Potential sources of stem cells as a regenerative therapy for Parkinson's disease

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Abstract: Stem cells are believed to hold enormous promise as potential replacement therapy in the treatment of neurodegenerative diseases such as Parkinson's disease (PD). Stem cells were investigated to be the alternative therapeutic source capable of differentiating into dopamine (DA) neurons. Multiple important signaling factors were recorded for the induction of DA neuronal traits from mouse embryonic stem cells (ESCs) such as fibroblast growth factor 8, sonic hedgehog, and Wnt 1. Recent protocols were described for the differentiation of human ESCs into DA neurons, achieving high efficiency of DA neuronal derivation. Despite that, the use of human ESCs is still ethically controversial. The transcription factors necessary for DA neuron development from adult neural stem cells (NSCs), such as Pitx3, Nurr1, En-1, En-2, Lmx1a, Lmx1b, Msx1, and Ngn2, were investigated. In addition to replacement of lost DA neurons, adult NSCs were recorded to provide neuroprotective and neurogenic factors for the mesencephalon. In addition, induced pluripotent stem cells and bone marrow-derived mesenchymal stem cells represent reliable stem cell sources of DA neurons. Future studies are recommended to provide further insight into the regenerative capacity of stem cells needed for the treatment of PD.

Keywords: dopamine, embryonic stem cells, neural stem cells, Parkinson's disease, induced pluripotent stem cells, mesenchymal stem cells

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting the dopamine¹ (DA)-producing neurons in the substantia nigra.² The efficacy of the pharmacological treatment with L-DOPA gradually decreases with the development of side effects.³ Deep brain stimulation was applied as an alternative therapy for symptom relief in some advanced PD patients, although its effect is variable and it does not address the cause.² Neural protection with neurotrophic factors of the remaining DA neurons was proved to be effective in a small, open-labeled trial.⁴ Neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF)⁵ and neurturin have been shown to be neuroprotective, reducing dopaminergic cell death following toxic challenges.⁶ Nevertheless, these factors do not cross the blood-brain barrier. They require direct application into the relevant area, and when delivered directly into the brain the precise location of the cannula seems critical.⁷

A potential therapeutic approach studied recently is fetal cell transplantation. Transplantation of primary ventral mesencephalic tissue into the striatum⁸ aims to restore regulated DA release from grafted dopaminergic neurons. There is good evidence of graft survival and grafted neurons developing afferent and efferent projections with the

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host neurons of the human brain of PD patients. Moreover, positron emission tomography scanning has revealed significant increases in activation in the areas reinnervated by the grafted cells, and longitudinal clinical assessments indicate significant functional recovery for motor control, in some cases for more than 10 years.⁹ There have been no reported cases of overt immunorejection⁸ even after several years of withdrawal from immunosuppression.¹⁰ However, two National Institutes of Health-sponsored double-blinded trials reported severe graft-induced dyskinesias. It was suggested to be related to the quality of dissected tissue and the excessive DA release from dense hyperdopaminergic areas identified within the graft of some patients.¹¹ Histologic examination of the postmortem brain of a 68-year-old man who underwent transplantation revealed Lewy bodies in pigmented transplanted DA neurons in the substantia nigra, suggesting that the disease process would affect the donor cells.¹² In addition, the limited availability of tissue and the need for multiple donors for single patients led to problems of coordinating and storing tissue prior to transplantation.⁸ Fetal neural transplantation studies provided the first proof-of-principle experiments demonstrating cellular replacement as a feasible therapy for PD. However, poor clinical outcomes, the most concerning being the development of graft-induced dyskinesias, and the limited efficacy of this technique should be stressed as the reasons why alternative stem cell sources for PD are sought.

Stem cell-based regenerative medicine offers great hope for patients affected by a neurodegenerative disease such as Parkinsonism as potential sources of dopaminergic neurons. The aim of this review is to highlight the recent investigations demonstrated to differentiate and transplant stem cells from various sources such as those derived from the early developing mouse embryo (mouse embryonic stem cells [ESCs]), early developing human embryo (human ESCs), adult brain (neural stem cells [NSCs]), reprogrammed somatic cells (induced pluripotent stem cells), or mesenchymal stem cells (MSCs).

