.

Sources of neural stem cells

A recent PubMed search with the key words “epilepsy” and “stem cell” leads to 446 references. Accordingly, there are already a number of excellent studies and review articles that nicely address and summarize this topic (Loscher et al., 2007; Maisano et al., 2009; Naegele et al., 2010; Shetty and Hattiangady, 2007). In this review, therefore, we will not reiterate all relevant issues nor discuss all articles and perspectives on the roles for stem cells in epilepsy, but rather focus on a few key issues that hold promise for applying regenerative medicine to new therapeutic approaches.

Embryonic

There has been a great deal of attention focused on ESNPs as a potential powerful source of cells for neuroprotection and repair for a variety of neurological disorders. In particular, relevant to stem cell transplantation in the hippocampus, rodent-derived ESNPs that give

rise to glial- or neuronal-restricted precursor cells have been grafted into early postnatal rodent hippocampal slices in culture in order to monitor their survival, differentiation, and potential functional integration within established circuitries (Benninger et al., 2003). These studies, like many similar stem/progenitor cell transplant studies, have looked at directed differentiation of the donor-derived cells and their incorporation into brain circuitries that could be monitored over time using slice recordings and even the application of new optogenetic technologies (Tønnesen et al., 2011) to be able to observe the interactions of donor-derived cells with host neurons and glia. ESNPs hold great promise because of their protean nature and the possibility that cues derived from host neural circuitries might help direct their fate choice and differentiation toward needed populations of cells for replacement therapies as well as provide a therapeutic neurohumoral environment. However, populations of ESNPs still may be contaminated by undifferentiated embryonic stem cells, thus presenting the potential to generate inappropriate tissue constituents, including teratomas, following their grafting to the host CNS (Chen et al., 2009; Knoepfler, 2009; Wernig et al., 2004). New methods of purifying ESNPs, as well as providing a molecular micro-environment that favors controlled differentiation within established and even diseased circuitries, still place these cells at the top of most cellular therapeutic lists as being the most promising of transplantable cells. Like all donor-derived embryonic cells, without somatic cell nuclear transplantation to achieve a patient-oriented personalized approach, grafted ESNPs for epilepsy should require immuno-suppression to avoid rejection. Future advances in the immunology of stem and progenitor cells may afford ex vivo genetic engineering of even universal ESNP donor cells for transplantation into compromised, seizure-prone forebrain circuitries. It also still remains to be determined if the original source of ESNPs give rise to heterogeneous populations of precursor cells that exhibit distinctive differentiation potential that ultimately could affect fate choice and integration within mature and reorganizing host circuitries. Most studies to date however treat ESNPs as a single type of stem/progenitor cell whose fate and differentiation potential is largely if not exclusively driven by geography; i.e. the host tissue location represents the most important determinant of fate choice and differentiation. That said, what one would assume to be a most receptive host brain environment for appropriate and functional assimilation of ESNPs does not always work out to be true. Chen et al. (2009) have recently shown that a granule cell-depleted cerebellum in the weaver mutant mouse does not provide enough differentiation cues or presents a toxic environment that does not support the complete differentiation of a proven mouse ESNP line. New insights gained from studies of neural development and neuronal differentiation will undoubtedly help guide us to methods of better priming of stem/progenitor cells toward particular classes and subtypes of neurons for achieving their complete and functional integration into established and compromised neural circuitries (Peljto and Wichterle, 2011).

Fetal

Fetal stem cells are those derived from tissue-specific precursors that have many morphogenetic attributes in common with their embryonic, blastocyst-derived ancestors. Fetal NSC/NPs have also been studied in numerous neurological disease and injury models, and these cells can exhibit impressive differentiation and integration potential following their grafting to many different CNS sites. In particular, many of the cells that have been

used or proposed in clinical trials for spinal cord injury (Wirth et al., 2001), stroke (STEP Participants, 2009) and pediatric metabolic disorders (Tamaki et al., 2009) are fetal neural precursor cells that have been harvested from the rodent or human forebrain and expanded ex vivo to provide transplantable populations of primary or immortalized cell lines (e.g., De Filippis et al., 2008). These cells usually differentiate into interneuron populations although fetal human neural stem/progenitor cells have also exhibited lineage diversity in their production of neurons with a variety of transmitter phenotypes (Ourednik et al., 2001). A common site for harvesting fetal NP/NSCs is the ganglionic eminence. This structure (divided into medial, lateral, and caudal subdivisions) in the immature striatum produces most of the GABAergic neurons of the forebrain (Wonders and Anderson, 2005). As mentioned for ESNPs, fetal-derived NSC/NPs are generally assumed to possess impressive potency and plasticity in their fate choice and differentiation regardless of their original site of origin; however, the fetal cells would presumably harbor more “memories” of their most recent morphogenetic habitat because of potential site-restricted developmental programs that were more recently at play before these cells were isolated and prepared for transplantation.

