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The Role of Stem Cells in Parkinson's Disease

Amitabh Gupta, MD, PhD[a], Ted M. Dawson, MD, PhD[b,*]

[a]*Department of Neurology, John Hopkins Hospital, Pathology 509, 600 North Wolfe Street, Baltimore, MD 21287, USA*

[b]*Institute for Cell Engineering, Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, 733 North Broadway, Suite 731, Baltimore, MD 21205, USA*

Traditionally, the symptoms of Parkinson's disease (PD) have been described as a triad of resting tremor, rigidity, and akinesis, reflecting the thought that PD is a disease of the extrapyramidal motor system. Over the years, however, it has become evident that PD is a disease that is much more encompassing, involving vast parts of the central nervous system and even the peripheral nervous system. Hence, aside from motor symptoms, PD shows a multitude of nonmotor symptoms, including sensory symptoms, autonomic symptoms, symptoms of sleep disturbances, and neuropsychiatric symptoms. In fact, as PD progresses, it is the nonmotor symptoms that can be the most disabling. Common problems encountered are loss of smell, vaguely described pain, dementia with marked loss of executive functioning and bradyphrenia, psychotic episodes that are interspersed with anxiety attacks or apathy, orthostatic hypotension, erectile dysfunction, neurogenic bladder disturbances, posture and balance problems, and, finally, a broad spectrum of rapid eye movement (REM) sleep disorders that can culminate in a person acting out his or her dreams in the early morning hours. A recent meta-analysis has shed light on poor prognostic factors of PD [1]. Predictive factors for more rapid motor progression, nursing home placement, and shorter survival time include older age at onset of PD, associated comorbidities, presentation with rigidity and bradykinesia, and decreased dopamine responsiveness. Pathologically, the symptoms are reasonably well correlated with degenerative changes in corresponding neural structures. Pathology studies indicate that the principal defect, which underscores the triad of motor symptoms, lies in the degeneration of dopaminergic cells in the substantia nigra pars compacta (SNc), thereby compromising their functional projections to the striatum. With respect to the nonmotor symptoms, the neurodegeneration of multiple neurotransmitter systems and structures was discovered, including the norepinephrine neurons of the locus ceruleus, the serotonin neurons of the raphe nuclei, the cholinergic neurons of the nucleus basalis and pedunculopontine nucleus, the olfactory bulb, the vagal nuclei, and broad circuits within the limbic system and neocortex. Even sympathetic neurons in the peripheral nervous system are affected, likely explaining some of the gastrointestinal and cardiac symptomatology seen in patients with PD. As such, degeneration of the myenteric plexus is thought to contribute to constipation and drooling, which are so frequently seen in advanced disease.

Genetics

From the genetic perspective, PD is quite heterogeneous. The most common form of PD is idiopathic, which is characterized pathologically by Lewy bodies in the various structures mentioned. Lewy bodies are intracytoplasmic inclusions made up of several components, including, most prominently, α-synuclein. In approximately 10% of the PD population, however, five specific genetic defects have been identified. The best-characterized genetic defect is the mutation in α-synuclein, which supports the notion that Lewy body formation is intrinsically connected to

* Corresponding author.
E-mail address: tdawson@jhmi.edu (T.M. Dawson).

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disease mechanism. Another gene identified is Parkin, which causes autosomal recessive juvenile-onset parkinsonism. The gene was originally identified in a Japanese cohort, in which patients presented less commonly with resting tremor, and, most interestingly, Lewy body formation was usually absent. Other important genes that are linked to familial PD comprise PINK1, DJ1, and LRRK2. Notably, LRRK2 mutations have also recently been found in approximately 4% of sporadic (nonfamilial) cases of PD.

Disease mechanism

Given the genetic clues, an understanding of disease mechanism is progressively advancing. The α-synuclein mutations are gain-of-function mutations, which, in animal models, drive Lewy body–like inclusion formation and diffuse neuron degeneration, including degeneration of dopaminergic neurons in some models. Whether inclusion body formation is protective or toxic to the neuron, however, remains a topic of intense debate. One school of thought is that the different familial-associated mutations promote formation of α-synuclein oligomers, protofibrils, and fibrils, which renders α-synuclein toxic, whereas Lewy body formation is protective because it sequesters the α-synuclein toxic species. Conversely, arguments for a toxic effect of Lewy body formation can also be made, because it is hard to envisage that inclusions the size of a nucleus are not harmful to a neuron.

Aside from the research on α-synuclein, the discovery of Parkin highlighted the role of aberrant protein folding in PD. Parkin is an E3 ligase, which ubiquitinates proteins that are targeted for destruction by the proteosome [2,3]. Recent studies indicate that the p38 subunit of amino-acyl transfer RNA (tRNA) synthetase and fuse-binding protein-1 are pathogenic substrates that accumulate in mice and human beings lacking Parkin [4,5].

PINK1 is believed to be a mitochondrial kinase [6]. Given that many of the PINK1 mutations in PD patients are located in the PINK1 kinase domain and apparently disrupt its kinase activity, it is conceivable that altered phosphorylation of PINK1 target proteins is partly responsible for neuronal degeneration. Accordingly, the identification of downstream targets of PINK1 is an active undertaking.