Mouse ESCs

Researchers have been studying the mechanisms involved in embryonic development in rodents and trying to understand the cues and signals directing the early inner cell mass toward the neuroectodermal lineage and maturing into fully differentiated nerve cells.¹³

Mouse ESCs were differentiated to neural progenitors using the expansion with basic fibroblast growth factor (FGF2) and inducing dopaminergic differentiation by

withdrawal of the mitogens. Moreover, the addition of molecules known to be involved in the development of midbrain dopaminergic neurons significantly raised the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, with an efficiency of 30%.¹⁴ These molecules represent the extrinsic signaling factors such as sonic hedgehog (SHH), a glycoprotein secreted from the floor plate cells, and fibroblast growth factor 8 (FGF8) secreted from the mid-hindbrain boundary. These signaling factors instruct the midbrain identity of neural progenitors.² The induced cells, resulting from the addition of these factors, expressed specific markers of DA neurons, secreted DA in response to depolarization, and showed electrophysiologic properties similar to neurons.¹⁴ However, FGF8 and SHH did not provide the DA neurons with the sufficient midbrain phenotype.¹⁵ It is recommended that additional signaling factors are needed for patterning the midbrain identity. Prakash et al¹⁶ demonstrated that Wnt 1, which is another extrinsic signaling factor, is essential for midbrain DA neuron specification in addition to FGF8 and SHH. Thereafter, Rodriguez-Gomez et al¹⁷ showed that nearly all the DA neurons exhibited a midbrain phenotype by involving the expression of En-1, which is a transcription factor expressed in midbrain identity. Another factor is Ptx3 transcribed for synthesizing and metabolizing DA.^{17,18}

An additional important signaling factor detected for the induction of DA neurons is Lmx1a. Lmx1a is a homeodomain transcription factor that has been shown to be induced in response to early signaling in the ventral midbrain and is selectively expressed in proliferating DA progenitors. Lmx1a was proved to be an important determinant of DA neurons during embryonic development.^{19–21} Forced expression of Lmx1a can promote the differentiation of DA neurons in mouse ESC cultures.²¹ Stably transformed mouse ESCs were generated using a nestin enhancer (NesE)-driven expression vector, which is a neural progenitor marker. NesE directs Lmx1a expression to the neural progenitor stage of ESC differentiation. Moreover, after transplantation of NesE–Lmx1a mouse ESC-derived progenitors into neonatal rats, surviving tyrosine hydroxylase positive (TH⁺) neurons extensively innervate the striatum and appear identical to primary mouse DA neurons.²¹ Therefore, it is suggested that Lmx1a could be used for the effective generation of functional and transplantable DA neurons from differentiating mouse ESCs.

In addition to the chemically inducing methods for the differentiation of mouse ESCs into DA neuronal traits, recent protocols reported that DA neurons can also be efficiently differentiated from mouse ESCs by coculturing method.

ESCs were cocultured with stromal cells (PA6 cells) followed by treatment with FGF8 and SHH. The effect of PA6 cells was believed to promote neural differentiation from ESCs, influencing the midbrain patterning.²² It is recommended that both chemical and coculturing methods require further studies to direct the development and differentiation of ESCs as a regenerative cell therapy for PD.

Human ESCs

Massive advancement in embryology and neurogenesis has helped ESCs researchers to develop protocols that generate neural progenitors capable of differentiating into neurons, astrocytes, and oligodendrocytes from human ESCs.^{23,24} FGF8 and SHH, as in mouse ESCs, are employed to pattern the human ESC-derived neuroepithelial cells. The previous yield was about 30% TH-expressing DA neurons among all differentiated progenies.^{25–27} Possible explanations for these disappointing results could be the low survival rate of TH⁺ neurons due to host immune response. An additional factor is the apoptotic behavior of dopaminergic cells in the host brain, possibly due to limited success of the differentiation protocol.¹³ Moreover, some of these studies have also reported ectopic nonneural protein expression of the transplanted cells in the brain.^{28,29} Transplantation studies have shown an incidence of teratoma formation following transplantation of predifferentiated human ESCs in the brain. These tumors were suggested to arise from residual undifferentiated ESCs or precursor cells that maintain their proliferative capacity *in vivo*.³⁰ Selective apoptosis of tumor-inducing cells³¹ and genetic modification³² were tested to overcome tumor-inducing activity in human ESCs. Purifying cells prior to transplantation using sorting technology was also examined to prevent teratoma formation.³³ Magnetic sorting of early postmitotic neurons using antibodies to eliminate contaminating cells has been used to isolate neurons from differentiating ESCs.³⁴ The establishment of a pure and safe differentiated human ESC population with reduction of the risks associated with tumor overgrowth following transplantation is recommended to be an important research goal for regenerative medicine.

A much higher yield of DA neurons was reported when cocultured with immortalized mesencephalic astrocytes.³⁵ But, once again, the proportion of midbrain DA neurons is generally low and did not assess the expression of midbrain markers such as En-1 and Ptx3 in the DA neurons. The combination of FGF8 and SHH alone, as is the case in mouse ESC differentiation, is not optimal for patterning the human ESC-generated neuroepithelial cells to midbrain progenitors.²

The reason for less efficient midbrain patterning of the human ESC-derived neuroepithelial cells than that from mouse ESCs is that human ESCs almost exclusively differentiate to neuroepithelia with a forebrain identity.³⁶

