Stem Cell Technology for Neurodegenerative Diseases

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Over the past 20 years, stem cell technologies have become an increasingly attractive option to investigate and treat neurodegenerative diseases. In the current review, we discuss the process of extending basic stem cell research into translational therapies for patients suffering from neurodegenerative diseases. We begin with a discussion of the burden of these diseases on society, emphasizing the need for increased attention toward advancing stem cell therapies. We then explain the various types of stem cells utilized in neurodegenerative disease research, and outline important issues to consider in the transition of stem cell therapy from bench to bedside. Finally, we detail the current progress regarding the applications of stem cell therapies to specific neurodegenerative diseases, focusing on Parkinson disease, Huntington disease, Alzheimer disease, amyotrophic lateral sclerosis, and spinal muscular atrophy. With a greater understanding of the capacity of stem cell technologies, there is growing public hope that stem cell therapies will continue to progress into realistic and efficacious treatments for neurodegenerative diseases.

ANN NEUROL 2011;70:353–361

Neurodegenerative diseases are characterized by the loss of neurons in the brain or spinal cord. Acute neurodegeneration may result from a temporally discrete insult, such as stroke or trauma, leading to a localized loss of neurons at the site of injury. Chronic neurodegeneration may develop over a long period of time and results in the loss of a particular neuronal subtype or generalized loss of neuronal populations. In the brain, Alzheimer disease (AD) and Huntington disease (HD) result in widespread loss of neurons, whereas Parkinson disease (PD) involves the specific and localized loss of dopaminergic (DA) neurons in the substantia nigra. In the brainstem and spinal cord, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) involve the degeneration and loss of motor neurons (MNs). Although these conditions all exhibit unique neuronal pathologies, the exact mechanisms for neuronal loss are complex, making the identification of efficacious treatments elusive.

The lack of effective therapies for these neurological diseases creates an enormous burden on society. In the USA, approximately 7 million people are living with AD, HD, PD, ALS, or SMA (Fig 1A; http://www.alz.org/, http://www.apdaparkinson.org/, http://www.hdsa.org/, http://www.alsa.org/, http://www.smafoundation.org/). Projected research spending by the National Institutes of Health (NIH) in 2011 for these 5 diseases totals $768 million, and spending allocation is proportional to the number of individuals affected by each disease (see Fig 1A; http://report.nih.gov/rcdc/categories/). In the USA, an estimated 5.3 million individuals suffered from AD in 2010, making it by far the most prevalent neurodegenerative disorder, carrying an estimated health care cost of $172 billion (http://www.alz.org/). PD affects up to an estimated 1.5 million people in the USA, with approximately 50,000 new cases diagnosed each year, and the estimated costs of PD stood at $11 billion per 500,000 affected Americans in 2009.[1] Furthermore, HD and ALS each affect 30,000 Americans, and SMA affects 25,000 Americans.

Despite the destructive nature of these diseases, the number of affected individuals, and health care costs surpassing billions of dollars, there is a stunning lack of treatment options. Recently, cellular therapies have earned increased attention as potentially feasible novel therapies. Analysis of published scientific articles demonstrates that <2% of all papers per disease field examine the application of stem cells (see Fig 1B). However, it is

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22487

Received Dec 2, 2010, and in revised form May 10, 2011. Accepted for publication May 16, 2011.

