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Stem cell sources and therapeutic approaches for central nervous system and neural retinal disorders

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Abstract

In the past decades, stem cell biology has made a profound impact on our views of mammalian development as well as opened new avenues in regenerative medicine. The potential of stem cells to differentiate into various cell types of the body is the principal reason they are being explored in treatments for diseases in which there may be dysfunctional cells and/or loss of healthy cells due to disease. In addition, other properties are unique to stem cells; their endogenous trophic support, ability to home to sites of pathological entities, and stability in culture, which allows genetic manipulation, are also being utilized to formulate stem cell–based therapy for central nervous system (CNS) disorders. In this review, the authors will review key characteristics of embryonic and somatic (adult) stem cells, consider therapeutic strategies employed in stem cell therapy, and discuss the recent advances made in stem cell–based therapy for a number of progressive neurodegenerative diseases in the CNS as well as neuronal degeneration secondary to other abnormalities and injuries. Although a great deal of progress has been made in our knowledge of stem cells and their utility in treating CNS disorders, much still needs to be elucidated regarding the biology of the stem cells and the pathogenesis of targeted CNS diseases to maximize therapeutic benefits. Nonetheless, stem cells present tremendous promise in the treatment of a variety of neurodegenerative diseases.

Keywords

embryonic stem cell; neurodegenerative disease; neuroregeneration; somatic stem cell; stem cell therapy

In a broad sense, stem cells are a population of cells capable of indefinite self-renewal that give rise to “daughter” cells committed to specific differentiation lineages through asymmetrical cell division. Their ability to control proliferation, differentiation, and apoptosis distinguishes them from neoplastic cells. Embryonic stem cells are primordial cells of the developing blastula capable of generating an entire organism, while somatic stem cells reside within individual organs and usually only give rise to cell types specific to that tissue. The normal function of stem cells includes the maintenance of homeostasis mediated by providing trophic support, as well as serving as a reservoir for replacing dysfunctional and senescent cells throughout the lifetime of the organism.[153] The fact that stem cells have the potential to differentiate into various cell types and tissues is the principal reason they are being explored in treatments for diseases in which there may be dysfunctional cells and/or loss of healthy cells due to disease.

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The CNS, traditionally believed to have limited regenerative capabilities, retains a limited number of stem cells in adulthood, particularly in the dentate gyrus of the hippocampus and the subventricular zone that replenishes olfactory bulb neurons.[138,180] However, these cells have little capability to participate in CNS repair in the presence of degenerative diseases or traumatic injury,[113] and they also present limitations as a cell source for transplantation therapies. This implies that new potential sources of stems cells for CNS therapies need to be considered. Ultimately, the goals are to replace lost neurons, reestablish functional neural circuitry, and restore neurological function. As such, the investigation and development of stem cell–based therapies aimed at the CNS have received a tremendous amount of attention.[108,116,125]

In this paper, we start by first reviewing some fundamental characteristics of ESCs and somatic (adult) stem cells, using NSCs and BMSCs as examples. We then consider in more detail 3 main approaches to neurological stem cell therapies: cell transplantation, neuroprotective strategies, and gene therapy approaches. Finally, we discuss in depth the recent advances made in stem cell–based therapies for treatment of progressive neurodegenerative diseases in the brain and the neural sensory retina, as well as neuronal degeneration that is secondary to other disease.

Stem Cell Sources

A wide range of stem cells from various sources are currently being investigated for their potential in stem cell–based therapies for CNS disorders. Although a detailed discussion of their properties and characteristics is beyond the scope of this review, we present here a broad introduction and provide extensive references for the interested reader.

Embryonic Stem Cells

Embryonic stem cells are pluripotent cells isolated from the inner cell mass of Day 5–8 blastocysts with indefinite self-renewal capabilities as well as the ability to differentiate into all cell types derived from the 3 embryonic germ layers. This has been demonstrated with mouse ESCs, first isolated in 1981, in which in vitro cultures of mouse ESCs could be propagated indefinitely without telomerase-mediated cell senescence and were able to give rise to all cell types of the body both in vitro and in vivo.[52,100,110]

Successful isolation of human ESCs was achieved in 1998, and they were shown to share similar self-renewal and differentiation properties to mouse ESCs.[154] Since then, human ESC work has relied heavily on previous studies done on mouse ESCs and embryonic carcinomas, cells derived from testicular teratocarcinomas.[45,46] While identifying precise markers that define undifferentiated ESCs is still a very active area of investigation, it is generally accepted that the transcription factors Oct-4, Sox-2, Nanog, Rex-1, and myc are important for maintaining the pluripotency and self-renewal properties of mouse and human ESCs.[22] However, several lines of evidence caution against overextrapolating mouse ESC results to human ESCs. For example, unlike mouse ESCs, which express stage-specific embryonic antigen–1, human ESCs express stage-specific embryonic antigen–3 and –4.[64] In addition, surface antigens associated with matrix keratin sulphate/chondroitin sulphates (TRA-1-60, TRA-1-81, GCTM-2, TG-30, and TG-343) are expressed by human ESCs but not mouse ESCs.[89] Finally, in contrast to mouse ESCs, human ESCs have been shown to express CD9, Thy1, and Class 1 major histocompatibility complex.[64]

Other research is focusing on identifying signaling pathways necessary for sustaining stem cell pluripotency so that undifferentiated human ESCs can be maintained and expanded in vitro without potential transspecies contamination associated with xenogeneic feeder layers[7,170] or animal-derived reagents.[146] It has been shown that while leukemia inhibitory factor and

bone morphogenetic protein inhibit neural differentiation of mouse ESCs and promote self-renewal under feeder-free conditions,[140,175] they are not sufficient to support undifferentiated human ESCs.[6,42,68,127] There is evidence that inhibition of bone morphogenetic protein and FGF-2 signaling is important to maintain undifferentiated human ESCs without a feeder layer.[172]