Currently, there are a number of established human ESC lines originally propagated on mouse feeder layers, although several have recently been transferred to feeder-free support systems.^{37,38} These protocols involve the prolonged use of complex media containing serum or other undefined reagents and/or cell-conditioned media or coculture with PA6 mouse stromal cells.^{39,40} Media additives such as B27 and Matrigel[®] have been used by other protocols. These additives contain undefined components as well as hormones and growth products of animal origin.³⁸ The use of these animal compounds ultimately can cause immune rejection after their transplantation into the brain, as animal cells contain immunogenic antigens that can be incorporated into human ESCs.⁴¹ In addition, the prolonged time course required for cells to adequately develop expression of DA traits in culture elicits an extensive, highly branched network of processes that produce irreparable mechanical damage to cells during harvesting.³⁸ Iacovitti et al⁴² studied several well-characterized (H9, BG01) and several new uncharacterized (HUES7, HUES8) human ESC lines. The authors investigated the capacity of these lines to differentiate into DA neurons in cultures. They examined a novel rapid protocol that uses only chemically defined human-derived reagents in a simple serum-free media supplemented with 1 mM dibutyryl-cAMP (dbcAMP). Within 3 weeks, cells from all four cell lines progressed from the undifferentiated state to β -tubulin III positive cells expressing DA traits such as TH, L-amino decarboxylase acid (AADC), Ptx3, Lmx1b, Nurr1, and dopamine transporter (DAT) in culture. All four lines produced a comparable degree of TH differentiation: H9, 60%; HUES7, 78%; HUES8, 81%; and BG01, 56%. Importantly, TH expression was maintained in cells 5 days after removal of dbcAMP from the media, suggesting that cells had permanently adopted a DA phenotype. Moreover, transplantation of these cells into the striata of 6-hydroxy-dopamine (6-OHDA)-treated rats at the neuronal progenitor stage resulted in the appearance of differentiated DA traits *in vivo* 2–3 weeks later.⁴² These results give a great hope for the improvement of the DA neuron differentiation capacity of the newly examined factors using only human-derived reagents. However, it is recommended that the quantitative data showing the number of functional TH⁺ cells that had survived transplantation and data of functional efficiency following transplantation should be reported. In addition,

it is suggested that the percentage of surviving TH cells detected *in vivo* will be lower than the percentage of TH⁺ cells generated in culture due to transplantation survival. Furthermore, the cultured cells still require feeder layers to support cell maintenance.

Cho et al⁴³ introduced a method that allows differentiation of human ESCs into functional TH⁺ neurons up to approximately 86% of the total human ESC-derived neurons. Achieving high efficiency of DA neuronal derivation would not only increase the efficacy of the therapy but also minimize potential disastrous side effects such as teratomas resulting from undifferentiated residual ESCs. They generated pure spherical neural masses (SNMs), which could be expanded for long periods without losing their differentiation capability. The expanded SNMs can be stored frozen and thawed at any time. On the other hand, SNMs could be coaxed into DA neurons efficiently within a relatively short time (2 weeks) when needed. The SNM culture and DA neuron derivation from the SNMs do not need feeder cells, which would save a lot of time and effort required for handling feeder cells and reduce the risk of contamination of unwanted cells and pathogens.⁴³

Concerning the clinical improvement of signs and symptoms after transplantation of human embryonic DA neurons in patients with severe PD, Freed et al¹² proved that transplanted cells can survive and result in more clinical benefits in younger but not older patients.

It could be concluded that ESCs are currently the most promising donor cell source for cell replacement therapy in PD. However, the use of human ESCs is still ethically controversial. In addition, using human ESC-derived DA neurons have not been as favorable when compared with mouse ESC-derived neurons. Although some progress has been made in the generation of DA neurons from these cells, many technical studies are recommended to be made before the application of human ESCs to treat PD. Examples involve increasing the purity of DA neurons and overcoming the reduced survival of transplanted cells and limited functional recovery. Moreover, supplying sufficient numbers of TH⁺ cells need to be grafted for an effect to be detected when using human ESCs. In addition, decreasing tumor formation after transplantation and clearly demonstrating the fate of human ESC-derived DA neurons in the brain of PD models are suggested. It is recommended that more optimization is needed to show that human ESCs could be used efficiently.

Adult NSCs

NSCs have been of great interest to scientists seeking a cure for neuronal damage. Implanting ESCs will always involve

allograft transplantation, raising immunologic consequences, whereas the ultimate use of adult stem cells will provide the possibility of autologous cell transplantation, supported by their very nature of being more committed than ESCs.⁴⁴ In addition to replacement of lost neurons, NSCs were proved to be important cellular factories providing neuroprotective, anti-inflammatory, angiogenic, and neurogenic factors to the brain, consequently creating a reparative and homeostatic microenvironment.^{45,46} The use of fetal-derived NSCs has shown significant promise in rodent models of PD, and the potential for tumorigenicity appears to be minimal.⁴⁷

