Published in final edited form as:
Cell Stem Cell. 2012 February 3; 10(2): 151–155. doi:10.1016/j.stem.2012.01.009.

Clinical Translation of Stem Cells in Neurodegenerative Disorders

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Abstract

Stem cells and their derivatives show tremendous potential for treating many disorders, including neurode-generative diseases. We discuss here the challenges and potential for the translation of stem-cell-based approaches into treatments for Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis.

Introduction

"Neurodegenerative disease" is a term used for a wide range of acute and chronic conditions in which neurons in the brain and spinal cord die. In these disorders, there is a core loss of cells with a secondary population of "at risk" cells that may succumb to the ongoing disease process, possibly driven or exacerbated by a local inflammatory response. Stem-cell-based therapies could potentially be beneficial by acting though several mechanisms:

cell replacement, where transplants of cells are given to directly replace those that are lost;

trophic support, where the cells are used to promote survival of affected neurons and endogenous repair of the diseased brain areas;

modulation of inflammation, which may be involved in the disease process.

Any stem-cell-based approach for treating a neurodegenerative disorder must be proven to work through one or more of these mechanisms. No application in patients can be justified on the basis that there is no alternative effective therapy if that new experimental treatment has neither proven efficacy in the laboratory nor any evidence to show a mechanism of action. Trials showing safety alone without any scientific grounds for their use are unethical. Here, we discuss the clinical translation of stem cells in the treatment of three

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neurodegenerative disorders: Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Comprehensive reviews covering this topic have been published and are recommended for further reading (see Table 1).

PD causes deficits in the control of movement secondary to the degeneration of the dopaminergic nigrostriatal pathway, but other dopaminergic and nondopaminergic systems are also affected, with the development of many nonmotor problems. Whereas many motor features respond rather well to dopaminergic medication and deep brain stimulation, effective therapies for the nonmotor symptoms, such as dementia, are lacking, and the progression of the disease is not slowed by available treatments. In experimental clinical trials, transplants of human fetal ventral mesencephalic tissue (containing developing nigral dopaminergic cells) can produce dramatic motor improvements in some patients. Stem cell transplants should be able to, in theory, help patients with PD by either replacing the dopaminergic neurons or restoring and maintaining the integrity of the nigrostriatal pathway through the release of trophic factors. Stem cells can only be said to be curative if they (1) differentiate into appropriate midbrain dopaminergic neurons and (2) integrate and send axons into areas with dopaminergic axon and synapse loss. No uncontrolled cell proliferation should occur, as this would pose a tumor risk. Based on the available data, stem cells have the potential to help restore or preserve striatal dopaminergic innervation; however, there are few cell differentiation protocols that enable derivation of human dopaminergic neurons with an authentic midbrain phenotype.

HD is an autosomal dominant disease that causes cellular dysfunction and loss at numerous central nervous system (CNS) sites including the striatum. The disease is fatal and is characterized by chorea, psychiatric problems, and progressive dementia. There is currently no effective treatment. Reparative strategies for HD using cell replacement or trophic factor support have concentrated on the striatum. Preclinical studies in rodents and nonhuman primates have suggested that intra-striatal transplants of fetal striatal tissue may have restorative effects. While the clinical translation of these studies in small Phase I trials has not resulted in the types of dramatic improvements as seen for PD, there are some early data suggesting that intra-striatal implants of human fetal striatal allografts can ameliorate some aspects of the disease. More preclinical work remains to be done, and the final outcome of the already-initiated human transplant studies needs to be assessed before considering additional clinical trials for HD. With regard to stem cell transplants, there is some evidence that neural progenitor cells can ameliorate functional deficits in animal models of HD, perhaps through the generation of astrocyte support cells and the release of growth factors.