Another protein worth mentioning in the setting of disease mechanism is LRRK2. In patients with PD, the LRRK2 mutations are gain-of-function mutations. The LRRK2 protein is a cytoplasmic protein [7] and a member of the ROCO protein family. Among the multiple functions that are predicted based on its structure, a Ras/guanosine triphosphatase (GTPase) domain suggests a role in vesicular trafficking and cytoskeletal assembly, and it likely plays a role in signal transduction because it seems to function as a mixed lineage–like kinase [8]. The familial-associated mutations lead to enhanced kinase activity [8], and overexpression of mutant LRRK2 leads to neuronal cell death, which is attenuated by blocking its kinase activity as well as its GTPase activity [9]. Thus, drugs aimed at inhibiting LKKR2 kinase or GTPase functions are attractive neuroprotective agents.

Finally, in sporadic PD, the role of mitochondria dysfunction is important, because there is defective respiratory chain protein complex I activity in the substantia nigra (SN) of patients with PD [10], consistent with the finding that complex I inhibitors, such as rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), can cause parkinsonism. Fig. 1 summarizes the current understanding of disease mechanism and envisioned treatment interventions that arise from this knowledge [11].

Current management

Medical treatment

Given the aging of the population, PD is likely to become an even greater health care problem over the next decades, with estimations ranging from a two- to fourfold increase in disease prevalence. Therefore, recognition and treatment of this disease is a major focus in neurology. Given that research has provided substantial progress in understanding disease mechanism and pathologic findings, treatment approaches have been evolving at an encouraging rate. The classic treatment approach is to replete dopamine loss in the basal ganglia network. After it was realized that dopamine does not cross the blood-brain barrier (BBB), the first effective treatment was accomplished with administration of levodopa (L-dopa), which successfully passed the BBB and was subsequently metabolized to dopamine in the central nervous system. The peripheral production of dopamine, in turn, had unwanted side effects, including those that affect the gastrointestinal system. Next, the effectiveness of L-dopa