NSCs isolated from the developing ventral mesencephalon (VM) were expanded *in vitro* to give rise to neurons, astrocytes, and oligodendrocytes.⁴⁸ However, their ability to retain their DA phenotype following expansion was limited.⁴⁹ Transplantation of mouse NSCs into the intact or 6-OHDA-lesioned rat striatum exhibited neuronal markers and expressed the DA enzymes TH and aromatic AADC.⁵⁰ Nevertheless, in a significant number of these grafts, no cells expressed the enzyme TH, suggesting that other factors may have been more influential in deciding the fate of transplanted stem cells. Yang et al⁵¹ investigated C17.2 NSCs derived initially from the external germinal layer of mouse neonatal cerebellum. The cells were maintained in culture at differing levels of confluence (30% to >100%). Low confluence (<50%) cultures exclusively comprised flattened polygonal cells, which, when transplanted, migrated widely in the brain but did not express TH.^{51,52} In contrast, high confluence (>100%) cultures, containing both polygonal cells and an overlying bed of fusiform cells, spontaneously differentiated to express TH. Possibly, highly confluent cells manufacture and secrete growth factors in sufficient quantity to promote their own maturation, causing a change in their appearance and potentiality after transplantation.⁵¹ When these NSCs were maintained for 12–20 passages and then transplanted, virtually all engrafted cells in 65% of the grafts expressed TH. These observations suggested that high confluence and high-passage cultures are able to respond to TH-inducing cues *in vivo*, possibly as they develop the appropriate receptors and signaling molecules.⁵¹ These findings raise the question about the *in vivo* factors that are capable of TH expression. It has been suggested that the loss of a particular cell type, such as DA neurons in a 6-OHDA-lesioned brain, guides transplanted stem cells in the appropriate differentiation.^{53,54} Other elements have been shown to influence the survival, migration, and DA differentiation of stem cells following transplantation. These elements are supposed to be growth factors secreted from injured neurons, reactive glia, and

inflammatory agent cytokines secreted from monocytes and macrophages as local injury-induced agents.^{51,55}

Recent studies have focused on identifying the factors necessary for normal DA development, as demonstrated in ESCs, including FGF8, SHH, and Wnt1. In addition, the transcription factors necessary for DA neuron differentiation, such as Pitx3, Nurr1, En-1, En-2, Lmx1a, Lmx1b, Msx1, and Ngn2, were studied.¹⁸ Pitx3 is a paired-like homeobox protein expressed exclusively within the central nervous system (CNS) in DA neurons of the substantia nigra, zona compacta, and ventral tegmental area.⁵⁶ In mice, which lack detectable levels of Pitx3 transcripts, A9 DA neurons (those located in the substantia nigra) degenerate and TH expression is lost. However, A10 DA neurons (those located in the ventral tegmental area) are less affected.^{57,58} These data show that Pitx3 is important for A9 DA neuron specification and survival.^{59,60} The particular time point of induction of DA neurogenesis in NSCs was shown to be very critical. Therefore, cocultured neurospheres (NSs) expressing either Nurr1 or Pitx3 with an E11 rat VM (equates to E9.5 in the mouse) led to an increase in DA neurogenesis in vitro.⁶¹ Interestingly, only coculture with Pitx3 overexpressing NSs resulted in a significant increase in TH⁺ neurons. Nevertheless, this was not the case in the Nurr1 overexpressing group. Nurr1 alone is not sufficient to induce TH in NSCs. They need to be in contact with astrocytes from E16 VM, proving that astrocytes from older embryos elicit the correct signals. The developmental age of E11 is prior to the neuronal-glia switch.⁶² An additional important factor is the regional specification. Cells overexpressing Nurr1 need to be in direct contact with the astrocytes to affect TH expression. These results indicate that the required signals are not diffusible and are contact mediated or highly labile.⁶¹ Postmortem analysis of the recovered Parkinsonian primates demonstrated that a marked number of human NSCs' progeny were astrocytes, which were found juxtaposed with the remaining host nigrostriatal pathway. These astrocytic cells presumably expressed neuroreparative factors, including GDNF, and provided homeostatic adjustments to the microenvironment.⁶³

An important contribution to the previous studies is allowing the brain to determine the fate of the donor cells and their corresponding reparative properties. If the neurodegenerative microenvironment is not reduced or stabilized, it is possible that the disease process will continue to adversely affect both the donor and host DA cells,^{64,65} which raises an important question: does the microenvironment of the lesioned part of the brain affect the phenotype of the transplanted cells or do the new cells affect the microenvironment of the diseased

brain? It is hypothesized that the microenvironment should be stabilized to allow the brain to determine the fate of the donor cells.