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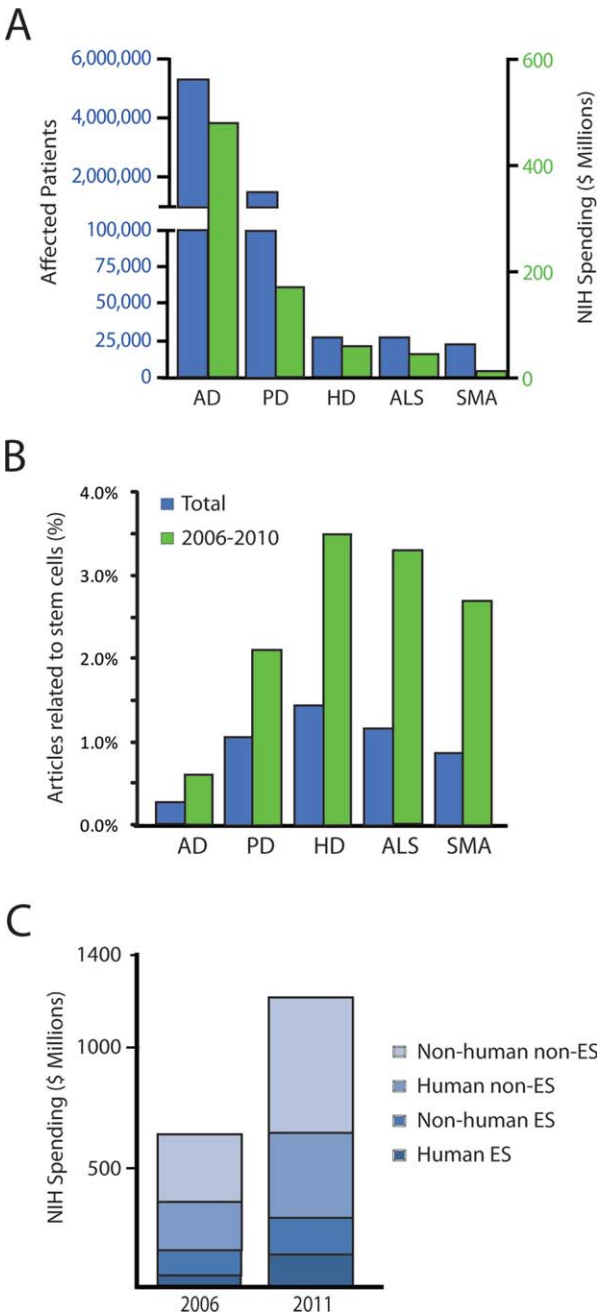

FIGURE 1: Analysis of the current state of stem cell research for neurodegenerative diseases. (A) Comparison of approximate number of affected individuals in the USA (blue) and anticipated breakdown of National Institutes of Health (NIH) spending on diseases in 2011 (green). (B) Portion of published literature focusing on stem cell technologies for individual neurodegenerative diseases overall (blue) and between 2006 and 2010 (green). Literature mining was performed using the MeSH terms "Alzheimer disease," (AD) "Parkinson disease," (PD) "Huntington disease," (HD) "amyotrophic lateral sclerosis," (ALS) "muscular atrophy, spinal," (SMA) and "stem cell." (C) NIH spending on stem cell technologies in 2006 and 2011 by stem cell type and species. ES = embryonic stem.

likely that these figures will increase as the field of cellular therapy advances. In the past 5 years this percentage rose for each neurodegenerative disease analyzed (see Fig 1B), and NIH spending on stem cell research doubled between 2006 and 2011 (see Fig 1C; http://report.nih.gov/rcdc/categories/). Although stem cell therapies in the USA are methodically examined via cautiously designed trials and basic laboratory science, the lack of available and effective treatments has prompted people suffering with neurologic diseases to turn elsewhere for "stem cell treatments." Reports of "stem cell treatments" from clinics in China and India are followed by glowing patient testimonials; however, these treatments are not the result of rigorous trials investigating safety and efficacy. Analysis of 7 spinal cord injury patients receiving treatment at a clinic in China included uncertainties concerning the type of cells utilized, appropriate delivery for the level of injury, and ultimate presence of any clinical benefit.[2] Since this analysis, improvements in cell quality, cell identification, delivery accuracy, and delivery targeting and reduced postoperative complications may have been made but have clearly not been documented.