To date, human ESCs have been shown to differentiate into various derivatives of the ectoderm including neural precursors,[126,134,178] dopamine and motor neurons,[101,118,121,174] retinal cells,[13] keratinocytes,[62,73] and melanocytes.[53,178] In addition, they have also been differentiated into connective and structural tissue cell types as well as cell subpopulations from blood and cardiovascular tissues normally derived from the mesoderm.[15,79,80,92,120,123,145,163] Finally, they have also been shown to give rise to cell types from the endoderm including pancreatic, hepatocyte, and lung epithelium.[10,133,137] However, it should be appreciated that most of these differentiated cell types were assessed morphologically using immunohistological methods only, so that their functional characterization and therefore physiological potential remain to be determined.

In addition to the aforementioned challenges for using ESCs in transplantation therapies as well as the associated ethical concerns, there are a number of other obstacles that need to be overcome before ESCs can be considered for clinical use. First, the most widely used method today for differentiating ESCs involves culturing them as free floating embryoid bodies that inevitably give rise to cell types of different lineages derived from all 3 germ layers.[135] This approach, however, does not allow efficient production of mature, functional, and homogeneous cultures of the desired cell type with reliable precision and accuracy. Again, this highlights the need for better isolation, identification, and maintenance of undifferentiated human ESCs as well as the development of better differentiation protocols. Secondly, since Class 1 major histocompatibility complex is upregulated during differentiation,[49] clinical applications of human ESCs could lead to host immune rejection in the absence of immunosuppressive therapy. To address this issue, therapeutic cloning via somatic cell nuclear transfer, in which the nuclear genome of the host is introduced into an enucleated oocyte to generate human ESC lines that are histocompatible with the patient, is currently being explored.[14,67] Finally, as demonstrated by the formation of teratocarcinomas following injection of mouse ESCs into the striatum of parkinsonian rats,[18] unregulated cell growth of undifferentiated cells in vivo poses major challenges for the safety of using ESCs in any kind of transplantation therapy.

Adult (Somatic) Stem Cells

The discovery of somatic stem cells, sources of multipotent stem cells in adult animals with the capacity to differentiate into tissue-specific cell types, has caused much excitement because these cells represent a potential source of autologous cells for transplantation therapies that eliminates immunological complications associated with allogeneic donor cells as well as bypasses ethical concerns associated with ESCs. The realization that a cell source must exist in the adult animal for cell replacement in normal physiology and following injury was first recognized in the hematopoetic system with the development of bone marrow transplantations. Hematopoetic stem cells in the bone marrow transplanted to lethally irradiated subjects have the capability to reconstitute all the lineages of the blood system (erythroid, lymphoid, myeloid, and megakaryocytic).[107,177] Interestingly, in the wake of these findings, the discovery of unexpected plasticity and regenerative capabilities in the adult CNS made it the first solid organ system shown to possess somatic stem cells, later termed neural stem cells.[141] While it may not be surprising to find stem cells in the bone marrow that would support the rapid turnover of blood cells in the hematopoetic system, the discovery of stem cells in the brain—an organ that was previously thought to be largely immutable following embryogenesis—revolutionized

traditional principles of adult organogenesis and cell replacement and opened new avenues of investigation in regenerative medicine.

Stimulated by studies involving the CNS and blood, other investigations soon revealed multipotent somatic stem cells in other tissues and organ systems including adult mammalian testis, epidermis, gut, heart, pancreas, lung, retina, vasculature, and breast.[41,109,143] Unlike ESCs, which are able to generate all cell types of the body, somatic stem cells are largely believed to be only capable of differentiating into cell types from their tissue of origin, although recent work on "trans-differentiation" of BMSCs is challenging such notions.[20,32,129] To date, the general consensus is that these somatic stem cells originate from pluripotent ESCs during development and are stored in specific stem cell niches to participate in organogenesis and support cell turnover throughout the lifetime of the organism. Since a lot of the current somatic stem cell work on CNS regeneration involves the use of NSCs and BMSCs, these 2 cell types will be discussed in more detail in the following sections as examples of somatic stem cells.

Neural Stem Cells

The concept that the adult mammalian CNS contains NSCs was first inferred from evidence of neuronal turnover in the olfactory bulb and hippocampus in the adult organism.[4,5] Since then, NSCs and neural progenitor cells, cells with more restricted neural differentiation capabilities committed to specific subpopulation lineages, have been generated from human ESCs[37] or directly isolated from neurogenic regions of fetal and adult CNS, such as the subventricular zone, which provides neuroblasts to replenish inhibitory interneurons in the olfactory bulb.[55,130,136,141] The multipotency of NSCs was demonstrated in vitro in the 1990s by their ability to differentiate into neurons, astrocytes, and oligodendrocytes as well as various forms of neural precursors.[55,58,117,149,164] In addition, in vivo delivery of these cells to animal models of neurodegenerative diseases was associated with varying degrees of functional recovery (discussed below).[115]

Currently, there is still no set of markers or protein expression profiles that precisely define and fully characterize undifferentiated NSCs. To date, they are primarily isolated and propagated in vitro as cells that form free-floating neurospheres when cultured in serum-free medium on non-adherent surfaces in the presence of mitogenic factors such as basic FGF or FGF-2 and epidermal growth factor,[63,84,128,141] although there have also been reports of monolayer cultures.[58,76] However, the neurospheres are by no means homogeneous. They consist of heterogeneous cells expressing a continuum of surface markers and transcription factors that change during long-term culture. Furthermore, the clonality of individual neurospheres is not absolute; time-lapse video microscopy has captured merging and exchanging of cells between neurospheres.[138,139] While it has been shown that undifferentiated fetal NSCs can be maintained in vitro for up to 6 months[27] and that the derived neurons preferentially differentiate down the GABAergic lineage,[75] there is evidence that the fraction of neuron-toglia generation from NSCs declines with extended culture time.[74,81,168] Adult-derived NSCs exhibit similar properties in vitro to fetal NSCs but with more limited expansion potential. The data and results to date suggest that the development of a fully validated protocol to characterize and extensively propagate undifferentiated NSCs is still much needed before there is any hope of their clinical use.