ALS causes the dysfunction and degeneration of motor neurons in the spinal cord, cerebral cortex, and brainstem, leading to rapidly progressing muscle weakness and death within a few years of onset. No effective treatment exists. To date, there is no proof-of-concept study demonstrating that cell therapy can reduce the symptoms or extend life in people affected with ALS. The most discussed use of stem cells is to replace dying motor neurons in the brain and spinal cord. However, there are major technical hurdles to overcome including the delivery of cells to all of these sites and the requirement of axonal growth over long distances to the muscles. Because glial cells may also contribute to the pathology in ALS, leading to a secondary loss of neurons, a more practical approach may be to provide stem-cell-derived astrocytes to degenerating regions to protect dying motor neurons. Combining these approaches with the delivery of growth factors may lead to further neuroprotection. However, the progression of motor neuron diseases may also be driven by inflammatory cascades, and the careful consideration of how such processes may influence the survival of transplanted cells and the outcome of neuroprotective approaches is needed.

Details of Current State of Knowledge: Unmet Needs and Prioritized Target Indications

Preclinical Models

Animal models of PD concentrate on lesions of the nigrostriatal dopaminergic pathway (e.g., by 6-hydroxydopamine, MPTP, and rotenone) and studies of sensorimotor functions. While this is a useful starting point, these models do not imitate the clinical disorder, which has many nonmotor and motor features with nondopaminergic, extranigral pathologies. Nevertheless, these dopaminergic lesion models are useful in evaluating the utility of dopaminergic therapies such as stem cell grafts. Attempts to develop transgenic models of PD have been pursued in recent years, but these also represent useful but partial models of the core pathologies.

The fact that HD is fully transmitted through a family gene mutation has allowed transgenic models to be developed. Several mouse models of HD exist, but overall cell loss is limited, and thus the use of transgenic mice for neuronal replacement has met with limited success. However, all current transgenic HD models can serve, to an extent, as experimental paradigms for studying the possible trophic actions of stem cells, and a delay in disease-like signs in these models would serve as a useful outcome measure. For the replacement of cells or neurons lost in the HD circuitry, excitotoxic/metabolic models likely serve as more direct tests of the restorative potential of cell grafts.

The primary animal model of ALS involves the overexpression of mutated superoxide dismutase gene one (SOD1). Mice, rats, or pigs expressing this mutation show no phenotype until young adult ages are reached, at which time motor neurons gradually die in the ventral spinal cord, leading to paralysis and death. This progression is accompanied by inflammation. While very accurately portraying the motor neuron loss seen in patients with an SOD1 mutation, it is unclear how this model relates to cases of the much more common sporadic ALS, in which the SOD1 gene is unaffected.

Clinical Studies

Clinical trials using stem cells or their derivatives have not been performed thus far for PD or HD. Following the intra-striatal transplantation of human fetal mesencephalic tissue, which is rich in dopaminergic neuroblasts, the dopaminergic neurons that form from the transplanted tissue reinnervate the denervated striatum and become functionally integrated, restoring striatal dopamine release and, for some PD patients, giving rise to clear clinical improvement for over a decade. A subgroup of transplanted patients developed troublesome involuntary movements (dyskinesias), probably due to graft-derived striatal serotonergic hyperinnervation or uneven distribution of the grafts across the striatal complex. Grafts of human fetal striatal tissue placed in the striatum of patients with mild HD have, in some cases, given rise to long-term functional benefit for both motor and cognitive features.

Hematopoietic and mesenchymal stem cells have both been delivered to ALS patients to alter the inflammatory environment and were reported to provide either no or some clinical benefit. However, the preclinical data regarding safety, dosage, long-term survival, differentiation, and functional efficacy are insufficient, and without a control group, the clinical evidence of improvement is weak. It is important to note, however, that no serious side effects were observed after injecting cells into the spinal cord of ALS patients. More preclinical studies are needed prior to further patient applications. Recently, neural stem cells that were expanded from human fetal spinal cord tissue were transplanted into the spinal cord of ALS patients as part of a new FDA-approved Phase I trial. The rationale for this study is not motor neuron or astrocyte replacement. Rather, the new cells may form

interneurons, which might affect motor neuron output through poorly understood presynaptic mechanisms and perhaps release growth factors to protect the remaining motor neurons.