Neurodegenerative diseases create a tremendous burden on society, and despite decades of research, effective treatments do not exist. Cellular therapies are attractive options, and the application of stem cell research to neurodegenerative diseases is rapidly expanding. In the current review, we detail the current state of stem cell research for neurodegenerative diseases, beginning with a brief introduction to the various stem cell technologies available. We also describe the rationale and remaining hurdles associated with transitioning stem cell therapies from bench to bedside. Finally, we discuss the current data and progress for translational stem cell therapies for AD, PD, HD, ALS, and SMA.

Stem Cell Technology

Stem cells have the capacity to proliferate and differentiate into multiple cellular lineages. There are different classifications of stem cells that reflect the range of possible cell types they can produce and the ways in which the stem cells are derived. These include embryonic stem (ES) cells, progenitor cells, mesenchymal stem cells (MSCs), and induced pluripotent stem (iPS) cells (Fig 2). To appreciate the potential applications of stem cell technology to neurodegenerative diseases, it is important to understand the characteristics of the various stem cell types available and the potential impact of cellular therapies on disease mechanisms.

Stem Cell Classifications

Each stem cell type possesses certain qualities and advantages, and the rationale for utilizing each depends on the desired applications and outcomes. ES cells are derived

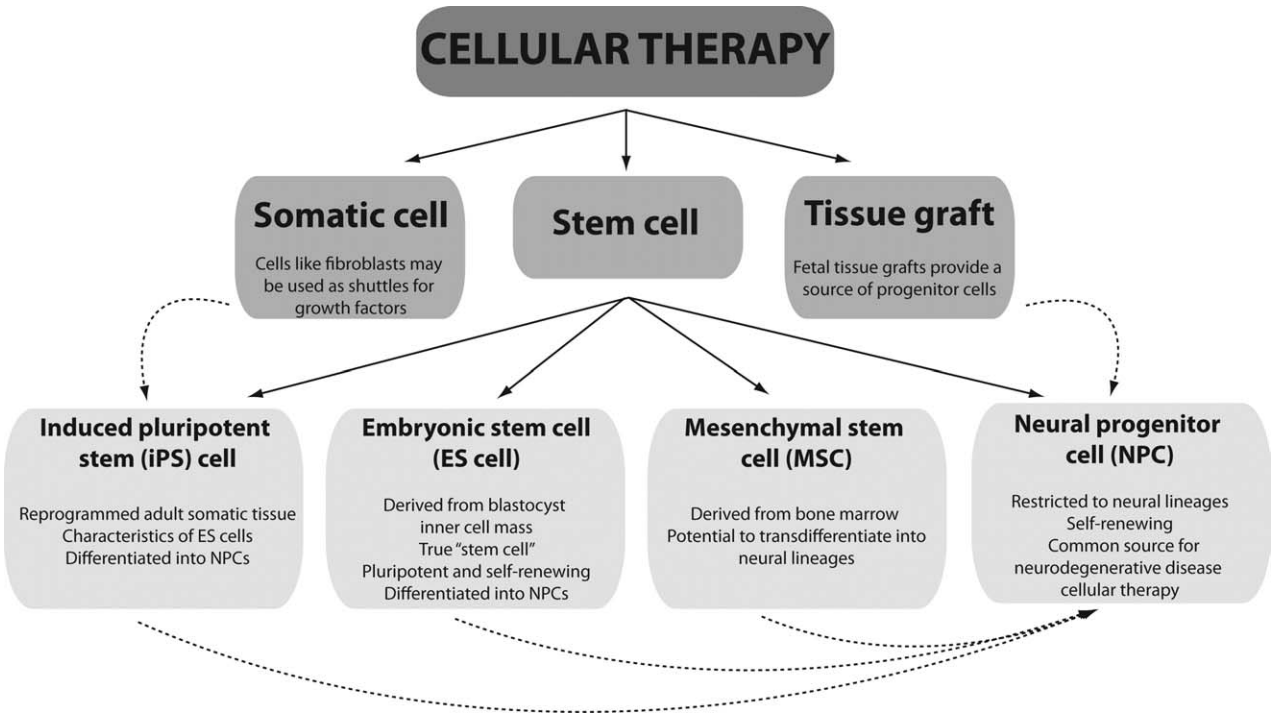

FIGURE 2: Stem cell technology and cellular therapy classifications. Cellular therapy involves the treatment of diseases using cells or tissue grafts. Various types of stem cells may be utilized, each possessing unique characteristics and advantages depending on the desired outcomes. Selecting the appropriate stem cell and treatment mechanisms for each disease is necessary to support the translation of cellular therapies for neurodegenerative diseases from bench to bedside. Solid arrows represent divisions within each category. Dashed arrows represent sources of iPS cells and NPCs.

from the inner cell mass of a developing blastocyst and are pluripotent, possessing the capacity to give rise to all 3 germ layers. Progenitor cells, which are derived from more developed fetal or adult tissues, are multipotent, meaning they give rise to more restricted lineages than ES cells. These potential lineages are usually determined by the germ layer of origin. For example, neural progenitor cells (NPCs), or neural stem cells (NSCs), are capable of differentiating to cell types within a neural lineage. NPCs may be derived directly from fetal or adult neural tissue, or by directed differentiation of ES cells via cell culture manipulation.[3,4] Along with this limited differentiation potential, NPCs also appear to have a more restricted self-renewal potential; although the self-renewal state may be maintained in culture, cells stop proliferating and start to differentiate when transplanted in vivo.[5] MSCs are an alternative source of multipotent self-renewing cells and are derived from adult bone marrow. Naturally, they differentiate to produce osteoblasts, chondrocytes, and adipocytes; however, there is evidence that they can transdifferentiate to a neural lineage.[6] MSCs provide an accessible alternative to ES cells and potentially circumvent the need for immunosuppression in cellular therapies because they are derived from an autologous source. Using autologous cells, however, may be less desirable when dealing with genetic diseases because the cells may possess the same genetic predisposition to disease. For example, MSCs derived from ALS patients exhibit diminished growth and differentiation capacity; however, these issues may be circumvented by enhancing cell culture techniques and establishing optimal cell passage numbers.[7,8]