Transplantation of both fetal and adult NSCs in vivo has so far showed few tumorgenic complications; however, there has also been limited differentiation of tissue-specific mature neurons (also further discussed below). The underlying problem is believed to be the short time window following neural induction during which the cells can be directed toward specific neuronal lineages.[21] Functional recovery following NSC transplantation is probably due to neuroprotective effects of the grafted cells rather than the replacement of lost neurons.

Nevertheless, because they can potentially be an autologous stem cell source for transplantation with minimal risk of tumor formation, the therapeutic use of NSCs is being actively investigated.

Bone Marrow-Derived Mesenchymal Stem Cells

Bone marrow-derived mesenchymal stem cells, also termed bone marrow stromal cells, are another example of a somatic stem cell being studied for its therapeutic potential in the CNS and in other tissues. They are a population of adherent cells in the bone marrow, independent of the hematopoietic system, that normally give rise to osteoblasts, chondrocytes, and adipocytes.[1] In comparison to NSCs, an additional attractive quality of BMSCs is the relative ease of clinically viable cell isolation. They can then be further expanded and differentiated in vitro using various media formulations and culture surface conditions to direct them to different cell lineages.[66] Like other stem cells, BMSCs have been shown to be able to migrate to areas of injury, even crossing the blood-brain barrier.[3,150] Although the reproducibility and efficiency of BMSC therapies need to be carefully examined, these early experiments suggest that BMSCs can be administered intravenously to CNS targets.

Despite the relative ease of isolation and migration potential of BMSCs, there is considerable debate in the literature regarding the true plasticity of these cells and therefore their clinical utility. First, BMSCs are normally obtained from the bone marrow by plastic adhesion assay, but there has been no characterization of the full spectrum of adherent cells that reside in the bone marrow. Whereas the authors of some studies have used a fluorescence-activated cell-sorting assay to obtain a more homogeneous population of cells, there is no consensus on the set of surface markers that characterize undifferentiated BMSCs, making it difficult to directly compare studies. In fact, one current school of thought in the field is that the bone marrow may serve as a reserve for a wide variety of stem cells, such that BMSCs obtained by plastic adherence assay alone may actually include heterogeneous subpopulations of multipotent stem cells with differing preferences for varying cellular lineages.[129] Second, original reports of in vivo BMSC differentiation into neurons and glia,[24,167] termed “transdifferentiation” because BMSCs do not normally differentiate down neural lineages,[144] were challenged by confounding results claiming transdifferentiation to be the result of cell fusion.[151] However, recent evidence seems to show that BMSCs can generate neurotransmitter-responsive cells with electrophysiological properties similar to neurons.[165] In addition, there are reports of the coexpression of markers from different neural phenotypes in BMSCs following putative transdifferentiation events, including GABA and tyrosine hydroxlase,[147] nestin and GFAP,[166] nestin and β-tubulin,[156] GFAP, and GALC.158 Still, most of these coexpression patterns have not been observed in primary neural cells or in cells derived from NSCs; therefore, whether these observations actually support the neurogenetic plasticity of BMSCs remains to be determined. Finally, an additional potential therapeutic application of BMSCs is in neuroprotection. These cells have been shown to release a variety of neurotrophic growth factors that can enhance cell survival in injured CNS regions,[124] although again the effectiveness of any neuroprotective applications needs to be further investigated and ultimately assessed in vivo.

In Vivo Stem Cell Transplantation Therapeutic Strategies

To date, there are 3 major classes of applications being explored for functional restoration of the CNS that in part make use of stem cells—namely, cell replacement strategies, neuroprotection, and gene therapy. Each of these takes advantage of the unique properties and capabilities offered by stem and progenitor cells, and they are often used in combination to maximize therapeutic effects.

Cell Replacement: Restoring Lost Neurons and Glia

Arguably the most desired outcome of stem cell–based transplantation therapies is to provide injured areas of the CNS with a population of cells, potentially engineered, that can use local biochemical and mechanical cues to differentiate into cell types that have been lost or altered by disease mechanisms and ultimately restore neurological function. Snyder et al.[142] demonstrated the potential use of stem cells in cell replacement therapies in 1997 when they found that grafting C17.2 cells, an NSC cell line derived from the external germinal layer of neonatal mouse cerebellum and immortalized with the *v-myc* gene,[84] into areas of photolysis-induced apoptotic pyramidal neurons in the adult mouse neocortex led to integration into regions of cell death, differentiation into pyramidal neuron-like cells, and extension of neurites that established appropriate afferent synaptic contacts.[142] These results suggest that there may be local environmental cues during injury in the adult neocortex that can direct the differentiation of NSCs to regenerate areas of damage even though the normal developmental window has passed. The sensitivity to differentiation cues was emphasized in Snyder and colleagues' study by the preferential differentiation of NSCs into glia in areas outside the photolytic lesion.[142] This in itself raises technical issues related to how one controls the proliferation and differentiation of transplanted cells outside target areas. One potential strategy may be to use local cues induced by injury mechanisms or other secondary mechanisms that regulate proliferation and differentiation processes under the control of specific inducible promoters although this is in itself is challenging task.