Redmond et al⁶⁶ reported no dyskinesias, tumors, deformations, or overgrowth in the transplanted primates in patients in an earlier stage of the disease. The authors found that grafted cells could survive, migrate, and induce behavioral recovery of Parkinsonian symptoms. The neuronal cells in the early stage of the disease can protect and repair more of the DA nigrostriatal system. Efforts have been extended to stimulate and recruit endogenous precursors residing within the adult CNS in PD models. In addition to the generation of a small population of dopaminergic neurons, other cells within the grafted NSCs were found to be releasing growth factors exerting neuroprotective or neuroregenerative influences.^{66,67} Growth factor treatment activates endogenous neurogenesis and migration of precursor cells from the subventricular zone (SVZ), an area known to support persistent generation of NSCs even in the adult brain.^{68,69} Recent studies demonstrated that the fate of stem cells depends critically on its 'niche' or local environment.⁷⁰ Because endogenous NSCs are also present in the environment of transplanted NSCs, interactions between these two cell types may play a critical role in determining the behavior and fate of both, and ultimately the impact on neural protection and repair.⁷¹ Stimulation of endogenous neurogenesis after transplantation of human NSCs cloned by v-myc gene transfer (HB1.F3 cells) was observed to be a feasible therapeutic option for PD.⁷² The study recorded about a 140% increase in endogenous neurogenesis but with no migration, differentiation into TH⁺ neurons, or indication of participation of the endogenous NSCs in the neuroprotection. Madhavan et al⁷¹ demonstrated that graft-expressed GDNF, SHH, and stromal cell-derived factor 1 alpha (SDF-1 α) can stimulate significant endogenous NSC proliferation, migration, and neuronal differentiation in association with nigrostriatal protection in a rat model of PD. Moreover, they observed a significant preservation of striatal TH expression and substantia nigra TH cell number. In addition, grafted NSCs stimulate the release of growth factors and chemokines that induce plastic responses from the host, such as endogenous neurogenesis. NSCs have been shown to spontaneously produce a variety of growth- and plasticity-promoting factors, including GDNF, neurotrophin-3, brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF).^{72,73} Other studies indicate that GDNF and SHH can spread considerable distances in the striatum to stimulate cells in the SVZ, and SDF-1 α has been shown to mediate long-distance NSC migration toward injury sites, where it

has been expressed.^{74,75} Regarding these expressed factors, GDNF has been shown to have neuroprotective and trophic actions on dopaminergic neurons when injected into the striatum or substantia nigra.⁷⁶ GDNF was also shown to promote SVZ neurogenesis and migration of newly born neuroblasts into the striatum.⁷⁴ SHH has been proved to be a critical factor for the survival and differentiation of dopaminergic neurons during development and in protecting them against toxic insults in the adult brain. In addition, SHH displays a chemoattractive activity *in vitro* on SVZ-derived neuronal progenitors and is also known to control stem cell behavior in the postnatal and adult brain.⁷⁷ The chemokine SDF-1 α has been tested to be able to direct migration of NSCs to sites of injury and also play neuroprotective roles.⁷⁸ Other factors, such as activation of toll-like receptors, contributed to neurogenesis and NSC interactions and triggered the production of neuroprotective mediators.⁷⁹ A novel and important finding in the studies of Madhavan et al⁷¹ is that they point to the likelihood of synergistic interactions between endogenous and exogenous NSCs after transplantation. They reported that endogenous NSCs proximal to the graft expressed SHH, which possibly contributed to the observed neuroprotection and NSCs' regulating abilities. Such expression of SHH by the endogenous NSCs in combination with SHH from the grafted ones strengthened the neuroprotective response. Moreover, endogenous NSCs, via their expression of SHH, might have supported the survival of the grafted cells.⁷¹ Future studies are recommended to investigate the signals involved in directing DA neuron differentiation and maturation. DA neuron transcriptional factors such as Nurr1, Pitx3, En-1, En-2, Lmx1a, Lmx1b, Msx1, and Ngn2 should be expressed either simultaneously or sequentially. The role of potential endogenous precursors for neuroprotection, the survival and fate of the transplanted NSCs, and the efficiency of synergistic exogenous and endogenous interactions need to be addressed in the future.

Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients represent one of the most reliable stem cell sources of DA neurons. Somatic fibroblast cells were reprogrammed to generate iPSCs by forced expression of transcription factors such as OCT4, SOX2, NANOG, KLF4, c-MYC, and LIN28.^{80,81}

Wernig et al⁸² reported the differentiation of mouse iPSCs to DA neurons efficiently by retroviral transduction of the transcription factors. Transplanted cells migrated into various brain regions and differentiated into glia and neurons

when injected into embryonic cerebral ventricles. The cells synaptically integrated into the host brain and showed active action potential. In addition, tested rat models of PD presented high numbers of TH⁺ cells that showed complex morphologies and were also positive for En-1, VMAT2, and DAT. Transplanted animals showed a marked behavior recovery 4 weeks after transplantation.