More recently, the development of iPS cells has provided an additional source of autologous stem cells for modeling and treating diseases. iPS cells are generated from somatic tissue such as fibroblasts and are reprogrammed into ES-like cells by the addition of select transcription factors. The original approach utilized Oct 3/4, Klf, Sox2, and c-Myc,[9] and multiple research groups have now accomplished successful reprogramming of fibroblasts using various combinations of factors delivered by vector-, virus-, protein-, or RNA-mediated approaches.[10–13] Although many neurological disorders rely on complex genetic rodent models or chemical treatments that may not fully represent human neurodegenerative diseases, these cells afford options for disease modeling and provide novel sources for autologous cellular therapies. It should be noted that residual alterations from the genetic reprogramming required to induce pluripotency are possible[14,15]; therefore, careful characterization of patient iPS lines must be performed. With the continued advancement of iPS technology, however, directed differentiation of patient iPS cells

may be utilized to model human disease processes for mechanistic and therapeutic discovery.

Cellular Therapy Strategies and Applications

Cellular therapies utilize cell or tissue grafts to treat diseases or injury (see Fig 2). Treatment objectives of stem cell therapies typically center on cellular replacement or providing environmental enrichment. Cellular replacement for neurodegenerative diseases involves the derivation of specific neuronal subtypes lost in disease and subsequent grafting into affected areas of the nervous system. The newly transplanted neurons may then integrate, synapse, and recapitulate a neural network similar to that lost in disease. Alternatively, stem cells may provide environmental enrichment to support host neurons by producing neurotrophic factors, scavenging toxic factors, or creating auxiliary neural networks around affected areas. Many strategies for environmental enrichment utilize stem cells to provide de novo synthesis and delivery of neuroprotective growth factors at the site of disease. Growth factors such as glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-I (IGF-I), and vascular endothelial growth factor (VEGF) are protective in neurodegenerative disease models and provide in situ support at the main foci of disease.[16–21] The appropriate objective of cellular therapy for each neurodegenerative disease must be based on the specific neuronal pathology of each disorder. Whereas cellular replacement may be effective in diseases like PD where a specific neuronal subpopulation is lost, ALS is most likely to benefit from cellular therapies that enrich the local spinal cord environment to support the remaining MNs. Factors such as how well grafted neurons integrate and migrate within the host tissue, and the distances that axons must extend to reach their targets, must be considered when determining the potential efficacy of cellular therapies for neurodegenerative diseases.