Adult

Few would argue that the most successes to date with the use of stem cell transplantation for human disease therapy have come from adult stem cell sources, e.g. bone marrow hematopoietic stem cell transplants for a variety of cancer and other therapies. These cells offer autologous, non-immunocompromising, therapeutic options with the harvesting of either bone marrow-derived cells or using induced pluripotent stem cells (“iPSCs”, Takahashi and Yamanaka, 2006) to make different populations of somatic cells for transplant therapies. There is also a growing literature on the potential use of cord blood stem cells for a variety of protection and repair approaches for neurological disease; to date, we are not aware of breakthrough technologies for applying these adult stem cells as potential therapeutics in epilepsy. Mesenchymal stem cells, however, isolated from bone marrow and other tissues have been engineered to release neuroprotective and potential seizure-attenuating factors in epilepsy (e.g. adenosine, Ren and Boison, 2010).

The occurrence of neurogenesis in the adult mammalian brain is now well established. This has been most thoroughly documented in two areas, the dentate gyrus and the subventricular zone lining the lateral ventricles (Altman, 1969; Altman and Das, 1965; Lois and Alvarez-Buylla, 1993). These cells generate granule cells of the dentate gyrus and GABAergic interneurons of the olfactory bulb, respectively. Adult neurogenesis in humans has also been documented (Eriksson et al., 1998; Sanai et al., 2004). Other studies have identified cells from adult mammalian brain that can be maintained in vitro and give rise to neuronal and glial progeny (Morshead et al., 1994; Reynolds and Weiss, 1992). Similar neural progenitor cells have now been identified in adult human brain (Ayuso-Sacido et al., 2008; Kukekov et al., 1999; Moe et al., 2005; Nunes et al., 2003; Roy et al., 2000; Sanai et al., 2004; Walton et al., 2006). Scheffler et al. (2005) reported a method to maintain cells from the subventricular zone of adult mice in vitro. These cells were plated and grown as adherent monolayers. They were initially expanded in solution that contained growth factors (epidermal growth factor, EGF, and basic fibroblast growth factor, bFGF). They characterized, in vitro, three cell types

that had been described in the subventricular zone of rodents in vivo by Doetsch et al. (1997). A layer of cells with the features of type I protoplasmic astrocytes appeared to grow on top of a layer of small, transient amplifying cells. These cells corresponded to type B and type C cells as described by Alvarez-Buylla and colleagues (Doetsch et al., 1997). Upon withdrawal of growth factors, the cultured cells began to differentiate and form neuroblasts (corresponding to type A cells of Doetsch et al., 1997). When exposed to retinoic acid, these neuroblasts formed mature neurons that were exclusively GABAergic. Each of these cell types displayed physiological properties similar to those described for their corresponding cell types in vivo (Belluzzi et al., 2003; Wang et al., 2003).

Walton et al. (2006) applied similar techniques to isolate and expand AHNPs from mature human brain tissue. Fresh tissue specimens were taken from five patients undergoing anterior temporal lobectomy for the treatment of intractable epilepsy. Specimens for culture were taken from three locations: hippocampus, white matter lining the temporal horn of the lateral ventricle, and lateral temporal neo-cortex. Using similar culture conditions to those of Scheffler et al. (2005), AHNPs were successfully cultured and expanded to an average of 60 population doublings. Although the cell lines were not immortal, they exhibited impressive ex vivo expandability and it was calculated that one cell would have the potential to generate enough neurons to form roughly 4×10^7 adult brains. These proliferating cells remained dependent on externally supplied growth factors as well. In contrast to the murine subventricular zone cells used in Scheffler's study, expanded human AHNPs in this study did not readily form neurospheres. When injected into the lateral ventricles of postnatal day 3 (P3) mice, the transplanted cells were primarily incorporated into the lining of the ventricles and had morphological and chemical properties of astrocytes. However, when transplanted into cortex of newborn and adult NOD-SCID mice, these cells could form mature neurons primarily in the cortex and, less frequently, in the hippocampus. These cells could also be made to differentiate in vitro by removal of growth factors and addition of cAMP, nerve growth factor (NGF), and IBMX. Most of the criticisms of adult NSC/ NP approaches still, however, center on a perceived limited expandability and their potential harboring of genetic mutations or limited phenotypic plasticity because of their potential decades of aging in situ.