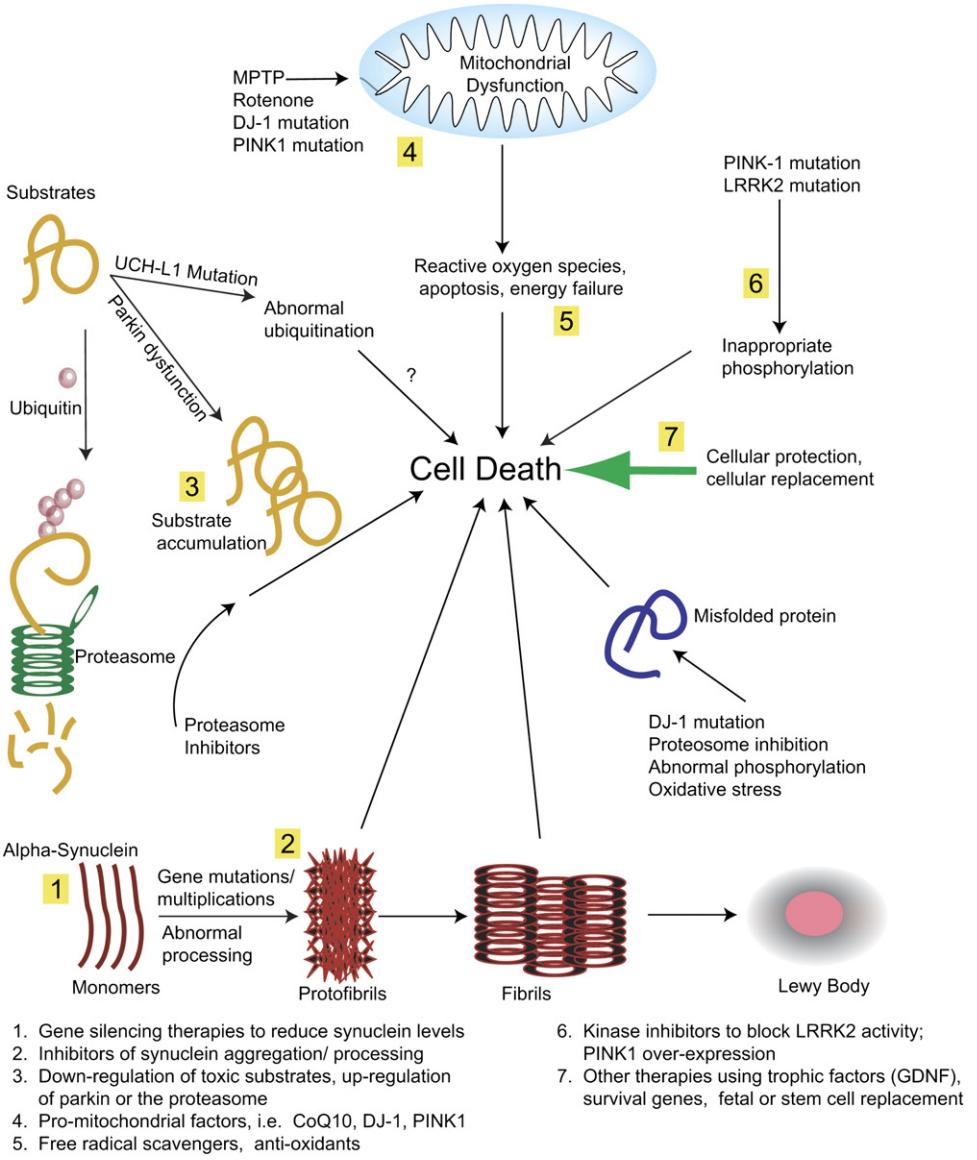

Fig. 1. Model of dopaminergic cell death and possible sites for therapeutic intervention in PD. Studies on inherited forms of PD have led to the identification of genes that lead to dopaminergic cell loss when mutated. These genes are involved in a variety of cellular processes that include protein ubiquitination and degradation by means of the proteasome, response to oxidative stress, protein phosphorylation, mitochondrial function, and protein folding. Potential points of therapeutic intervention are highlighted: (1) gene silencing therapies to reduce synuclein levels; (2) inhibitors of synuclein aggregation or processing; (3) interventions to downregulate toxic substrates or upregulate Parkin or proteasomal function; (4) interventions to enhance mitochondrial function with factors like CoQ10, DJ-1, or PINK-1; (5) free radical scavengers and antioxidants; (6) kinase inhibitors to block LRRK2 activity or interventions to increase PINK-1 function; and (7) other therapies using trophic factors, such as glial-derived neurotrophic factor (GDNF), survival genes, or fetal or stem cell replacement, that would protect or replace susceptible cells. (*From* Savitt JM, Dawson VL, Dawson TM. Diagnosis and treatment of Parkinson disease: molecules to medicine. J Clin Invest 2006;116:1748; with permission.)

for PD was optimized with the concomitant administration of carbidopa, an inhibitor of L-dopa degradation, after it became evident that much of the L-dopa was peripherally metabolized before passing the BBB. Carbidopa also effectively reduces or eliminates the peripheral side effects of L-dopa. Unfortunately, as the L-dopa/carbidopa approach was providing reasonable treatment of PD motor symptoms, two new hurdles arose: one was the development of motor fluctuations, and the other was the realization that the nonmotor symptoms of PD were not dopamine responsive.

Motor fluctuations mainly present in two forms, namely, off-time and dyskinesias. The first form relates to the fact that the effect of L-dopa becomes less effective over time as the disease continues to progress with further degeneration of dopamine neurons. Accordingly, the patient becomes less responsive, likely as the result of a decreased dopamine load over time because of decreased dopaminergic neurons or desensitization of the dopamine effect at the postsynaptic receptor. In those instances, the patient reverts to showing PD motor symptoms before the next dose of medication and demonstrates mostly resting tremor, rigidity, and akinesia. The second form, conversely, reflects a situation in which chronic treatment causes too much dopamine effect in the basal ganglia neuronal network, which is likely attributable to mechanisms of increased sensitization at the synaptic level that are not well understood. In this case, the patient presents with symptoms of continuous movements of the face, trunk, or limbs that vary in their appearance, being choreiform-athetoid, dystonic, or tremorous-myoclonic. Other forms of motor fluctuations occur and are reviewed in detail elsewhere [12].

With respect to off-time, a recent meta-analysis analyzed a variety of medications, among which entacapone (catechol-O-methyltransferase [COMT] inhibitor) and rasagiline (monoamine oxidase [MAO] B inhibitor) seemed to be the most reasonable therapies, reflecting the notion that decreasing the peripheral metabolism of L-dopa can avoid a situation in which dopamine in the brain is insufficient [13]. With regard to dyskinesias, after the arrival of dopamine receptor agonists, the debate has centered around the time point at which to start L-dopa, given that L-dopa continues to remain the most effective medication. Some believe that patients younger than 70 years of age who have mild PD with impaired daily functioning should be started on dopamine receptor agonists, such as ropinirole or pramipexole. In this manner, it is hoped that motor fluctuations are delayed, because administration of L-dopa is delayed until required with disease progression. Such an approach is supported by a recent randomized trial in which patients receiving dopamine receptor agonists had less frequent and severe dyskinesias. As others believe, however, the onset of motor fluctuations may partially be a feature of the disease itself, being associated more with L-dopa treatment simply because of the fact that these cases were too severe to be controlled by dopamine receptor agonists alone. Irrespective of when to start L-dopa treatment, amantadine offers some benefit in the treatment of dyskinesias [13]. Discussion of treatment of motor fluctuations in PD is beyond the scope of this article, however, and the reader is referred to several excellent reviews on this topic [13,14]. The same holds true with respect to controlling nonmotor symptoms, wherein medical approaches have been far less satisfying as compared with treating the motor symptoms (for reviews, see the articles by Pahwa and colleagues [13], Diamond and Jankovic [14], and Menza and Marsh [15]).

Neurosurgical treatment

Aside from medical management, there are also surgical interventions available to ameliorate the disease [16]. Notably, much like with medication, the surgical approach primarily addresses the motor symptoms. At the forefront of this field is deep brain stimulation (DBS). DBS is a technique whereby an electrode is stereotactically positioned in a desired brain structure and stimulated by means of an externally placed device through a connecting wire. DBS has been applied to the subthalamic nucleus (STN), the internal globus pallidum (GPi), and the ventral intermedius (VIM) nucleus of the thalamus. Recently, a comparative analysis of the available DBS data was conducted. The recommendations that arose from this analysis indicate that the STN may be considered the best target for improvement of motor function and reduction of off-time, dyskinesia, and medication use. Some evidence indicates that a better outcome can be predicted if preoperative responses to L-dopa are favorable.

Neuroprotection

Much thought has been devoted to devising treatment options that aim at preventing or

delaying the disease itself rather than at treating the symptoms. Emphasis is placed particularly on neuroprotection [16]. Unfortunately, in an overall assessment, evidence-based data for neuroprotection do not exist. In trials assessing medications, the hope that L-dopa therapy by itself is not only of symptomatic benefit but aids in disease delay cannot be supported by existing clinical data. In similar fashion, several compounds, including riluzole, pramipexole, ropinirole, and amantadine, have not demonstrated any statistical benefit. Nonetheless, mild optimism remains with reference to dopamine receptor agonists, rasagiline, creatine, and coenzyme Q10, although their role in neuroprotection has not panned out to be conclusive.