Cellular Therapy for Neurodegenerative Diseases

Selecting the appropriate stem cell type and understanding the desired mechanism of support is only 1 step in developing and translating cellular therapies to patients. The course from bench to bedside is long and complex; and although each disease and cellular therapy is unique, certain universal issues must be considered for a safe transition to patient therapies.[22–24] The Table describes some of these issues that are pertinent to the development of any clinical trial, and describes some of the issues that arise along these lines for stem cell therapies. This transition from bench to bedside may take well over a decade of in vitro, in vivo, and large animal studies (Fig 3). As we acknowledge and overcome these issues, however, advances in the field of translational stem cell therapy will continue to gain momentum. Next, we will discuss the potential for therapeutic success, supported approaches, and current progress in translating stem cells from bench to bedside for specific neurodegenerative diseases.

AD

AD, the most frequent form of dementia, is characterized by memory loss and cognitive decline.[25] As the disease progresses, there is a widespread loss of neurons and synaptic contacts throughout the cortex, hippocampus, amygdala, and basal forebrain.[26] Although the exact pathology of AD remains unclear, pathologic hallmarks include Aβ plaques and neurofibrillary tangles.[26,27] There is an increased risk of developing AD with age, and the majority of AD cases are late onset, developing after 65 years of age.[28,29] With an increasingly aging population, the burden from AD is anticipated to rise.

Current treatment options for AD are centered on regulating neurotransmitter activity. Enhancing cholinergic function improves AD behavioral and cognitive defects.[30] Targeting the cholinergic system using stem cell therapies may provide environmental enrichment. Neurogenesis in the hippocampus decreases as we age and is exacerbated in AD[31,32]; therefore, cellular therapies that enhance neurogenesis or replace lost neurons may also delay the progression of AD. Enhancing BDNF levels, which are decreased with age and in AD, promotes neurogenesis and protects neuronal function.[33] Rodent AD models receiving NPC grafts demonstrate increased hippocampal synaptic density and increased cognitive function associated with local production of BDNF.[19] Similarly, BDNF upregulation along with NPC transplants also improves cell incorporation and functional outcomes in an AD rat model.[21] Nerve growth factor (NGF) production is another mechanism of cellular therapy efficacy. Genetically engineered patient fibroblasts that produce NGF are currently being examined in a phase I trial for AD.[34,35] Integration of NGF fibroblasts into a major cholinergic center of the basal forebrain provided some benefit to AD patients.[34] The Danish company NsGene (http://nsgene.dk/) is currently developing an NGF-releasing therapy using encapsulated epithelial cells. Combining engineered growth factor overexpression with the benefits of NPC integration into neural networks may provide an enhanced approach to treating AD. Furthermore, given the widespread neuronal loss involved in AD pathogenesis, targeting multiple systems simultaneously may be advantageous.