Since this early work, many other studies have been focused on demonstrating and optimizing stem cell–based methods for the replacement of CNS cells lost to injury or disease. We discuss several of these in the next section. A number of challenges still need to be addressed before stem cell transplantation for cell replacement can be successfully translated to the clinical setting. With increasing knowledge of stem cell biology, there need to be developed ways to more reliably characterize the differentiation and cell-cycle states of stem cells prior to transplantation to better evaluate and control clinical outcomes. Also, there is evidence that differing transplantation techniques (for example, implanting multiple micrografts compared with a single large graft) may have a significant effect on the outcome and success of the procedure.[111,112] In addition, for grafts with allogeneic donor cells, establishing a postoperative immunosuppression regimen to avoid acute and chronic rejection of the graft is essential.[94]

Neuroprotection: Secretion of Neurotrophic Factors

The notion that stem cells may also play a neuroprotective role in vivo has been suggested in several studies that documented functional improvement following stem cell transplantation in the absence of any significant differentiation and reestablishment of functional synaptic connections. Ourednik et al.[115] first showed that undifferentiated NSC C17.2 cells produced GDNF in vitro, which may have contributed to the observed “rescuing” of dopamine neurons in regions of NSC transplantation in aged mouse brains in vivo. Human NSCs isolated from fetal spinal cord expressed and released GDNF as well as brain-derived neurotrophic factor when implanted into a transgenic rat model of ALS, leading to delayed onset of motor dysfunction and extended life spans.[171] Neural stem cells grafted into rat models of spinal cord injury have also been shown to express nerve growth factor, neurotrophin-3, and glial growth factor receptors, such as ErbB-2, and PASK, the mammalian homolog of the fray gene that is involved in axon ensheathment.[95,173] This list is by no means all inclusive, and it is conceivable that there may be additional unidentified neurotrophic factors that we are not yet aware of released by stem cells under stress of pathological conditions that may contribute to the neuroprotective effect. In addition to NSCs, this effect has also been found in stem cells from other organ systems. Human MSCs have been shown to secrete neurotrophin-3 in

coculture with neonatal cortical brain slices.[124] The same neurotrophin expression profile was observed at 45 days after transplantation of human MSCs in nude mice.[124]

Vehicles for Gene Therapy: Delivery of Factors to Delay, Arrest, and Reverse Injury

Contrary to localized and acute traumatic insults, in a number of degenerative disorders multiple subpopulations of cells and anatomical regions can be affected due to dysfunctional proteins (for example, lysosomal storage diseases) or inadequate synthesis of key factors (for example, dopamine in Parkinson disease). Stem cells possess several characteristics that make them promising delivery vehicles for gene therapy approaches aimed at reversing genetic mutations. Although most can be propagated for numerous passages in vitro to allow genetic manipulation prior to transplantation, there is evidence that at least some stem and progenitor cells tend to selectively target regions of injury or disease, seem to display intrinsic neurotrophic properties, and may have the potential to differentiate into cell types endogenous to the targeted regions. It has been shown, at least in NSCs, that a variety of molecules released during inflammation (such as stromal derived factor-1α) and chronic diseases can act as chemoattractants to guide stem cells to the site of injury.[71]

Stem Cell Therapies for CNS Disorders

In this final section, we summarize recent progress in stem cell therapies aimed at 6 major CNS disorders and illustrate how some of the aforementioned methods and strategies are being utilized to formulate clinically viable treatments.

Parkinson Disease

Parkinson disease is a neurodegenerative disorder caused by decreased stimulation of the motor cortex due to progressive degeneration of the nigral dopaminergic neurons of the substantia nigra, leading to motor function deficiencies characterized by muscle rigidity, tremor, bradykinesia, and akinesia.[78] To date, the main stem cell therapy strategy for Parkinson disease is cell replacement aimed at the restoration of dopaminergic neurotransmission in the striatum.

The results of animal model studies suggest that cellular transplantation may improve striatal function by normalizing dopamine receptor sensitivity, regulating dopamine receptor activity, and reconstructing both afferent and efferent connections in the striatum.[17,56] Functional improvements have been reported following the transplantation of fetal mesencephalic grafts into patients with Parkinson disease.[94] Clinical improvements have been observed to develop gradually over 6–24 months after the procedure and to last 5–10 years.[94] However, issues with fetal tissue availability, complications with optimizing treatment conditions, and variations in functional recovery following the procedure limit this approach from developing into a standardized therapy.

Embryonic stem cells and somatic NSCs may provide a reliable and more plentiful source of dopamine neurons needed for transplantation.[83,121,161] In vitro protocols have been shown to be able to differentiate mouse, monkey, and human ESCs into dopamine neuron-like cells expressing specific transcription actors (for example, Pax2, Pax5, and En1) indicative of dopamine synthesis[14,101,121,174] and electro-physiological properties of midbrain dopamine neurons.[82,83,148] In a recent study involving the transplantation of human ESC–derived dopaminergic neurons into the neostriata of 6-hydroxydopamine–lesioned rat model of Parkinson disease, the authors reported long-term restoration of motor function.[132] However, the grafts also showed expanding cores of undifferentiated mitotic neuroepithelial cells that could potentially become tumorigenic.

Although a great deal of progress has been made in this area, a number of challenges remain to be addressed before stem cell–based therapies can be used as standardized treatments for

Parkinson disease. First, differentiation protocols need to be further optimized to reliably produce highly purified and homogeneous populations of desired cell types. Second, the ability of transplanted stem cell-derived dopamine-producing neurons to survive, reinnervate, and restore dopaminergic neurotransmission in the striatum still needs to be fully assessed in vivo to ensure the long-term efficacy and safety of the procedure. Finally, treatment conditions such as patient selection, postoperative rehabilitation, and immunosuppression also need to be optimized to maximize therapeutic benefits.