Stem cells

In describing the current state of PD management, it becomes evident that much room for improvement exists. A cardinal problem is that medical treatment and even surgical approaches are not permanent solutions, given the increasing side effects that arise over time. In this respect, stem cell therapy holds tremendous promise for being one of the most exciting therapeutic avenues of the future [17]. In principle, the current practice uses cell culture systems in which stem cells are differentiated into dopaminergic cells that are then transplanted into the SN or its target areas. If appropriate integration of such transplanted cells is achieved, this may offer a stable long-term solution in terms of motor symptom suppression. Critically, such an approach is deemed to replace dopamine in a more physiologic fashion, and it lends hope to the notion of decreased motor complications. Additionally, should this transplantation approach prove successful, stem cells could be used as delivery tools for multiple modifying therapies, including the delivery of trophic factors that might aid in delaying, stopping, or reversing PD progression. Again, site-specific delivery provides promise for minimizing the side effect profile.

Tissue transplantation

The field of stem cell therapy in PD was initially realized by proof-of-principle studies with fetal brain transplants. In the case of transplanting fetal human midbrain tissue, which is rich in dopaminergic neurons, into the striatum of human subjects, encouraging findings were made, given that marked and long-lasting improvements were seen in several avenues in some patients [18]. Clinical improvement was observed with akinesia, with some patients being able to decrease their L-dopa dosage for several years [19,20]. Histologically, the grafted neurons survived for as long as 10 years in the setting of ongoing disease progression and concomitant endogenous dopaminergic cell death [19]. Further, given that dopamine release was increased after transplantation within the striatum, the argument was made that dopaminergic cells are able to functionally integrate into neuronal circuits of the basal ganglia system [20]. Of course, because such encouraging results are only applicable to a subset of patients, much work allowing more broad and reproducible application of the benefit needs to be done to overcome key problems. Two key problems are limited cell survival [21] and dyskinesias in 7% to 15% of grafted patients [21–23]. The former problem may reflect adverse immune reactions, whereas the latter problem may arise from incorrect connectivity or an intrinsic property of the graft itself, such as a suboptimal ratio between dopaminergic and nondopaminergic neurons. Moreover, transplantation into the striatum (rather than into the SN) may interfere with optimal results; the use of transplants bears the intrinsic problem of variability; and the use of human fetal tissue certainly is restricted by limited availability, let alone the moral and ethical questions that this therapy provokes.

Generating dopaminergic neurons from stem cells

Despite the innumerable problems attached to the stem cell approach, the proof of principle in patients successfully laid the foundation for the next steps in stem cell research. Not surprisingly, given the need for reproducibility and availability, the notion of using a pure population of dopaminergic neurons became increasingly attractive. Given the experience with human transplant tissue, key requirements have to be attached to the desired cell population. These requirements, which are inadvertently interconnected, include that the cells be able to:

1. Reliably survive in a high percentage
2. Secrete a standardized amount of dopamine for consistent efficacy
3. Avoid rejection or induction of an inflammatory response
4. Appropriately connect with the target cells in the striatum to minimize dyskinesias as well

as to optimize replacement not only of transmitter but of destroyed extrapyramidal neuronal circuitry

Having defined the desired features of transplantable dopaminergic neurons, much effort has been devoted to investigating how best to obtain these cells. Three major types of precursor cells are discussed as a possible source from which dopaminergic cells can be obtained through cell culture differentiation, namely, embryonic stem (ES) cells, neuronal precursor cells (derived from fetal or adult brain), and other tissue stem cells (derived from bone marrow or umbilical cord). Most experience so far has supported the notion that only ES cells and fetal brain neural stem cells (NSCs) are reliable sources for dopaminergic neurons, with the former source showing the more promising results. Differentiation protocols vary and continue to require optimization.

Embryonic stem cells

With respect to ES cells, in general principle, the creation of neurons requires two consecutive phases, namely, the induction of a neural fate and neuronal differentiation. Neural fate itself can be obtained in two ways: generation of adherent neuroblasts (or neurospheres) or generation of neuroectodermal cells [18]. Neuroblasts are obtained by culturing ES cells in the absence of leukocyte inhibiting factor (LIF) and in the presence of retinoic acid, which induces embryoid body formation, and subsequent treatment of the embryoid bodies with epidermal growth factor (EGF) and fibroblast growth factor (FGF)-2 or alternative culture with stromal cells. Neuroectodermal cells, conversely, are generated by maintaining ES cells in adherent cultures, which induces neural fate, and subsequently treating them in ways similar to treatment of the embryoid bodies. Finally, once neuroblasts or neuroectodermal cells are created, removal of mitogens induces neuronal differentiation, which can yield more than 50% neurons by way of a default pathway.

Given that less than 5% of neurons show dopaminergic characteristics, a major undertaking has been to increase the proportion of dopaminergic cells within the neuronal pool that is desired to be transplanted. Over the years, several strategies have shown promise. In mice, ES cells overexpressing Bcl-XL [24] or being cocultured with stromal cell lines [25] can significantly increase their differentiation into dopaminergic cells. More impressively, overexpression of nuclear receptor related-1 (Nurr 1) [26,27] protein during murine ES cell differentiation in combination with exposure to FGF8 and sonic hedgehog (SHH) yielded approximately 80% dopaminergic neurons [28]. Notably, aside from optimizing neuronal subtype-specific differentiation, enrichment of dopaminergic neurons can also be achieved by specifically isolating them from a given neuronal pool before transplantation. For example, it has been shown that when differentiating mouse ES cells are targeted by a green fluorescent protein (GFP) reporter gene, which is driven by a dopaminergic neuron–specific promoter, subsequent isolation by fluorescence-activated cell sorting (FACS) can yield up to 98% dopaminergic neurons in the eventual transplantation cell population [29]. Finally, monkey ES cells have been successfully differentiated into dopaminergic neurons, as ES cell–derived neurospheres yielded approximately 25% dopaminergic neurons, when exposed to FGF2 and FGF20 [30].