TABLE: Common Considerations When Translating Stem Cell Therapies to Neurodegenerative Disease Patients

Inclusion/exclusion criteria	Enrolling late stage patients may prevent loss of quality of life
	Late stage patients may mask any positive effects due to the intervention occurring too late in the disease course
Realistic expectation	Informed consent forms must clearly illuminate the goals of the study
	Safety trials vs efficacy trials
	Expectations of therapeutic effects based on disease state at intervention
Controlled study	Ideal study is a double-blind placebo study
	Late stage patients may mask any positive effects not observed due to the intervention occurring too late in disease
	Original PD studies offered control arm treatment after a 1-year follow-up, which confuses interpretation of efficacy
Immunosuppression	Although the brain remains an immunologically privileged site due to the blood–brain barrier, there is evidence that this barrier can be compromised in disease
	Studies of cell graft survival demonstrate that immunosuppression increases the survival of graft tissue
Potential side effects	Prevent/minimize potential side effects (ie, meningitis, fever)
	Avoid exacerbation of disease and tumor formation
	Risk vs quality of life
Safety of cellular therapy administration	Consider CNS accessibility and safety of delivery methods
	Pros/cons of systemic delivery, lumbar puncture, or stereotactic injection are important

CNS = central nervous system; PD = Parkinson disease.

PD

PD results from the progressive loss of DA neurons in the substantia nigra.[36] Patients suffer from severe motor deficits manifesting as tremors, muscle rigidity, and unstable gait and posture. Current treatment options include deep brain stimulation or therapies that aim to increase dopamine levels by providing a dopamine precursor, L-dopa, or providing dopamine agonists.[37–39] These treatments are effective early in disease to alleviate symptoms, but long-term efficacy is uncertain; they do not correct the deficit, have long-term side-effects, and become increasingly ineffective with PD progression. Cellular approaches for PD, on the other hand, focus on the replacement of lost DA neurons. Initial cellular therapies for PD utilized fetal ventral midbrain tissue as a source of DA neurons. Clinical trials have had varying degrees of success, but they supported cellular therapies for a potential functional benefit in PD.[23] Potential limitations of utilizing fetal tissue, however, include ethical concerns, and the ability to obtain adequate amounts of tissue for treatment. Alternatively, ES cells offer sources for large-scale production of neurons that acquire a midbrain DA phenotype.[40,41] Grafting both ES- and MSC-derived DA neurons into rat PD models results in functional recovery.[42,43] The ability to produce patient-specific DA neurons has recently been demonstrated using iPS cells.[44] Transplantation of these cells into a rodent PD model improved functional deficits and demonstrated cell integration in the host tissue.[45] These reports are among the first to demonstrate a therapeutic use for iPS cells in a neurodegenerative disease.

Although studies maintain cellular replacement as a viable approach for treating PD, environmental enrichment may also support existing DA neurons and slow or prevent further degeneration. Growth factor therapy through direct delivery or viral-based systems protects against neuronal decay in PD.[46,47] MSCs and NPCs engineered to produce growth factors such as BDNF, VEGF, GDNF, and IGF-I provide prolonged local growth factor production in situ. Transplantation of growth factor-producing MSCs and NPCs protects DA neurons and promotes functional recovery in rodent