Huntington Disease

Huntington disease or Huntington chorea is an inherited autosomal dominant disorder caused by a repeating CAG mutation in the Huntington gene (chromosome 4) leading to abnormal processing of the defective protein, accumulation of its fragments in cellular compartments, and eventual loss of GABAergic medium spiny neurons in the striatum as well as neurons in the cortex.[78] In patients with Huntington disease, progressive loss of these neurons disrupts functions in the cortico-striatal-pallidal circuit through disinhibition of pallidal signaling output, leading to severe physical and cognitive impairments as well as psychopathological symptoms. Currently, there is no treatment for Huntington disease. The stem cell therapy strategy most explored for this disease has been cellular transplantation of somatic stem cells of adult and fetal origin in an attempt to restore the inhibitory connections. This is based on promising results obtained in animal studies[106,160] and subsequent Phase I clinical trials[12, 122] of intrastriatal implantation of fetal striatal primordium from the fore-brain.

Huntington disease is uniquely suitable for fetal striatal neural grafts because the treatment involves populations of cells that are just entering active developmental growth and maturation into the exact cell type that needs to be replaced. As has been shown in animal models, varying levels of restoration—from the establishment of efferent and afferent connectivity to the development of complex frontostriatal neural circuitry—result in different degrees of functional improvement. These range from reduced chorea to recovery of complex motor/cognitive abilities[50] and even restoration of habit-learning capabilities.[23] In a Phase I clinical trial, 3 of the 5 patients with Huntington disease who underwent fetal neural cell transplantations had metabolically active grafts (demonstrated using fluorodeoxyglucose positron emission tomography scans) as well as clinical improvements in motor and cognitive functions for up to 6 years following the procedure.[12] In 1 report on a patient with Huntington disease who died 18 months after transplantation of a graft placement of unrelated causes, the authors found that the implanted neural grafts had successfully integrated into the host tissue, had extended neurites, and had innervated dopaminergic fibers of the host tissue.[57]

Although still under investigation, it is thought that the native population of NPCs in fetal tissues is the critical element responsible for the observed improvements. Therefore, it is presumable that NSCs may offer a treatment option to stop or even reverse the progress of Huntington disease by providing a reliable source of homogeneous NPCs for transplantation. There are numerous studies on stem cell grafts transplanted into different animal models of Huntington disease, most of them using NPCs expanded as neurospheres and differentiated in vitro prior to transplantation. In a recent study involving the injection of adult NPCs isolated from the subventricular zone into a rodent quisqualic acid lesion model of Huntington disease, investigators showed good graft viability with extensive cell migration at 8 weeks with the presence of differentiated cells positive for DARPP-32 and GAD67 (markers for GABAnergic spiny striatal neuron) as well as reduced functional impairment determined by apomorphine-induced rotational asymmetry and spontaneous exploratory forelimb use.[159] Studies done with fetal human and rodent NPCs have also demonstrated good graft survival and integration into host tissue with varying degrees of differentiation,[106,160] but further assessment of functional recovery is needed. In addition, transplantation of grafts with stem cells of mesenchymal origin

(that is, bone marrow and human umbilical cord blood cells) showed longer survival[51] as well as improved motor function[29] and working memory.[91] Compared with NPCs, fewer studies have been conducted using ESCs. Recent work by Dihné et al.[43] have demonstrated ≥ 75% ESC differentiation into β-tubulin–positive immature neurons in vitro prior to transplantation with no tumor growth following the procedure. However, no functional assessments were undertaken in these studies.

While tremendous progress has been made, there are still challenges to be addressed before stem cells can replace fetal neural tissue as cell transplantation therapy for Huntington disease. While a GABAergic phenotype appears to be the default differentiation pathway of NPCs,[74] protocols need to be developed that can more precisely direct differentiation into the striatal medium spiny projection neurons and interneurons needed for transplantation. Also, despite evidence that differentiated cells from grafts sprout neurites and make synaptic contacts with host tissue, observations of extensive axonal outgrowth have been limited, and there is no conclusive evidence of reconstruction of neural circuits in damaged areas.[50] Because restoration of the corticostriatal circuit is crucial for long-term functional recovery, more work is needed to optimize graft–host connectivity as well as the development of more rigorous tests to accurately assess motor and cognitive functions.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis is a progressive neurodegenerative disease caused by degeneration of motor neurons in the cortex and ventral grey matter of the spinal cord, leading to muscular atrophy throughout the body and, eventually, death.[78] Unlike Parkinson disease and Huntington disease, ALS has a more rapid clinical progression in which glial cells may play a key role.[77] In fact, experimental evidence of wild-type nonneuronal cells ameliorating degeneration of SOD1 mutant–expressing motor neurons in a chimeric mouse model of ALS composed of a mixture of normal and mutant cells suggests that neurotrophic support by stem cells could provide an effective treatment even without neuronal differentiation.[35] As a result, stem cell therapeutic strategies for ALS involve transplantation aimed at neurotrophic support and neuroprotection in addition to the replacement of lost motor neurons. In animal studies of stem cell therapies for ALS, genetically transplanted modified human fetal NSCs into the spinal cord of a mouse ALS model have been found to survive and secrete GDNF at the 11-week end point of the study with some differentiation into astrocytes.[85] However, the cells were not able to completely halt neurodegeneration and the rapidly progressing disease. Similar decreases in the rate of disease progression were observed in a recent study involving the transplantation of NSCs that had been first differentiated in vitro into cholingeric motor neuron–like cells and transplanted into the spinal cord of the same animal model.[39]

In addition to NSCs, reconstitution of wild-type BMSCs following irradiation of the SOD-1 transgenic mouse model of ALS showed delayed disease onset and increased life span.[38] Similar results have been obtained by intravenous infusion of human umbilical cord blood cells.[60] Although direct delivery of cells into the CNS may circumvent complications associated with peripheral injections, the more widely distributed and extensive disease related to ALS presents a unique surgical challenge in terms of cellular transplant delivery. Instead, the disease may benefit from a therapeutic strategy that utilizes the intrinsic capability of stem cells to migrate to areas of damage in order to provide neurotrophic support.