Cells Used

For PD, cells with the properties of dopaminergic neuroblasts have been generated in vitro for preclinical transplantation from stem cells of several different sources and species, including humans. For example, they have been derived from embryonic stem cells (ESCs), therapeutically cloned ESCs, neural stem cells (NSCs) and progenitors from the embryonic ventral mesencephalon, adult NSCs from the subventricular zone, and fibroblast-derived induced pluripotent stem cells (iPSCs). The yield of dopaminergic neuroblasts with the correct substantia nigra phenotype can be increased by improved differentiation protocols that utilize specific transcription factors that determine mesencephalic dopaminergic neuron specification or maturation during normal development.

For HD, attempts to generate striatal cells for transplantation from NSCs expanded in vitro face a challenge in that prolonged propagation in the presence of growth factors may impair the maintenance of a proper regional phenotype. Recently, a multistep protocol was established that permits the generation of striatal neurons from human ESCs. Upon transplantation into quinolinic acid-lesioned rats, these cells differentiated into DARPP32+ neurons and extended axons into the host fiber tracts. However, despite the fact that the cells were committed to a neural lineage, the grafts exhibited massive proliferation, resulting in overgrowth, indicating the necessity for a better control of donor cell differentiation after transplantation.

Motor neurons and astrocytes, which are potentially useful for preclinical applications for ALS, have been generated in vitro from mouse and human ESCs and iPSCs and from human fetal brain tissue. In one study, stem-cell-derived motor neuron precursors and neuroblasts established functional synapses with muscle fibers in vitro and, in a few cases, extended axons to ventral roots after transplantation into the spinal cord of adult rats with motor neuron injury. However, such axon targeting requires the presence of multiple complex factors, including GDNF, within the fiber tracts to attract donor axons. In recent experiments, mouse ESC-derived motor neurons that were transplanted into transected tibial nerves of adult mice established normal motor units and attenuated muscle atrophy. The main problem with this method is how to regulate the function of these motor neurons to affect movement when they are placed within the nerve rather than the spinal cord. While the death of motor neurons in ALS leads to paralysis, dysfunctional astrocytes may also play a role in the overall health and survival of sick motor neurons. Therefore, new strategies to transplant stem-cell-derived astrocytes that could protect dying motor neurons in ALS are also underway in many laboratories.

Future Directions

Preclinical Studies

Human stem-cell-derived dopaminergic neuroblasts, which will be required for patient applications, can survive in animal models of PD, and, after maturation, exert functional effects. However, some properties of stem-cell-derived dopaminergic neurons that are fundamental for successful clinical translation need to be confirmed in further studies, including evidence that they can substantially reinnervate the striatum, restore dopamine release in vivo, and markedly improve deficits that resemble the symptoms experienced by PD patients. Experimental work establishing these properties remains to be performed before a human stem-cell-derived dopaminergic neuroblast can be selected as a candidate cell for patient application. More specifically, these cells should be tested in a range of

appropriate animal models, which in the first instance will be the unilateral 6-hydroxydopamine lesion model of PD in rats. The cells must be shown to survive long-term and differentiate into the appropriate neuronal phenotype, i.e., midbrain doepaminergic neurons. Ongoing cell division must not be present beyond 1–2 months after transplantation, and the migration pattern of the cells should be defined. The connectivity of cells to the host should be demonstrated using standard immunohistochemical staining and in selected cases be combined with anterograde and retrograde tracers. A major portion of the striatum (>50%) should be evenly innervated by graft-derived dopaminergic fibers. The ability of the grafted cells to mediate sustained functional behavioral effects in the animal model of PD must be shown by the following ways:

- drug-induced rotation with a 100% reduction in amphetamine-induced rotation, which can be achieved by relatively low numbers of dopaminergic cells (~300) as is seen with fetal mesencephalic tissue grafts;
- significant recovery in performance in stepping, placing, or cylinder tests in rats; and
- the characterization of any changes in skilled reaching as compared with the limits of efficacy using primary fetal mesencephalic tissue grafts.