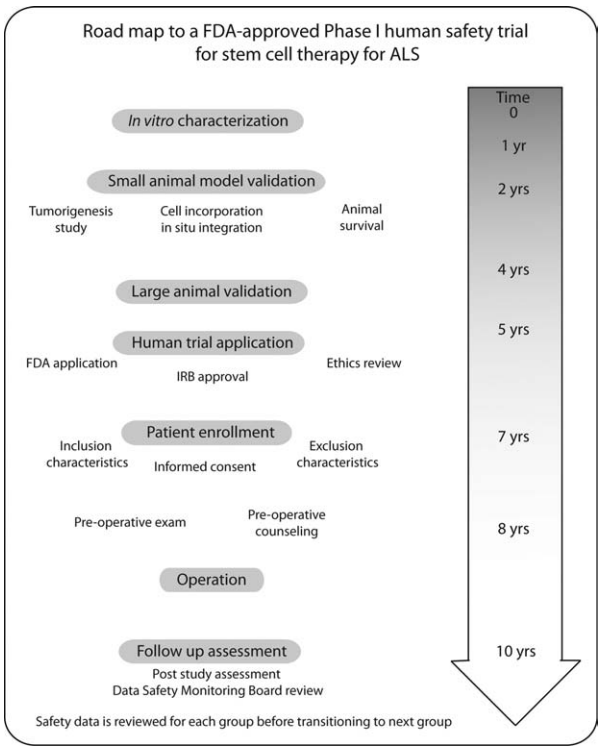

FIGURE 3: Overview of the stages involved in translating a stem cell therapy from the bench to patients, using the current amyotrophic lateral sclerosis (ALS) stem cell trial as a representative timeline. Supporting studies began in the late 1990s with the development of the HSSC line utilized in the trial and transitioned from HSSC characterization, through validation in animal models and stages required for human applications,[5,72,80,81] and finally to clinical trial approval in 2009.[5,78–80] The timeline in this figure reflects more than a decade of preclinical supporting studies for the current ALS trial. This road map provides an outline of the rigorous stages required for the translation of stem cell therapies from the bench to the bedside that may be applied to multiple neurodegenerative diseases. FDA = US Food and Drug Administration; IRB = institutional review board.

models of PD.[18,48–54] Taken together, the combination of cellular replacement and environmental enrichment may improve the efficacy of cellular therapies for PD.

HD

HD is an autosomal dominant polyglutamine disease caused by the accumulation of CAG repeats in the huntingtin gene.[55] Onset typically occurs in the 4th to 5th decade of life, with a disease course of approximately 20 to 30 years.[56] HD manifests with involuntary motor activity, dementia, personality changes, and cognitive impairment associated with the progressive loss of medium spiny neurons (MSNs).[57] Loss of these GABAergic neurons in the striatum is also accompanied by degeneration in the cortex, brainstem, and hippocampus.[57] Despite the known genetic basis for HD, insight into disease mechanisms and identification of effective therapies remain elusive.

Cellular therapies have provided some of the only positive treatment outcomes for HD. Initial therapies utilized fetal-derived tissue,[58] and grafting using the whole ganglionic eminence offered an optimal source of MSNs for HD.[59] The transplantation of neural cells and striatal grafts into rodent HD models demonstrated that MSNs integrate and form circuitry in the host.[60,61] Translation of fetal tissue grafting into HD patients prompted slight transient improvements and a period of stabilization prior to the inherent decline.[62] Key issues still remain, based on the ethical implications of utilizing fetal tissues and the dangers associated with cellular therapies such as graft overgrowth and the presence of non-neuronal cells within grafts.[62] Overall, the relative safety of the technique has been demonstrated in trials for both HD and PD.[22,23,58,59]

Stem cells also have the potential to restore functional loss of MSNs in HD. Striatal injections of NPCs into HD rodents demonstrated incorporation as well as migration to secondary sites associated with the disease.[63] The resulting functional improvements confirmed that isolated cell types provide similar functional benefits to those observed with fetal tissue, although mechanisms of cellular therapy protection were not examined. To address the role of environmental enrichment in cellular therapy for HD, NPCs engineered to overexpress GDNF were transplanted into HD rodents. Whereas unmodified NSCs provided no neuroprotective effects, NPCs expressing GDNF protected neurons and promoted functional recovery.[18,20] This study validates that environmental enrichment and protection of endogenous neurons may lead to functional recovery.

ALS

ALS is an adult onset disorder involving the degeneration and loss of MNs. Patients present with loss of coordination and muscle strength with transition to complete loss of muscle control. Death typically results from respiratory failure within 2 to 5 years of diagnosis. Multiple cell types and mechanisms are likely involved in ALS pathogenesis,[64] which makes the development of conventional drug therapies difficult. Cellular therapies for ALS provide both an integrating neural component and environmental enrichment to support and protect MNs from degeneration.[16,17] Assessment of several stem cell types, including NPCs and MSCs, in ALS rodent models demonstrates that systemic and direct intraspinal injection ameliorates disease progression,[5,65–71] suggesting that intervention prior to the irreversible loss of critical MN numbers may improve outcomes. Because it is crucial to protect the remaining MNs in ALS, the ability of stem cells to provide environmental enrichment via GDNF, VEGF, and IGF-I expression has also been examined, as