It should also be noted that since potential causes of ALS could have a hereditary component, it is unclear whether autologous stem cells can provide therapeutic benefits. Despite the fact that many treatment parameters await elucidation, clinical trials are underway based on the transplantation of autologous BMSCs into spinal cords of patients with ALS.[104] Cells isolated and expanded for 32 days in vitro were transplanted into 7 patients with rapidly progressing ALS. No major complications occurred post-procedure and no abnormal cell proliferation was

observed on magnetic resonance imaging 4 years postsurgery. Four of the 7 patients exhibited significant reduction in the rate of decline of respiratory function and in scores on the ALS functional rating scale.[105] However, 1 noted problem with using autologous stem cells is the variation in expansion potential between different patients dependent largely on age, and this may significantly affect the efficacy and outcomes of the treatment.

Retinal Degenerative Diseases

Another area where stem cell–based therapies are being actively pursued as a potential alternative is in the treatment of retinal degenerative diseases. In comparison with other CNS targets, the retina offers some unique advantages that make it considerably more favorable for the development of transplantation and stem cell therapies. The neural sensory retina is a unique structure of the CNS in that all of the essential cell types and neuronal circuitry are contained within its 5 primary layers spanning ~ 200 μm in thickness.[78] As such, with the exception of therapies targeting RGCs and optic nerve degeneration (such as in glaucoma), functional neuronal synapses that need to be established between host and transplanted cells are local and short-range connections with minimal need for guidance. The primary flow of information is essentially unidirectional from photoreceptors to the RGCs, although there are lateral connections mediated by different classes of horizontal cells, amacrine cells, and inner plexiform cells that synapse with photoreceptors and bipolar cells to filter and preprocess visual information before it passes on to the brain. Despite the cellular heterogeneity of the neural retina, most of the neuron types have been identified morphologically and/or physiologically.[102,103] Furthermore, the retina is the most accessible part of the CNS, which facilitates surgical delivery of cells and grafts via transplantation or injection, and does not require compromising or crossing the blood–retinal barrier because any cells or materials directly transplanted into the vitreous or subretinal space are on the immunologically protected CNS side of the barrier. Furthermore, the retina can be noninvasively assessed anatomically and physiologically by using tools such as optical coherence tomography and various electroretinography modalities.

While glaucoma, which primarily affects RGCs and the optic nerve, and some forms of vitreoretinal degeneration affect the inner retina, age-related macular degeneration, retinitis pigmentosa, and the numerous forms of retinopathy are all characterized by progressive loss of retinal photoreceptors and retinal pigment epithelial cells, resulting in remodeling of neural retinal circuitry and eventually vision loss. Stem and progenitor cells isolated from a number of sources including embryonic tissue, adult brain, adult bone marrow, and even the retina itself are being explored for their potential as stem cell–based therapies for these diverse classes of disorders.[59,96,97,99]

Of the numerous studies being conducted, there have been 2 general approaches to the transplantation of stem cells or stem cell–derived cells to rescue the degenerating retina: 1) photoreceptor survival promoted by restoring the supportive functions of the retinal pigment epithelial RPECs through subretinal grafts of stem cell–derived RPE-like cells; and 2) directly replacing lost photoreceptors with transplanted stem cells and retinal precursor cells coaxed to differentiate and integrate into the outer nuclear layer of the degenerating retina. In contrast to therapies for Huntington disease and ALS, which have already entered clinical trials, and Parkinson disease, for which graft viability and host integration have been investigated and verified, the potential of these cell-based therapies for retinal degenerative disorders is still being determined in animal models.

A recent study of subretinal injections of human ESC–derived RPECs into the Royal College of Surgeon rat model of retinal degeneration (caused by inhibition of RPEC phagocytosis of photoreceptor outer segments due to mutation in the receptor tyrosine kinase *mertk* gene[40]) reported rescue of visual response and acuity assessed using electroretinography, optomotor

acuity thresholds, and luminance threshold recordings in the superior colliculus.[96] However, there is no direct evidence that the observed functional improvements were due to rescue of RPEC phagocytosis rather than a general neuroprotective effect brought on by the transplanted cells. Indeed, a variety of cell types have been shown to provide temporary rescuing effects through secretion of neurotrophic factors when transplanted into the retina,[59,97] and the human ESC–derived RPECs have been shown to produce pigment endothelium–derived factor,[86] a trophic factor with protective and morphogenetic effects on photoreceptors.[11,16,70] Furthermore, the grafted cells were observed to aggregate at the injection site instead of integrating into the host retina with poor long-term cell retention that parallels the decline in rescue effects at 4 weeks after transplantation.[96]