Since the discovery and first publications of induced pluripotency in somatic cells (Takahashi and Yamanaka, 2006; Yu et al., 2007), there has been an unprecedented interest in the concept of generating embryonic stem cells and tissue-specific progenitor cells from these for repair and replacement in human neurological diseases. The methods first described by the Yamanaka and Thomson labs relied on viral vectors carrying combinations of embryonic morphogenetic factors (e.g. *Oct4*, *SOX2*, *c-Myc*, *KLF4*, *NANOG*, *LIN28*) where the vector backbone, along with the transgenes, are permanently integrated into the host cell genome. Since the original publications, there has been a great deal of interest in the use of induced pluripotent stem cells (iPSCs) for studying neurological diseases. Generating iPSCs from individual patients affords the opportunity of massive generation of cell types from that patient for bioassays including drug-screening on the at-risk cells (e.g. motor neurons in ALS, see Dimos et al., 2008), as well as for autologous cell replacement therapies, or true personalized medicine, using the patients re-engineered cells to not express

disease genes or to express neuroprotective factors. A flurry of publications have appeared since these first papers describing the utilization of iPSC technology for a variety of devastating human diseases, including other neurodegenerative diseases such as spinal muscular atrophy (Ebert et al., 2009) and Parkinson's disease (Park et al., 2008). Recently, iPSC technology has moved to a level where non-integrating viruses could be used to deliver the reprogramming transgenes (e.g. using adenoviruses, see Stadtfeld et al., 2008) proving that insertional mutagenesis is not necessary for reprogramming in vitro. Most recently, fears of residual transgene expression affecting iPSC-generated cells and potentially leading to hyper- or neoplasia have been addressed with a new system where non-integrating episomal vectors (Epstein-Barr virus, oriP/EBNA1 plasmids) were exploited because of the ability to remove the episome and thus have complete absence of vector or transgene sequences that still confer ES cell pluripotency in human fibroblasts (Yu et al., 2009). Silva et al. (2008) showed that neural stem cells can be reprogrammed at a very high frequency using a single round of transduction to achieve a stable, ground-state pluripotency.

Evidence for functional integration of neural stem cells

There are several ways that transplanted stem cells could influence host brain function. These include using the cells to deliver neuro-modulating compounds or neurotransmitters into the micro-environment, as a source of growth factors, as inciters and modulators of the inflammatory response, and through manipulation of the glial cells. Some of these strategies have been applied to epilepsy models and are the subject of a recent review by Loscher et al. (2007). The focus of the current review is on stem cell-based strategies that would alter brain function by creating new neurons that would integrate synaptically into the host network in such a way that a lost or malfunctioning class of neurons is replaced or augmented. Toward that end, it is pertinent to examine the evidence for functional integration of stem cell-derived neurons after transplantation. Benninger et al. (2003) transplanted ESNPs derived from the J1 ES cell line (Li et al., 1992) into long-term hippocampal slice cultures from P9 rats. Over 2–3 weeks, these cells developed into functionally mature neurons with action potentials and normal membrane properties. They received synaptic GABA and non-N-methyl-D-aspartate (NMDA) currents but NMDA currents were rarely found. They also received synaptic contacts from the host perforant path (the normal source of afferent connections for neurons in this area). Englund et al. (2002) transplanted immortalized neural progenitor cells from embryonic rat brainstem (RN33B) into the cerebral cortex and hippocampus of newborn rats on P1–2. These cells were studied 4–15 weeks after transplantation. The transplant-derived cells had morphologies indicative of neurons with pyramidal cells being more common than non-pyramidal neurons. Long-range projections from transplanted neurons to the host thalamus and contralateral hippocampus were demonstrated using fluorogold techniques. Whole cell recordings from transplanted cortical pyramidal-like neurons showed the presence of action potentials, NMDA and non-NMDA-mediated EPSCs, GABA-mediated IPSCs, and appropriate types of short-term plasticity for these synaptic currents. These findings suggest that neural progenitors possess a remarkable capacity for differentiation and functional integration when transplanted into the immature rat brain. Wernig et al. (2004) injected