For a cellular replacement approach to HD, it must be shown that the grafted cells survive long-term in appropriate animal models. The cells should differentiate into medium spiny striatal projection neurons. When integrated into the brain, the transplant should contain at least 30%–50% medium spiny neurons as seen for transplants of cells derived from the fetal ganglionic eminence. The migration pattern of the cells should be known in addition to their connectivity to the host. Ongoing cell division must not be present beyond 1–2 months after transplantation. The grafted cells should be able to induce behavioral improvements, and this functional recovery should be sustained. This should involve beneficial effects on the following deficits in animals with unilateral striatal lesions:

- paw reaching with the contralateral paw; and
- poor performance on tasks of sensorimotor integration such as contralateral neglect or a lateralized choice reaction time task;

and should provide beneficial effects in bilaterally lesioned animals with respect to:

- normalization of locomotor activity; and
- alleviation of deficits in a relevant cognitive task sensitive to fronto-striatal dysfunction, such as delayed alternation.

For neuronal replacement therapy for ALS, the stem cells must be competent to make functional motor neurons. This generally requires ESCs or iPSCs as the source and increases the risk of teratoma formation. Once the cells have been characterized in vitro, they must be tested in vivo and shown to be able to survive long-term at multiple CNS sites, integrate into existing spinal cord neural circuitries, and receive appropriate regulatory input and extend their axons over long distances to reinnervate muscles. Finally, the transplanted motor neurons must induce a clear functional benefit. While the SOD1 models provide a good preclinical base for moving these types of studies forward, the environment of this model may not be representative of that found in sporadic human ALS patients. Therefore, new genetic models or models based on motor neuron lesions using toxins should also be considered.

The transplantation of stem cells for increased neuroprotection through the production of healthy astrocytes and/or the release of neurotrophic molecules, the modification of the inflammatory environment, or the generation of interneurons is a more realistic near-term

clinical goal for ALS. Preclinical data must show the protection of motor neurons in the SOD1 model and/or other motor neuron lesion models using the strategy suggested for patients. Ideally, this should be associated with functional improvement in the animals, although simply protecting the motor neurons in these animal models may be enough evidence to support moving forward to the clinic, because these models clearly do not represent sporadic ALS.

Clinical Studies

To be clinically competitive as a treatment for PD, stem-cell-based dopaminergic cell replacement therapy must induce the substantial (at least 50%–70%) amelioration of motor symptoms without significant side effects. This could be achieved if patients are carefully selected and the dose and site of implantation of the dopaminergic cells are based on preoperative imaging. Strategies to prevent the occurrence of graft-induced dyskinesias following cell therapy must be developed, e.g., by minimizing the number of serotonergic neuroblasts in the transplant material and by distributing the dopaminergic neuroblasts evenly over the striatum.

The use of stem-cell-derived neurons for replacement in HD is on less secure footing, given that it is still unclear whether primary allografts of fetal striatal tissue provide substantial and prolonged clinical benefits to patients with HD. Nevertheless, there is sufficient data from open-label studies to suggest that transplants containing striatal tissue can ameliorate and reverse some aspects of the disease. As such, stem-cell-derived transplants for HD could be useful in two major ways: (1) by replacing lost striatal projection neurons; and (2) by providing a local source of neurotrophic factors to support this same population. Stem cells may be capable of doing both of these functions at the same time. The successful administration of stem cell transplants will critically depend on them being implanted at the optimal stage of the disease course, which is likely to be in early stage patients.

Motor neuron replacement in ALS will probably be attempted first at the level of the spinal cord. These initial studies will clearly be “proof of concept,” be limited to a few spinal cord segments, and must be based upon extensive preclinical data. These studies need to be designed to show that the motor neurons survive transplantation without forming teratomas, that they can project axons into the ventral roots, and that some reach the muscle and make connections. The challenge will be that the disease progresses so rapidly that the patient may die before the growing axons reach their target.