Human ES cells have also been used to derive dopaminergic neurons. One approach used SHH to drive human ES cells into neuronal differentiation and then exposed these cells to βFGF to promote the development of the dopaminergic cell type. The yield of dopaminergic cells was approximately 40% [31]. Another approach used stromal feeder cells to induce neural fate and then optimized differentiation into dopaminergic neurons with exposure to SHH and FGF8, followed by withdrawal of these factors and addition of transforming growth factor-β (TGFβ) and glial cell line–derived neurotrophic factor (GDNF). Remarkably, up to 80% dopaminergic neurons were created in this fashion [32].

Neural stem cells

NSCs from fetal brain have also shown promise. Interestingly, in contrast to ES cells, the default pathway of NSCs is differentiation primarily toward astrocytes. Nonetheless, in principle, cultured NSCs can be differentiated into neurons along three alternative routes. Notably, each of these pathways has an intermediate stage, designated as direct isolation, cell line generation, and neurosphere creation. Direct isolation uses FACS in the setting of having reporter genes driven by neural promoters or having cells with defined antigenic features selectively gated. Cell line creation is accomplished through the use of cell cycle–controlling genes and mitogens. Finally, neurosphere generation is characterized by mitogen-dependent enrichment of NSCs.

Regardless of which route of differentiation is followed, once an intermediate step is attained, specific neuronal phenotypes can be induced using in vitro–guided genetic modification or extracellular signaling approaches. Some of these approaches, which have been optimized for obtaining dopaminergic neurons in mice, include overexpression of Nurr-1 and coculture with ventral mesencephalic astrocytes [33]; neurosphere differentiation with LIF, GDNF, and cytokines [34,35]; and expansion of isolated committed dopaminergic neuron precursors with FGF2, low oxygen, and ascorbic acid, followed by differentiation in low-oxygen conditions [36–38]. Human NSCs can also be preferentially driven into the dopaminergic cell fate, using expression of the antiapoptotic factor Bcl-XL [39]; neurotrophic factor neurotrophin-4 [40]; 17β-estradiol [41]; or a differentiation cocktail of forskolin, FGF8, and GDNF [42]. In the latter case, the yield of dopaminergic neurons from mesencephalon-derived human fetal neural precursor cells (NPCs) was increased by 20-fold. Lately, strategies are being developed to attempt to expose NSCs in their in vivo environment to various combinations of growth and trophic factors before their isolation and differentiation in vitro [43]. In this manner, it is hoped that NSCs can be "primed" toward a more effective dopaminergic pathway.

Other stem cells

Stem cells as a source for dopaminergic neurons can potentially be obtained from a variety of organs. The umbilical cord and bone marrow may be attractive because they provide an easy accessible source of a large amount of cells. Furthermore, harvesting cells from these organs does not seem to cause an ethical dilemma, particularly when this approach can be optimized to transplantation with autologous cells, which additionally minimizes the risk for rejection and the need for immunosuppression. This research is still in its early stages, however [44–48]. Encouragingly, when undifferentiated umbilical stem cells were transplanted into the striatum of striatal-lesioned rats, approximately 5% to 10% of stem cells had attained a dopaminergic cell fate at 8 weeks after transplantation [48]. Although this percentage is low, it is remarkable that a dopaminergic cell fate can be established endogenously from an undifferentiated state without evident host rejection or another type of inflammation. In vitro differentiation approaches of the future should shed light on the question of how much dopaminergic enrichment can be achieved to optimize transplantation efficacy.

Evaluation of stem cell–derived dopaminergic neurons in animal models

Are these stem cell–derived dopaminergic neurons beneficial in animal models of PD? Overall, the results are variable. In this section, some representative results are discussed, organized according to which stem cell species and stem cell type the transplanted dopaminergic neurons were originally derived from.

Most animal experiments were performed in a rat model of PD, in which 6-hydroxy dopamine (6-OHDA) administration into the striatum results in effective killing of dopaminergic neurons and their projections from extrastriatal areas, with the emergence of motor symptoms through pharmacologic challenge with dopaminergic agents. Subsequent striatal transplantation of engineered cells is followed by assessing functional microscopic and macroscopic recovery. Microscopically, circuit incorporation, transplant survival, and cell fate maintenance have been assessed. The latter is accomplished by observing the expression of tyrosine hydroxylase (TH), an intracellular enzyme that is specific to the dopamine synthesis pathway. Macroscopically, functional recovery can be investigated by a wide variety of electrophysiologic, behavioral, and motor tests.

Nurr 1–expressing dopaminergic neurons derived from mouse ES cells have proved to be quite promising [26,27]. Striatal transplantation of these cells into 6-OHDA–lesioned rats demonstrated effective integration into the basal ganglia network, with the acquisition of electrophysiologic properties that are characteristic of endogenous midbrain dopaminergic neurons. Survival of the transplanted cells was unchanged at 4 and 8 weeks after transplantation (cells were not followed for a longer period), indicating that once they were incorporated, survival seemed to be largely constant. Importantly, behavioral and motor tests demonstrated significant recovery, as compared with sham-treated animals, 9 weeks after cell grating. This is promising, given that only 5% to 10% of transplanted cells were present in the target area, that the transplanted cell population was only 50% enriched for the dopaminergic cell fate, and that effects were observed a few weeks after transplantation.