ESNPs into the ventricles of fetal (E16.5) rats. The cells migrated widely throughout the host brain and developed into pyramidal cells and GABAergic interneurons. At P10–21 the neurons showed intrinsic membrane properties appropriate for their cell type and received non-NMDA-mediated EPSCs and GABA-mediated IPSCs; but no NMDA-mediated currents were seen. Alvarez-Dolado et al. (2006) transplanted neural progenitors from the medial ganglionic eminence of fetal (E12.5–13.5) mice into the brains of newborn (P3–4) mice. These cells matured into GABAergic interneurons with firing properties and post-synaptic current appropriate for their cell type. In addition, nearby host pyramidal cells in the neocortex showed increased levels of synaptic inhibition compared to pyramidal cells in areas without transplanted interneurons. This study is important because it demonstrates the ability of the transplanted neurons to alter host neuronal function through synaptic connections. Tønnesen et al. (2011) used optogenetic methods to demonstrate reciprocal synaptic connections between graft and host neurons. They used DA-producing neural stem cells from fetal (E10.5) mice and studied them after engraftment onto striatal organotypic cultures and transplanted into 6-OHDA-lesioned adult mice (a model of PD). Both transplant and host cells were transfected with light-activated rhodopsins that could either hyper- or depolarize the cell. Whole cell recordings were then performed that documented reciprocal connections between the transplanted and host neurons in both preparations. Although many questions remain unanswered, these elegant studies have demonstrated the ability of stem cell-derived neurons to mature into functional cells that integrate into the host brain circuitry using reciprocal synaptic connections. Recent experiments in our laboratory studied AHNPs (Walton et al., 2006) that were transplanted into the neocortex and ventricles of P1 rats and suggest a high degree of integration into the host rat brain (Chen et al., 2010, abstract; and Chen et al., in prep). It should be noted that most functional studies have been performed within a few weeks of transplantation. Questions concerning deleterious long-term effects on host circuitry and the durability of any beneficial effects by the transplant-derived neurons remain largely unanswered at this time.

Stem cell transplants in epilepsy models

To date, research on stem cell replacement therapy for epilepsy has primarily focused on temporal lobe epilepsy using status epilepticus (SE)-based models that produce chronic, spontaneous seizures. Rüschen Schmidt et al. (2005) used ESCs derived from the J1 cell line as donors that were transplanted into the hippocampi of pilocarpine-treated adult rats. They found that the cells developed into mature neuron with action potentials, EPSCs, and IPSCs. Although the transplanted neurons showed little migration away from the site of implantation, they did send extensive projections into the host brain tissue raising the possibility that they could still exert control over host brain function. Carpentino et al. (2008) used ESCs to produce neural progenitors that were transplanted into the hippocampi of adult mice 1 week after kainic acid-induced SE. Some of these cells produced mature-appearing neurons in the host dentate gyrus. In contrast, the cells made benign tumors when transplanted into control mice. This suggests that the host hippocampus after SE may provide environmental cues that favor differentiation and maturation of transplanted neural progenitors.