Generated human iPSCs (hiPSCs) using retrovirus⁸⁰ and lentivirus⁸³ were proved to be differentiated to TH-expressing neurons. Transplanted cells showed ESC-like properties based on expression of surface markers, gene expression profiles, and formation of embryoid bodies.^{84,85} Recently, Okita et al⁸⁶ and Stadtfeld et al⁸⁷ studied reprogramming of mouse somatic cells without stable integration through the use of transient transfection or adenoviral infection to deliver the reprogramming factors. However, similar approaches were not proved to be successful in the hiPSCs as a result of the lower efficiency of these methods. Soldner et al⁸⁸ generated hiPSCs that are free of the reprogramming factors. These factor-free hiPSCs maintain a pluripotent ESC-like state and represent a more suitable source of cells, as the residual transgene expression in virus-carrying hiPSCs can affect their molecular characteristics.

The use of viruses represents a major limitation because they randomly integrate into the genome and can alter the differentiation of the iPSCs and induce cancerous transformation.⁸⁹ In addition, unknown genetic factors that resulted in the patient's disease could lead to degeneration of the reprogrammed cells. It is suggested that hiPSCs will provide a powerful tool for biomedical research in replacement therapy of degenerated neurons. However, long-term observation of the safety issues of this therapeutic approach need to be investigated.

MSCs

Mesenchymal cells are primordial cells that are capable of multipotency. MSCs could differentiate not only to osteogenic, adipogenic, and endothelial lineages but also to hepatocyte-like cells, neural cells, and erythroid cells.⁹⁰ They can differentiate into dopaminergic neurons under appropriate conditions.⁹¹ MSCs obtained from the patient bone marrow could be expanded on a large scale and then allowed to differentiate using an induction medium. MSCs can express several specific neuronal markers and transcriptional factors.⁹² MSCs were proved to produce NTFs that promote neuronal survival, endogenous cell proliferation, and nerve fiber regeneration.⁹³ NTFs, such as BDNF, NGF, and GDNF, can produce neuroprotective effects by slowing

the degeneration and stimulation of endogenous regeneration after MSC transplantation.⁹⁴ In addition, MSCs were found to possess immunoregulatory properties.⁹⁵ In vitro studies suggested that MSCs can release soluble factors involved in their immunosuppressive activity.⁹⁶ Therefore, it is suggested that MSCs can produce neuroprotective effect through an anti-inflammatory action. Clinical improvement after MSC transplantation was observed.^{97,98} The authors concluded that this improvement was due to the supporting effect of the MSCs and the secretion of neurotrophic factors more than cell replacement. Although there is no ethical or immunological controversy concerning MSCs, in contrast to ESC therapy, future studies are needed to provide sufficient knowledge about the safety and efficacy of MSC applications.

Conclusion

The applications of embryonic, adult neural, induced pluripotent, and MSCs in treatment for PD serve as a template for identifying or developing therapeutics for slowing, stopping, or reversing the disease process. The expression of DA phenotypic traits in transplanted stem cells is regulated through multiple factors, including the development of cell intrinsic mechanisms and the influence of outside environmental cues. Consequently, further studies are needed to identify the optimal conditions and specific factors that allow stem cells to someday fulfill their promise as a source of replacement therapy for the treatment of PD and to serve in other biomedical applications.