The problem of enrichment for dopaminergic neurons was addressed in another trial in which the transplanted cells were 98% enriched for dopaminergic neurons before transplantation, using a green fluorescent protein (GFP)–mediated FACS technique [29]. In this case, animals only exhibited mild recovery from their parkinsonian behavioral deficiencies, which was consistent with limited graft survival and circuit incorporation. Hence, even with a relatively pure dopaminergic cell population, results can be incomplete, which emphasizes that all aspects of the transplantation procedure have to yield optimal results to achieve sustainable and significant functional recovery. Alternatively, a pure cell population may not survive well after transplantation, perhaps indicating the importance of supporting cells in the transplant.

Monkey ES cells have also been tested in a primate model [30]. TH-positive cells were derived from monkey ES cells treated with FGF2 and FGF20. Bilateral transplantation of up to 600,000 of these cells into the striatum of an MPTP-lesioned monkey resulted in significant functional recovery of motility and posture 10 weeks after transplantation. This was associated with increased striatal uptake of a dopaminergic radiodye on positron emission tomography (PET; 14 weeks after transplantation) in the absence of appreciable dyskinesias or overgrowth of the transplanted cells. This result is one of the more encouraging because it makes a strong case in primates for proof of principle for the utility of transplanting dopaminergic cells derived from stem cells. Although data on long-term functional recovery are pending, major hurdles for more successful short-term results hinge on improvement of cell survival and circuit integration. In particular, the fact that less than 5% of transplanted cells survived in vivo acts as a constant reminder as to how much work still needs to be done, which is corroborated by the impression that insightful methodology of improving cell survival is still in the early stages. Nonetheless, if only 5% of transplanted cells can cause significant recovery, this again argues strongly for the great potential that stem cell approaches (especially ES cells) have.

Among ES cells, human ES cells constitute the final choice for the disease, of course. When using a differentiation protocol involving SHH and basic fibroblast growth factor (bFGF) that yields approximately 40% TH-positive neurons [31], these neurons show classic dopaminergic electrophysical properties, as reflected in the timed release of dopamine as well as reuptake of dopamine with potassium chloride–induced depolarization. After grafting into the striatum of 6-OHDA–treated rats, however, the transplanted cells lost their TH immunoreactivity over time, which indicates that they do not survive or alternatively lose their dopamine phenotype. Because behavioral or motor improvements were not monitored in this study, it is not clear whether human ES cells are capable of restoring function in animal models of PD. Given that human cell–derived dopaminergic neurons are not prone to survive in rodent tissue or at least have sustained TH expression, it may be difficult to apply results from such experiments to the human disease situation, irrespective of whether results are positive or negative.

Aside from ES cells, trials with NSCs are worthwhile mentioning as well. In the mouse, one approach has been to use dopaminergic neurons that were derived from expansion and differentiation of committed dopaminergic neuron precursor cells of the fetal mesencephalon [37]. This approach increased the number of transplantable dopaminergic neurons 30-fold (10 times from expansion, 3 times from enrichment), before transplantation into the striatum of 6-OHDA–treated rats. The results from this study are promising, because functional recovery of behavior and motor function averaged 75% at 80 days after transplantation, with histologic evidence of successful graft survival, maintenance of the dopaminergic cell fate, and fiber outgrowth. Particularly encouraging is the observation that graft survival and fiber outgrowth were comparable between grafts of differentiated dopaminergic neurons and primary fetal mesencephalic tissue, indicating that differentiation does not have to reduce neuronal survival and plasticity while reducing the risk of uncontrolled graft growth. Conversely, because the survival rate of grafted dopaminergic neurons was only 3% to 5%, the recurrent theme of improving the proportion of surviving cells continues to be a major issue. Nonetheless, given a stable in vitro expansion and differentiation system, future genetic manipulation of the cultured cells bears the potential of optimizing plasticity, survival, cell fate, and other key parameters. Furthermore, it should be interesting to see whether this concept of expanding and differentiating dopaminergic NSCs can be successfully applied to the primate model.

Human fetal NSCs have also been tested in 6-OHDA–treated rats [42]. Similar to the mouse

approach, mesencephalon-derived fetal dopaminergic NSCs were expanded and differentiated with a growth factor cocktail to yield a 20-fold increase in the absolute number of dopaminergic neurons. When these cells were transplanted into the striatum of 6-OHDA–lesioned rats, as compared with transplantation of undifferentiated NSCs, behavioral improvement and increased dopamine secretion from the striatum were seen at 12 weeks after transplantation. In fact, the control NSCs essentially did not differentiate in vivo and had the tendency to migrate away from the transplantation site, which was suggested to at least partially contribute to their lack of effect on recovery. Given that any stem cell treatment for PD requires transplantation material from a human source, this study is critical in the sense that it demonstrates the feasibility of using human NSCs for that goal, specifically by demonstrating the efficacy of in vitro predifferentiation. Accordingly, an important part of current research is focused on improving in vitro differentiation protocols, with some of them encompassing strategies that use endogenous neural differentiation and migration to maximize efficiency.

In the setting of the current evidence presented, a comparison of the various stem cell approaches is outlined in Table 1. In the future, it should be important to contrast the functional outcome between NSC and ES cell transplants in animal models of PD directly, which should aid in defining the stem cell source that holds the most promise in human disease. As progress is made, using stem cells from the bone marrow or umbilical cord may become a plausible alternative.