Hattiangady et al. (2008) obtained neuronal precursors from the lateral ganglionic eminence of fetal (E15) rats. They were injected into the hippocampi of adult rats 4 days after kainic acid-induced SE. The cells showed good survival and differentiation into various types of inhibitory interneurons, but they showed little capacity for migration in the adult brain. The transplanted cells did not prevent the occurrence of chronic motor seizures, but they did produce a significant reduction (67–89%) in the frequency of the seizures. A follow-up study (Waldau et al., 2010) used fetal NSCs (E14 rat) from the medial ganglionic eminence transplanted into the hippocampi of chronically epileptic adult rats (kainic acid model). The transplanted cells differentiated into neurons (13%, mostly GABAergic) and astrocytes (57%) and showed little capacity for migration away from the injection sites. Compared to pre-transplantation values, the animals showed a reduction of motor seizure by 43% and severe convulsive seizure (Racine V) by 90%. The authors attributed this effect to addition of new inhibitory neurons to the hippocampal circuitry and restoration of more normal levels of GDNF, a glial-derived protein with known anti-convulsive properties. Baraban et al. (2009) used fetal neural precursors (E13.5 mice) from the medial ganglionic eminence to treat seizures in a model of generalized epilepsy. Kv1.1 mutant mice have a defective potassium channel that affects GABAergic interneurons and leads to generalized epilepsy starting at 2–3 weeks of age. Neural precursors were transplanted into newborn mutant mice and resulted in an 86% reduction of electrographic seizures when compared to non-treated mutant mice at P32. Histological and physiological testing confirmed synaptic integration of the transplanted GABAergic neurons into the host circuitry. This is an impressive result given that the genetic defect in this model is not focal and the transplanted cells presumably had to exert an effect over large volumes of host brain to control seizure activity.

Chu et al. (2004) used human neural stem cells derived from the ventricular zone of a 15 week fetus injected into adult rats 1 day after pilocarpine-induced SE. Of note, the NSCs were injected into the tail vein of the host and then migrated almost exclusively to the limbic areas of the host brain. This appeared to be a special property conferred by the recent SE since NSCs injected into the tail vein of control rats showed little to no transplanted cells in the host brain. They found a significant reduction in the percentage of animals that showed spontaneous motor seizures 1 month later in the transplanted group (13.3% vs 86.7%) as well as reduced seizure frequency and severity. Grafted cells were detected throughout the hippocampi and about 30% showed markers for interneurons. However, only 1–2.5% of the transplanted cells contained NeuN, a general marker for mature neurons. This makes the overall phenotype of the transplanted cells uncertain.

Another cautionary tale was provided by an early transplant study from Buzsaki et al. (1989). They transplanted solid blocks of fetal rat hippocampus (E15–16) into adult rat hippocampi that had undergone disruption of the fimbria/fornix, the primary conduit for subcortical efferent and afferent fibers for the hippocampus. The blocks of transplanted tissue survived and sent fibers into the adjacent host hippocampus. In vivo recordings documented seizures that originated in the engrafted tissue and spread into the host hippocampus. About half of the animals demonstrated behavioral seizures as well. The authors attributed this seizure activity to enhanced recurrent excitatory connectivity within the engrafted tissue. Although this type of grafting would not typically be considered for

therapeutic purposes, it serves as an important reminder that not all outcomes from NSC/NP grafting are beneficial for the host.

Future directions

The studies reviewed in this article highlight the encouraging potential for NSC/NPs in correcting abnormal circuits that result in epilepsy. But they also have delineated that many of the challenges must be addressed prior to successful clinical applications. Perhaps the most obvious is the limited ability of NSC/NPs to migrate in the adult host brain. Translational research going forward will need to refine our ability to generate specific neuronal (and glial) cell types and match them to specific epilepsy models in a rational fashion. A better understanding of how the host environment affects migration, differentiation, and integration of the transplanted cells is also critical. Presently, it is not clear which type of NCS/NP will be the best candidate for cellular therapy and it may be that this depends on the specific type of epilepsy or model that is being addressed.