these growth factors all confer neuroprotection to MNs.[17] MN axonal degeneration precedes symptom onset and loss of MNs in ALS; therefore, providing distal support to MNs at neuromuscular junctions may also prevent neurodegeneration. Distal production of GDNF in muscle protects neuromuscular junctions and promotes MN protection, likely by retrograde transport.[71] Overall, the literature supports targeting cellular therapies to maintain MNs in the spinal cord and provide environmental enrichment to MNs and neuromuscular junctions.[72]

These supporting studies have created the foundation for the first phase I trial for ALS using fetal spinal cord-derived NPCs (http://neuralstem.com/). The cells utilized in the trial integrate safely into the spinal cord, synapse, and interact with host MNs, and also provide a source of growth factors upon direct spinal cord injection in rodents.[5,73] Delivery optimization and safety was further established in minipigs.[74,75] NPCs are being delivered to nonambulatory, and ultimately ambulatory, ALS patients through direct lumbar and cervical intraspinal injections to demonstrate the safety of the procedure and lack of toxicity from the cellular therapy. Because cellular therapies for ALS are designed to provide support and enrichment to existing MNs, it is likely that treatment efficacy in future trials will be best examined in earlier stage patients.

SMA

SMA involves the selective loss of MNs and presents with a broad range of onset and severity. SMA type I is the leading genetic cause of infantile mortality[76] and is characterized by early onset severe muscle weakness and fatality within 2 years. SMA is caused by a mutation or loss of the *SMN1* gene,[77] and the resulting decrease in SMN protein levels contributes to MN loss. In humans, low levels of SMN protein may be produced by alternative splicing variants encoded by the *SMN2* gene. Current pharmaceutical developments and gene therapy treatments focus on regulating *SMN2* to treat SMA. Cellular therapies, however, have been examined in mouse models of SMA, where grafting of ES cell-derived NPCs protected MNs from degeneration and improved survival.[78,79] It is possible that for SMA, transient rescue of the developmental loss of SMN may be sufficient to confer efficacy, which may not be the case for other neurodegenerative diseases where long-term degeneration of the transplanted cells is a valid concern.

Future Challenges

Neurodegenerative diseases create a tremendous societal burden due to their devastating nature, cost, and lack of effective therapies. Cellular therapies offer great promise for the treatment of these diseases, and research progress to date supports the utilization of stem cells to offer cellular replacement and/or provide environmental enrichment to attenuate neurodegeneration. In diseases where specific subpopulations of cells or widespread neuronal loss are present, cellular replacement may reproduce or stabilize neuronal networks. In addition, environmental enrichment may provide neurotrophic support to remaining cells or prevent the production or accumulation of toxic factors that harm neurons. In many cases, cellular therapies provide beneficial effects through both mechanisms.

Many questions still remain unanswered, and certain issues must be addressed as we continue the translation of cellular therapies from the bench to the bedside (see Table). The pathophysiology of each neurodegenerative disease discussed in this review is unique, and thus requires careful attention to the following topics. Which type of cells offers the best approach to treat this disease? What do we expect the stem cells to do, and what outcomes are predicted? How do we anticipate patients early and later in the disease course will respond to treatments? As we begin to design clinical studies that take into account these questions and learn lessons from the trials currently underway, we are poised to maximize the potential of cellular therapies to provide much-needed treatments for neurodegenerative diseases.

Acknowledgment

Funding is provided by the A. Alfred Taubman Medical Research Institute and the Program for Neurology Research and Discovery. S.A.S. is supported by the NINDS (T32 NS007222-28).

We thank J. Boldt for excellent secretarial support during the preparation of the manuscript.

Potential Conflicts of Interest

J.S.L., S.A.S.: employment, A. Alfred Taubman Medical Research Institute. J.H.: grants/grants pending, Juvenile Diabetes Research Foundation.

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