Stem cells as delivery tools

It is appealing to think that stem cells may serve as vehicles to deliver a wide variety of proteins to specific areas in the brain in an attempt to repair defective neurons rather than replacing them. These proteins may include designed or chosen trophic factors, enzymes of the dopamine synthesis pathway, or proteins that are defective in the genetic causes of PD. Hope for this approach can be derived from two observations. For one, the umbilical cord stem cell strategy seems to underline the tremendous potential that stem cell–mediated delivery of trophic factors has for positively affecting PD. For another, the

Table 1
Summary of data on efficacy of indicated stem cell types to differentiate in vitro, survive in vivo after transplantation, and facilitate functional recovery in mice models of Parkinson's disease

Stem cell type	Differentiation efficacy	Survival in vivo	Functional recovery
ESC			
Mouse	20–98%	5–10%	Yes As early as 9 weeks Impaired when using highly enriched cells
Human	40–80%	Lost TH-IR	No[a]
NSC			
Mouse	5–80%	3–5%	Yes Sustained past 80 days
Human	4–28%	2–3%	Yes Sustained past 80 days
BMSC[b]	5–40%	NA	Yes[c]
UCSC[b]	5–10%[d]	Lost TH-IR	Yes[c]

Abbreviations: BMSC, bone marrow stem cell; ESC, embryonic stem cell; NA, not available; NSC, neural stem cell; TH-IR, Tyrosine hydroxylase immunoreactivity; UCSC, umbilical cord stem cell.

[a] If human ESC-derived dopaminergic neurons were transplanted.

[b] Research in very early stages.

[c] Significant migration away from transplantation site.

[d] In vivo differentiation after transplantation (no in vitro predifferentiation).

Data are based on publications referred to in text.

successful introduction of a variety of molecules into the central nervous system has already been demonstrated with a different kind of delivery tool, namely, with viral vector–mediated gene transfer, which undoubtedly has significant limitations. Hence, stem cells may be engineered to make them the most effective protein delivery tools of the future. Of course, this stem cell strategy bears the advantage of not having to face the potential introduction of viral disease into a human patient. Even worse, viral integration into human DNA could lead to neoplastic conversion of human cells.

Finally, with regard to maximizing functional recovery, which basal ganglia location should be chosen for transplantation of the engineered stem cells? Insight may be derived again from the virally mediated gene transfer experiments. Although data from the primate model demonstrate functional recovery as a proof of principle [49,50], the injection sites were actually chosen broadly, including the striatum and SNc. Experiments with rats, however, in which the striatum was lesioned with 6-OHDA, demonstrate that virus injections in the striatum and not in the SNc resulted in partial functional recovery [51]. Accordingly, as an initial step, it is likely that the striatum is chosen as the primary location for stem cell transplantation. Part of this conclusion is drawn from the fact that axonal growth from cells transplanted into the SNc is insufficient to establish a degree of neuronal connectivity that would be reflected in relief of symptoms.

Future directions of stem cell therapy

To define the future directions of stem cell therapy, it is important to assess the problems that are encountered when working on stem cell therapy. After all, it is these problems from which future ideas arise. Many of the shortcomings of stem cell therapy have been discussed in the direct context of animal experimentation, wherein they seem to be best addressed, given that they can be related to the study design and expected outcome. Major examples include cell survival, loss of dopaminergic phenotype, circuit integration, long-term recovery prospects, and side effects like dyskinesias. Nevertheless, there are problems that are ''less technical'' in nature, which give rise to more conceptual thought. They are discussed in the following paragraphs.

In contrast to manipulating NSCs ex vivo, what is the potential of endogenous NSCs for dopaminergic neuronal replacement? The notion has been to target endogenous NSCs in situ with growth factors, trophic factors, and genetic constructs for transcription factors so that endogenous differentiation into dopaminergic neurons and subsequent or concomitant migration to the SN could be triggered in an attempt to compensate for degenerating dopaminergic neurons in PD. Clearly, although this approach is evidently associated with a decreased risk of unwanted side effects, progress has been limited so far. Critically, the number of endogenous precursor cells is restricted in space and number, being confined principally to the subventricular zone and hippocampus. Hence, once such cells can be triggered to differentiate endogenously, the real challenge is to obtain a reasonable number of neurons, let alone the prospect of guiding their migration to the SN, which implies overcoming a large distance and various neuronal structures.

Another important aspect that needs to be addressed is immune suppression. The current stem cell approaches mostly use stem cells from nonautologous sources, which, in the long run, likely require some form of immune suppression to avoid rejection and, more importantly perhaps, autoimmunity. In particular, the latter problem carries the potential of exposing a patient to harm, given the inflammatory host reaction that could occur in the vicinity or distance of the transplanted cells. The entire spectrum of inflammation, ranging from local inflammation to diffuse encephalitis, can be imagined. Accordingly, the appropriate use and selection of immune-modulatory agents are investigative fields on their own that should certainly gain importance once the stem cell approaches are closer to successful implementation. Conversely, as alluded to previously, stem cells from the umbilical cord or bone marrow as well as stimulation of endogenous stem cell production for area-specific repair or nuclear transfer may obviate such concern, which fuels the argument of strongly continued research in these areas.