Induction of pluripotency in somatic cells is a breakthrough in stem cell research, but is not ready for clinical applications because of some of the issues already discussed above, mainly associated with the use of the retroviral vector and the potency of these cells that often exceeds a current ability to control their differentiation to desired cellular types. With current methods, investigators cannot likewise control for the integration site or number of copies of each transduced gene and uncontrolled genomic integration can potentially interrupt critical genes, such as tumor suppressors, or alter transcriptional regulation of other genes, including oncogenes. Additionally, the use of c-myc as a reprogramming factor, a known oncogene, may contribute to the finding that a significant percentage of the mice derived from iPSCs develop tumors. iPSCs have been successfully generated without the use of c-myc (see above), but with much reduced efficiency. Even with some of the new iPSC technologies discussed above, as long as DNA molecules are introduced the potential of DNA integration cannot be totally eliminated. Therefore development of alternative methods could lead to a safer clinical application of iPSCs. Soldner et al. (2009) generated iPSCs from PD patients using a Cre-recombinase excisable-virus approach to achieve pluripotency free of viral reprogramming; however, new approaches using chemically defined growth conditions to induce pluripotency may eliminate the need for viral transfection reprogramming methods (e.g., see Ichida et al., 2009; Lyssiotis et al., 2009; Zhu et al., 2010). That said, there are still issues associated with the variability and heterogeneity of clones generated from the same reprogrammed cell (Hu et al., 2010) and somatic coding mutations (Gore et al., 2011) as well as copy number variation and selection during the process of reprogramming to pluripotency (Hussein et al., 2011). iPSCs do show great promise for the generation of tissue-matched replacement cells for the epilepsies or other neurological disorders, and a growing literature points to improved technologies for both generating (as discussed) and modifying the cells to enhance their therapeutic capabilities and optimize their controlled differentiation and functional integration within compromised brain circuits (e.g., see Hargus et al., 2010). For now, there is still a need for head-to-head comparisons of iPSC-derived human neural precursor cells with their putative indigenous brain counterparts (e.g., AHNPs), to determine which of these adult NSC/NPs might be best designed and engineered for the neurological problem at hand. Finally, even though iPSCs

might seem obviously well suited for autologous therapeutic approaches, another recent study has revealed their ability to induce an immune rejection response following syngeneic transplantation (Zhao et al., 2011). More studies are therefore needed to perfect this clearly powerful but still young technology for restorative neurology.

Therefore a scenario for a most promising stem cell therapy for epilepsy to date should include an iPSC that more specifically can generate requisite classes of therapeutic neurons and glia. A recent technological breakthrough, induced neuronal or “iN” methods developed by Vierbuchen et al. (2010), is the ability to generate neurons directly from non-neural somatic cells (e.g. fibroblasts) using reprogramming with a handful of transcription factors to bypass the generation of ES cells and ESNPs. Krencik et al. (2011) have provided the transcriptional recipe for generating large numbers of astrocytes from embryonic and iPS cells. This raises the possibility of induced astrocytic “iA” technologies that could be equally important for neuro-restorative therapies.

The importance of the astrocyte in the generation of therapeutic neurons for epilepsy should not be overlooked because of its specialized ability to control the molecular microenvironment around neuronal circuits that is so crucial to their physiological state. Also, astrocytes now have been implicated in a variety of neurological disorders, including ALS, Huntington’s and Parkinson’s Disease, as well as epilepsy (for review, see de Lanerolle et al., 2010). Thus, a near-future approach for using stem cell therapy and regenerative medicine to treat epilepsy might look something like this: patient-specific iNs and/or iAs are generated from skin biopsy-derived fibroblasts, or peripheral blood or bone-marrow derived cells, and these cells are used to generate different classes of neurons and glia to graft within the seizure-prone cortical or hippocampal locus. Subsequent behavioral and rehabilitative conditioning and therapeutic approaches will enhance the survival, fate choice and differentiation, and establishment of appropriate and functional synaptic networks to and from these cells with at-risk host circuits. Equally possible is the discovery of neuropoietins (Steindler and Pincus, 2002), possibly from insights gained during the antecedent stem/progenitor cell growth and phenotyping studies, that will facilitate the expansion and controlled differentiation of populations of indigenous stem/progenitor cells within these at-risk circuitries (e.g., AHNPs) that could also prove successful for functionally integrating without the need for ex vivo NSC/NP manipulation and cell transplantation. Concurrent synaptogenesis-promoting factors may also have to be either introduced or endogenously stimulated to facilitate the development of appropriate connections between the grafted and host cells.

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Abbreviations

bFGF basic fibroblast growth factor

CD	cortical dysplasia
DGCs	dentate granule cells
EGF	epidermal growth factor
EPSCs	excitatory post-synaptic currents
ESC	embryonic stem cell
ESNP	embryonic stem cell-derived neural precursor
GABA	gamma-aminobutyric acid
HS	hippocampal sclerosis
iPSC	induced pluripotent stem cell
IPSCs	inhibitory post-synaptic currents
NSC	neural stem cell
NSC/NP	neural stem cell/neural progenitor
SE	status epilepticus
TLE	temporal lobe epilepsy

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