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Disclosure

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References

1. Raichur A, Vali S, Gorin F. Dynamic modeling of alpha-synuclein aggregation for the sporadic and genetic forms of Parkinson's disease. *Neuroscience*. 2006;142(3):859–870.
2. Xi J, Zhang SC. Stem cells in development of therapeutics for Parkinson's disease: a perspective. *J Cell Biochem*. 2008;105(5):1153–1160.
3. Olanow CW, Obeso JA. Levodopa motor complications in Parkinson's disease. *Ann Neurol*. 2000;47:167–178.
4. Gill SS, Patel NK, Hotton GR, et al. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med*. 2003;9(5):589–595.
5. Green-Sadan T, Kinor N, Roth-Deri I, Geffen-Aricha R, Schindler CJ, Yadid G. Transplantation of glial cell line-derived neurotrophic factor-expressing cells into the striatum and nucleus accumbens attenuates acquisition of cocaine self-administration in rats. *Eur J Neurosci*. 2003;18(7):2093–2098.
6. Patel NK, Gill SS. GDNF delivery for Parkinson's disease. *Acta Neurochir Suppl*. 2007;97(Pt 2):135–154.
7. Nutt JG, Burchiel KJ, Comella CL, et al. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology*. 2003;60(1):69–73.
8. Lane EL, Handley OJ, Rosser AE, Dunnett SB. Potential cellular and regenerative approaches for the treatment of Parkinson's disease. *Neuropsychiatr Dis Treat*. 2008;4(5):835–845.
9. Bjorklund A, Dunnett SB, Brundin P, et al. Neural transplantation for the treatment of Parkinson's disease. *Lancet Neurol*. 2003;2(7):437–445.
10. Olanow CW, Goetz CG, Kordower JH, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol*. 2003;54(3):403–414.
11. Winkler C, Kirik D, Bjorklund A. Cell transplantation in Parkinson's disease: how can we make it work? *Trends Neurosci*. 2005;28(2):86–92.
12. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med*. 2001;344(10):710–719.
13. Barzilay R, Levy YS, Melamed E, Offen D. Adult stem cells for neuronal repair. *Isr Med Assoc J*. 2006;8(1):61–66.
14. Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat Biotechnol*. 2000;18(6):675–679.
15. Zhao S, Nichols J, Smith AG, Li M. SoxB transcription factors specify neuroectodermal lineage choice in ES cells. *Mol Cell Neurosci*. 2004;27(3):332–342.
16. Prakash N, Brodski C, Naserke T, et al. A Wnt1-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors in vivo. *Development*. 2006;133(1):89–98.
17. Rodriguez-Gomez JA, Lu JQ, Velasco I, et al. Persistent dopamine functions of neurons derived from embryonic stem cells in a rodent model of Parkinson disease. *Stem Cells*. 2007;25(4):918–928.
18. Smidt MP, Burbach JP. How to make a mesodiencephalic dopaminergic neuron. *Nat Rev Neurosci*. 2007;8(1):21–32.
19. Andersson E, Tryggvason U, Deng Q, et al. Identification of intrinsic determinants of midbrain dopamine neurons. *Cell*. 2006;124(2):393–405.
20. Ono Y, Nakatani T, Sakamoto Y, et al. Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. *Development*. 2007;134(17):3213–3225.
21. Friling S, Andersson E, Thompson LH, et al. Efficient production of mesencephalic dopamine neurons by Lmx1a expression in embryonic stem cells. *Proc Natl Acad Sci U S A*. 2009;106(18):7613–7618.
22. Kawasaki H, Mizuseki K, Nishikawa S, et al. Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron*. 2000;28(1):31–40.
23. Reubinoff BE, Itsykson P, Turetsky T, et al. Neural progenitors from human embryonic stem cells. *Nat Biotechnol*. 2001;19(12):1134–1140.
24. Taylor H, Minger SL. Regenerative medicine in Parkinson's disease: generation of mesencephalic dopaminergic cells from embryonic stem cells. *Curr Opin Biotechnol*. 2005;16(5):487–492.
25. Perrier AL, Tabar V, Barberi T, et al. From the cover: derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci U S A*. 2004;101(34):12543–12548.
26. Yan Y, Yang D, Zarnowska ED, et al. Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells*. 2005;23(6):781–790.
27. Park CH, Lee SH. Efficient generation of dopamine neurons from human embryonic stem cells. *Methods Mol Biol*. 2007;407:311–322.