Another category of problems is related to the quality of the stem cells themselves. Despite tremendous advances, some basic imperfections of stem cells need to be acknowledged. First, in some cell differentiation protocols, human ES cells are contaminated when they are plated and differentiated on mouse fetal layers. This bears the problem of cross-species contamination, with the subsequent fear of unwanted immune reaction and rodent gene introduction into the human

patient. Second, much research remains to be performed to enhance our understanding of the molecular characteristics of stem cells. For one, there is only scarce knowledge of the surface antigens that are specific to mesencephalic NSCs. Progress in this field of study should prove crucial in approaches that aim at enriching NSCs, such as FACS. For another, the ideal environment for effective neuronal cell fate determination and neuronal terminal differentiation is only partly understood. For example, the observation that astrocytes specify neural cell fate in NSCs [33,52] implies that glial cells are important for fate decision before and after NSC transplantation, which is a concept that has yet to be fully incorporated into the differentiation protocols. Hence, only with more such molecular knowledge can it be possible to produce enriched and healthy dopaminergic neurons more efficiently and reliably that have the prospect of long-term survival in the basal ganglia environment. Third and last, despite the reports of genomic stability [53], human ES cells can be genomically unstable, as exemplified by observed chromosomal aberrations in midterm cultured human ES cell lines [54]. The consequences of such findings are grave, given the worry for neoplastic changes. Conversely, however, genetic manipulations may also provide significant benefits. For example, homologous transformation may be helpful in designing desired transgenic human ES cells or cell lines that have properties with great therapeutic advantages, ranging from delivery tools for trophic factors to designed neurons that reliably and chronically produce constant and high amounts of dopamine. Constant dopamine production, in turn, may provide a foundation for decreased side effects, avoiding especially the motor fluctuations that limit L-dopa therapy.

What about the choice of location for stem cell transplantation? In literally every animal experiment, it has become the standard to transplant cells into the striatum after that structure is lesioned. Crucially, however, the initial loss of neurons in PD occurs in the SN. Although the 6-OHDA–lesioned striatum in rats offers a good animal model, it has its limitations in recapitulating the human disease. Perhaps it is important in the long run to transplant dopaminergic cells into the SN. Perhaps such cells survive much better there in their native environment, which may, in turn, improve circuitry integration. For example, transplantation into the SN may avoid the excessive aberrant intrastriatal connectivity that accompanies striatal transplantation, which may result in decreased run-away dyskinesias. Additionally, the intrinsic challenge of having axons travel a longer distance to connect to the striatal neurons may be counterbalanced by the possibility that physiologic connections to other brain stem nuclei could be facilitated, which could complement a more physiologic recovery in motor function. For example, re-establishing connections from the SN to the pedunculopontine nucleus may aid in the recovery of posture and balance, in addition to tremor, rigidity, and akinesis. Hence, in an effort to optimize stem cell treatment for PD, these cells need to be put into the SN, which requires new PD models that better reflect the human disease state. Initial studies have been conducted in rodents [55,56] and human patients [57], with results being in the early stages. Essentially, there is some improvement in animals, but the striatonigral pathway is far from being reconstructed.

What about nonmotor symptoms? The entire effort is currently focused on alleviating motor symptoms, and the behavioral tests used in the animal models are designed to address mainly motor function. As mentioned in the introductory section of this review, nonmotor symptoms are profound in PD and become the primary problem as the disease progresses. The conceptual underpinning of addressing nonmotor symptoms implies the need for replacement of nondopaminergic neurons in a wide variety of areas across the central nervous system. This is a daunting task, particularly given the complex nature of the neuronal circuitry involved, the limited knowledge of the anatomy of those circuits, and the limited knowledge of the neuronal subtypes that occupy these areas, which includes their precise neurotransmitter profiles and the way in which these relate to the nonmotor deficits seen in PD. Despite these tremendous hurdles, the improvement in creating dopaminergic neurons from stem cells indicates the feasibility of this overarching approach, which is undoubtedly going to have a great impact on developing similar strategies for treating the nonmotor aspects of PD. To this end, recent studies have initiated the thought process that although stem cell transplantation into the postcommissural putamen may primary affect motor symptoms, transplantation into the anterior putamen or caudate nucleus, and perhaps even into the SNc itself, may be more prone to addressing nonmotor pathologic changes of PD.

Finally, and importantly, ethical issues need careful consideration. Isolation of stem cells encroaches on critical religious and cultural belief systems that are deeply embedded in our society and everyday life. This is particularly delicate in the current situation, in which ES cells seem to be perhaps the best-suited cells for PD treatment. The concept of respecting human life without jeopardizing meaningful medical advances is a continuous balancing act, which requires input not just from scientists but from society as a whole.

Summary

Stem cell therapy for PD is at the forefront of PD research. In the setting of long-term limitations of medical and surgical therapy, the long-term advantages of this approach are tremendous, given its conceptual foundation of replacing damaged neuronal tissue and restoring neuronal circuitry. The road to reasonable and sustainable success is filled with hurdles, but many hurdles have been overcome and the remaining ones are being constantly tackled. Part of the problem lies in the fact that the transplant procedure is a multistep process, indicating that all steps have to be optimized and work in synergy so that maximum efficacy is accomplished. Nonetheless, proof of principle has been obtained, with reasonable success achieved already in the primate PD model. Progress at a fairly rapid rate would not come as a surprise, given that different kinds of stem cells are being investigated, each with its own advantages and disadvantages. Despite the progress and promise at hand, however, attention has to be paid continuously to the ethical and moral issues at hand. After all, given the nature of the material, stem cell therapy concerns cultures and societies and not merely a confined scientific community. Optimism regarding a broad consensus in society can be drawn from the fact that there exists a real need for treatment of PD, especially in an era in which the elderly population is expected to grow at a rapid rate.

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