Attempting to rescue the degenerating retina by cellular replacement of lost photoreceptor neurons presents its own unique set of challenges. In cases of severe retinal degeneration, the retina may have already undergone substantial postreceptor remodeling[54] (in the early stages of these diseases the retina beyond the photoreceptor layer remains relatively anatomically and functionally intact). Therefore, grafted stem cells would need to differentiate into functional photoreceptor neurons and establish the intricate functional connections with bipolar and horizontal cells in order to restore the neural retinal circuitry. A recent study of subretinal injections of photoreceptor precursor cells (isolated from the retina during rod photoreceptor genesis) into mouse models of retinal degeneration demonstrated cell integration into the retina, expression of the synapse protein bassoon, formation of synaptic contacts with bipolar cells identified by immunostaining with phosphokinase C, positive rod photoreceptor differentiation based on immunostaining for proteins involved in the phototransduction pathway (phosducin and rhodopsin), and improved visual responses assessed with extracellular field potentials of the retinal ganglion cell layer and papillary reflex to light stimulation.[99] Progenitor cells harvested at earlier and later developmental stages were to achieve these results, which the authors attributed to a lack of *Nrl* transcription factor expression generally only observed in postmitotic immature rod precursors.[99] However, despite the promising results of this study, the availability of human fetal photoreceptor precursors (which would need to be harvested in the second trimester of fetal development) limits the feasibility of translating such a treatment into the clinical setting. A better understanding of the developmental processes that guide embryonic and adult stem cells toward photoreceptor differentiation will allow optimization of protocols to reliably direct stem cell differentiation for photoreceptor replacement therapies. Furthermore, advances in therapeutic cloning could lead to successful generation of stem cells from adult somatic cells to facilitate transplantation of autologous cells for treatment of patients with retinal degeneration.

In addition to treating pathological entities that primarily involve the death of photoreceptors in the outer retina, stem cell therapies are also being investigated for treating degeneration of the inner retina—specifically, diseases that cause the degeneration of the optic nerve and RGCs (for example, glaucoma, Leber hereditary optic neuropathy, and ischemic optic neuropathy) for which there are currently no treatments available to improve vision once vision loss has occurred.[26,114] Regeneration of the optic nerve after injury or disease is difficult for a number of reasons. Optic nerve degeneration is usually accompanied by RGC apoptosis, whether it is due to elevated intraocular pressure, as in glaucoma, or excitotoxicity, as in retinal ischemia and reperfusion injury.[47,48] Furthermore, as is the case with most CNS neurons, RGCs in the adult mammalian retina do not have the ability to reinitiate axonal growth after injury on their own despite having this capacity during development.[30] Finally, similar to other areas of the CNS, reactive gliosis and upregulation of factors such as myelin-associated proteins (for example, Nogo) in the local environment following injury inhibit regrowth of axons that need to reestablish synaptic connections with the lateral geniculate nucleus.[30] Overcoming these challenges by using stem cell therapies would likely require a combination of cell transplantation and neuroprotective strategies. One major advantage of the inner retina and

optic nerve is that molecularly the mechanisms that ultimately result in RGC apoptosis and optic nerve loss are the same classic mechanisms described for neurodegenerative events in other parts of the CNS.[28,98,152] As such, both basic and clinical stem cell therapy investigations that target the inner retina and optic nerve might provide results that translate to or, at least guide, further research into similar therapies for other neurodegenerative disorders.

In a different and intriguing line of research investigators are attempting to use ESCs to essentially grow eyelike structures rather than replace a lost cell type or provide a neuroprotective environment. Mouse ESCs have been reported to generate eyelike structures consisting of lens, neural retina, and RPECs when cocultured with PA6 cells and induced with basic FGF, dexamethasone, and cholera toxin.[65] Furthermore, these structures have also been found to harbor retinal progenitor cells that could be differentiated into specific retinal cell types.[8] A recent study that cocultured these ESC-derived eyelike structures with both normal adult mouse retinal tissue and retinas with *N*-methyl-D-aspartate–induced excitotoxicity damage developed Tuj1-postive neurons that migrated to the ganglion cell layer. Most of these neurons in *N*-methyl-D-aspartate–treated retina went on to express ganglion–cell-specific markers, Hu and Bm3b.[9] However, functional assessment in a more physiologically relevant in vivo model remains to be done, and the clinical impact of this approach, if any, remains to be determined.

Finally, the adult retina offers another unique potential source of transplantable cells. Although residual proliferative activity has been detected in the ciliary marginal zone of the mouse and human retinas,[36,157] recent reports suggest that the adult mammalian neural retina may contain another small population of regenerative cells of glial origin, possibly a subpopulation of Müller cells, which are normally quiescent in vivo but can be induced to dedifferentiate into a more progenitor cell–like phenotype following injury.[87] It has been recently reported that the Sonic hedgehog signaling protein can induce Müller glia dedifferentiation into a more progenitor-like state with expression of Pax6, Sox2, and nestin both in vitro and in vivo.[162] Furthermore, these Müller glia–derived progenitor-like cells were shown to differentiate into rhodopsin-positive rod photoreceptor-like cells in a rat model of pharmacologically induced photoreceptor apoptosis.[162] Although considerably more work needs to be done, this approach is extremely fascinating and offers the potential of a clinically viable strategy involving a native population of endogenous cells to replace degenerated neurons in the retina and optic nerve.

Ischemia

Ischemia is due to inadequate oxygen supply and leads to activation of metabolic cascades that ultimately result in apoptosis of cells in the affected region.[31] In the brain, the effects of ischemia—the size of the lesion as well as the affected neuronal and glial cell types—can vary depending on the ischemic location and duration. Transplantation of naive and genetically manipulated stem cells are being explored as both a cell replacement strategy for lost cells and as a neuroprotective strategy. Transplantation of mouse ESC–derived neural precursors into an ischemic rat model of endothelial-induced middle cerebral artery occlusion showed graft survival up to 12 weeks, differentiation of neural precursors into immunohistochemically mature neurons and glia, and electrophysiological function.[25] Similarly, delivery of fetal human NSCs and human NPCs into focal ischemic lesions yielded differentiated cells that expressed neuronal and glial markers (MAP-2, NeuN, and GFAP), synaptic connections between graft-derived neurons and host tissue, and improvements in neurological functions.[72] Functional improvements were also observed with intravenously administered human NSCs in rat ischemic models that demonstrated the ability of these cells to migrate to lesion sites in the hippocampus.[33,34] However, despite promising results in the rodent models, differentiation of grafted stem cells and limited migration to injury sites have been observed to a much lesser extent in primates model.[131]