Cell Sources or Targets

After the transplantation of stem-cell-derived dopaminergic neuroblasts and their subsequent maturation, they must exhibit the properties of substantia nigra neurons to induce substantial benefit for treating PD. It must be possible to grow the cells in vitro in sufficient numbers to achieve a level of engraftment that makes those cells useful in the clinical setting. The definition of a dopaminergic nigral neuron derived from stem cell sources includes the expression by immunohistochemistry of phenotypic markers typical for those cells and the appropriate expression profiles of genes and transcription factors that define that specific region of the brain during normal nigral development. The cells need to exhibit excitability using appropriate neurophysiological measures and dopamine release in response to standard in vitro stimuli. All cell types in the cultures need to be identified, especially the number of nonnigral dopaminergic neurons, serotonergic neurons, other nondopaminergic neurons, glial elements, undifferentiated precursor/stem cells, and, in the case of ESC- and iPSC-derived preparations, nonneural cells. Recently, it has become possible to directly convert fibroblasts into functional “induced” neurons (iNs), including midbrain

dopaminergic neurons. It remains to be explored to what extent iNs can contribute to functional restoration in models of PD.

For HD, the cells grown in vitro must differentiate into striatal projection neurons (DARPP32+/GABA+) with a degree of efficacy that would make translation to the clinic practical. The proportion of other types of neurons should be determined using markers for striatal interneuron phenotypes (which may be critical for the grafts to achieve optimal function), in addition to markers of nonstriatal phenotypes (which may be considered to be contaminants). The definition of a striatal projection neuron includes the expression of typical phenotypic markers by immunohistochemistry, the profiles of genes/transcription factors that define that brain region during normal striatal development, and at least some of the receptor subtypes and neuropeptides typically found in striatal projection neurons. The cells should be able to release GABA and exhibit the excitability properties of neurons.

For ALS, the nature of the donor cells to be used for transplantation is still open to discussion. Whereas motor neuron replacement represents an ideal target, successful in vivo cell delivery, integration both into the motor cortex and across multiple levels along the anteroposterior axis, and targeted axon outgrowth currently pose insurmountable challenges. For such a neuronal replacement strategy, motor neuron progenitors would have to be delivered at a still immature, migratory stage to enable the donor cells to actively infiltrate the motor cortex and the ventral columns of the spinal cord and brainstem. Considering the recently discovered non-cell-autonomous effects of astrocytes from SOD1 mutants and sporadic variants on wild-type motor neurons, ALS might also represent a candidate disease for glial transplantation. However, further studies in animal models are required to explore whether wild-type astrocytes or glial cells engineered to express neurotrophic factors can home in on motor neuron territories and protect host cells from disease-related damage or death.

Acknowledgments

This work was supported by grants from the NIH (P01 NS057778), DFG (SFB-TR3 D2), the European Union (Neurostemcell and Eurostemcell), the BMBF (01GNO813, 01GN1009B, 0315799-BIODISC, and 0316020), BIO.NRW (StemCellFactory), the Hertie Foundation, and the NIHR award of a Biomedical Research Centre to the University of Cambridge/Addenbrookes NHS Trust. O.B. is a cofounder and shareholder of LIFE & BRAIN GmbH.

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Table 1

Recommended Additional Reading

Disease	Review Articles
General overview	Lindvall and Kokaia (2010)
Parkinson's disease	Arenas (2010); Lindvall and Kokaia (2009); Tsui and Isacson (2011); Wijeyekoon and Barker (2009)
Huntington's disease	Clelland et al. (2008); Kelly et al. (2009); Kim et al. (2008)
Amyotrophic lateral sclerosis	Hedlund et al. (2007); Mazzini et al. (2009); Nayak et al. (2006); Suzuki and Svendsen (2008); Thonhoff et al. (2009)