It was shown recently that the period of neurogenesis following stroke needs to extend to at least 4 month postinjury.[155] Subventricular zone NSCs form neuroblasts that migrate to lesion sites but are unable to repair damaged neural connections due to poor survival rates.[113] The observation that infusion of caspase inhibitors can prevent apoptosis of these neuroblasts caused by local inflammation suggests that stem cells may also be used for their neurotrophic properties to maximize the native regenerative potential of the CNS.[155] Growth factors and cytokines secreted by stem cells such as NSCs, BMSCs, and umbilical cord blood cells may aid in the survival of neurons and endogenous precursors by increasing proliferation and controlling inflammation.[19,119,169] Intravenous delivery of umbilical cord blood cells into a rat stroke model conferred short-term therapeutic benefits that were independent of stem cell migration to lesion sites.[19] These trophic effects were further enhanced by the overexpression of growth factors secreted by transplanted stem cells. Transplantation of BMSCs overexpressing brain-derived neurotrophic factor and GDNF,[88] hepatocyte growth factor,[179] and basic FGF/FGF-2[69] have been shown to improve functional recovery. In addition, it has been reported that while transplantation of NSCs into brains of postnatal mice subjected to unilateral hypoxic–ischemic injury showed targeted homing and integration into ischemic areas with ~ 5% differentiation into neuronlike cells, overexpressing NT-3 in these NSCs by retrovirus transduction enhanced these regenerative effects by increasing the fraction of NSC-derived neurons to 20% in infarct regions. These NSC-derived neurons reportedly encompassed a variety of neuronal subtypes appropriate to the cortex, including cholinergic, GABAergic, and glutamatergic neurons, with little glial differentiation and reduced astroglial scarring.[119] Furthermore, grafting of human NSCs genetically modified to overexpress vascular endothelial growth factor into mice cerebral cortex overlying intracerebral hemorrhage induced increased angiogenesis and behavioral recovery in animal models.[90]

Brain Tumors

Stem cells are also being used to improve the treatment of brain neoplasms, such as GBM, by improving understanding of the disease origin through studying stem cell biology, as well as by providing a therapeutic tool for targeted delivery of chemotherapeutic agents. Currently, the median survival rate of patients with GBMs is only 12–14 months following diagnosis despite aggressive treatment with radio- and chemotherapy and resection.[78] The primary difficulty in treating GBMs is the highly invasive nature of malignant cells, leading to diffuse and widespread distribution of tumor growth throughout the brain. In addition, limited knowledge regarding the initiating cells and subsequent carcinogenesis of the tumor limits our ability to accurately characterize the tumor and formulate effective therapeutic strategies.

Experimental data showing similar migratory, self-renewal, and molecular characteristics between NSCs and brain neoplasms suggest that the abnormal cells responsible for initiating tumorigenic growth may be stemlike cells that have lost proper control over proliferation and differentiation.[44,176] In fact, the presence of stemlike cells with uncontrolled growth and differentiation has been shown to correlate with malignancy of the tumor and its prognosis.[61] Therefore, it is conceivable that deciphering the differences between a normal stem cell and its tumorigenic counterpart may provide insights into the processes responsible for initiating the formation of brain tumors.

In addition to providing a better understanding of the origin of the disease, stem cells are also being employed as therapeutic tools to treat GBMs. Aboody et al.[2] found that murine and human NSCs grafted to different locations (intratumorally contralateral hemispheres and intraventricularly) with respect to the tumor as well as delivered intravenously showed remarkable abilities for targeting tumor sites, tracking down even individual cancer cells, and providing trophic support. Factors secreted by tumor cells for growth and angiogenesis as well as cytokines released by damaged cells are believed to be responsible for attracting the NSCs

to the neoplastic lesions. Specifically, chemokine receptor CXCR4 and its ligand stromal derived factor–1α have been shown to contribute to this targeting, along with stem cell factor, monocyte chemoattractant protein–1, and vascular endothelial growth factor.[176] Furthermore, grafting of NSCs modified to express cytosine deaminase allowed targeted delivery of chemotherapeutic drug through tumor site–specific conversion of prodrug to 5-flurouracil, resulting in dramatically reduced tumor mass. A variety of stem cell–based brain tumor therapeutics involving genetically modified and naive NSCs is currently being investigated.[93,176]

Conclusions

A variety of stem cells, embryonic and somatic, are being investigated for their potential to treat CNS disorders. Many studies in animal models are yielding promising results at the experimental level. However, clinical trials of stem cell transplantation for a number of CNS disorders have resulted in only moderately successful outcomes and have yet to demonstrate the ability of those cells to fully replace degenerating neuronal and glial cells in the CNS. Translational strategies must be based on a robust understanding of the fundamental principles of stem cell biology and CNS pathology to develop safe and effective stem cell–based therapeutics for CNS disorders, and this will undoubtedly take time. Much still needs to be elucidated regarding the biology of the stem cells as well as the pathogenesis of targeted CNS diseases to maximize therapeutic benefits. Still, stem cell–based therapies hold extraordinary promise for a wide range of neurodegenerative diseases.

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Abbreviations used in this paper

ALS, amyotrophic lateral sclerosis
BMSC, bone marrow–derived mesenchymal stem cell
CNS, central nervous system
ESC, embryonic stem cell
FGF, fibroblast growth factor
GABA, γ-aminobutyric acid
GBM, glioblastoma multiforme
GDNF, glial-derived neurotrophic factor
GFAP, glial fibrillary acidic protein
NPC, neural progenitor cell
NSC, neural stem cell
RGC, retinal ganglion cell
RPEC, retinal pigment epithelial cell

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