28. Park CH, Minn YK, Lee JY, et al. In vitro and in vivo analyses of human embryonic stem cell-derived dopamine neurons. *J Neurochem*. 2005;92(5):1265–1276.
29. Zeng X, Cai J, Chen J, et al. Dopaminergic differentiation of human embryonic stem cells. *Stem Cells*. 2004;22(6):925–940.
30. Brederlau A, Correia AS, Anisimov SV, et al. Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of in vitro differentiation on graft survival and teratoma formation. *Stem Cells*. 2006;24(6):1433–1440.
31. Bieberich E, Silva J, Wang G, Krishnamurthy K, Condie BG. Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. *J Cell Biol*. 2004;167(4):723–734.
32. Parish CL, Parisi S, Persico MG, Arenas E, Minchiotti G. Cripto as a target for improving embryonic stem cell-based therapy in Parkinson's disease. *Stem Cells*. 2005;23(4):471–476.
33. Pruszek J, Sonntag KC, Aung MH, Sanchez-Pernaute R, Isacson O. Markers and methods for cell sorting of human embryonic stem cell-derived neural cell populations. *Stem Cells*. 2007;25(9):2257–2268.
34. Schmandt T, Meents E, Gossrau G, Gornik V, Okabe S, Brustle O. High-purity lineage selection of embryonic stem cell-derived neurons. *Stem Cells Dev*. 2005;14(1):55–64.
35. Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat Med*. 2006;12(11):1259–1268.
36. Pankratz MT, Li XJ, Lavaute TM, Lyons EA, Chen X, Zhang SC. Directed neural differentiation of human embryonic stem cells via an obligated primitive anterior stage. *Stem Cells*. 2007;25(6):1511–1520.
37. Beattie GM, Lopez AD, Bucay N, et al. Activin A maintains pluripotency of human embryonic stem cells in the absence of feeder layers. *Stem Cells*. 2005;23(4):489–495.
38. Gerrard L, Rodgers L, Cui W. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. *Stem Cells*. 2005;23(9):1234–1241.
39. Itsykson P, Ilouz N, Turetsky T, et al. Derivation of neural precursors from human embryonic stem cells in the presence of noggin. *Mol Cell Neurosci*. 2005;30(1):24–36.
40. Lu J, Hou R, Booth CJ, Yang SH, Snyder M. Defined culture conditions of human embryonic stem cells. *Proc Natl Acad Sci U S A*. 2006;103(15):5688–5693.
41. Martin MJ, Muotri A, Gage F, Varki A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. *Nat Med*. 2005;11(2):228–232.
42. Iacovitti L, Donaldson AE, Marshall CE, Suon S, Yang M. A protocol for the differentiation of human embryonic stem cells into dopaminergic neurons using only chemically defined human additives: studies in vitro and in vivo. *Brain Res*. 2007;1127(1):19–25.
43. Cho MS, Lee YE, Kim JY, et al. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci U S A*. 2008;105(9):3392–3397.
44. Fujikawa T, Oh SH, Pi L, Hatch HM, Shupe T, Petersen BE. Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. *Am J Pathol*. 2005;166(6):1781–1791.
45. Einstein O, Ben-Menachem-Tzidon O, Mizrahi-Kol R, Reinhart E, Grigoriadis N, Ben-Hur T. Survival of neural precursor cells in growth factor-poor environment: implications for transplantation in chronic disease. *Glia*. 2006;53(4):449–455.
46. Lee JP, Jeyakumar M, Gonzalez R, et al. Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. *Nat Med*. 2007;13(4):439–447.
47. Uchida N, Buck DW, He D, et al. Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci U S A*. 2000;97(26):14720–14725.
48. Gage FH. Mammalian neural stem cells. *Science*. 2000;287(5457):1433–1438.
49. Chung S, Shin BS, Hwang M, et al. Neural precursors derived from embryonic stem cells, but not those from fetal ventral mesencephalon, maintain the potential to differentiate into dopaminergic neurons after expansion in vitro. *Stem Cells*. 2006;24(6):1583–1593.
50. Yang M, Stull ND, Berk MA, Snyder EY, Iacovitti L. Neuronal stem cells spontaneously express dopaminergic traits after transplantation into the intact or 6-hydroxydopamine-lesioned rat. *Exp Neurol*. 2002;177(1):50–60.
51. Yang M, Donaldson AE, Jiang Y, Iacovitti L. Factors influencing the differentiation of dopaminergic traits in transplanted neural stem cells. *Cell Mol Neurobiol*. 2003;23(4–5):851–864.
52. Akerud P, Canals JM, Snyder EY, Arenas E. Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. *J Neurosci*. 2001;21(20):8108–8118.
53. Doering LC, Snyder EY. Cholinergic expression by a neural stem cell line grafted to the adult medial septum/diagonal band complex. *J Neurosci Res*. 2000;61(6):597–604.
54. Nishino H, Hida H, Takei N, Kumazaki M, Nakajima K, Baba H. Mesencephalic neural stem (progenitor) cells develop to dopaminergic neurons more strongly in dopamine-depleted striatum than in intact striatum. *Exp Neurol*. 2000;164(1):209–214.
55. Jonakait GM, Wen Y, Wan Y, Ni L. Macrophage cell-conditioned medium promotes cholinergic differentiation of undifferentiated progenitors and synergizes with nerve growth factor action in the developing basal forebrain. *Exp Neurol*. 2000;161(1):285–296.
56. Nunes I, Tovmasian LT, Silva RM, Burke RE, Goff SP. Pitx3 is required for development of substantia nigra dopaminergic neurons. *Proc Natl Acad Sci U S A*. 2003;100(7):4245–4250.
57. Smidt MP, Smits SM, Bouwmeester H, et al. Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene Pitx3. *Development*. 2004;131(5):1145–1155.
58. Zhao S, Maxwell S, Jimenez-Beristain A, et al. Generation of embryonic stem cells and transgenic mice expressing green fluorescence protein in midbrain dopaminergic neurons. *Eur J Neurosci*. 2004;19(5):1133–1140.
59. Maxwell SL, Ho HY, Kuehner E, Zhao S, Li M. Pitx3 regulates tyrosine hydroxylase expression in the substantia nigra and identifies a subgroup of mesencephalic dopaminergic progenitor neurons during mouse development. *Dev Biol*. 2005;282(2):467–479.
60. Simeone A. Genetic control of dopaminergic neuron differentiation. *Trends Neurosci*. 2005;28(2):62–65.
61. O'Keeffe FE, Scott SA, Tyers P, et al. Induction of A9 dopaminergic neurons from neural stem cells improves motor function in an animal model of Parkinson's disease. *Brain*. 2008;131(Pt 3):630–641.
62. Kessaris N, Pringle N, Richardson WD. Specification of CNS glia from neural stem cells in the embryonic neuroepithelium. *Philos Trans R Soc Lond B Biol Sci*. 2008;363(1489):71–85.
63. Freeman MR. Sculpting the nervous system: glial control of neuronal development. *Curr Opin Neurobiol*. 2006;16(1):119–125.
64. Lindvall O, Björklund A. Cell therapy in Parkinson's disease. *Neuro Rx*. 2004;1(4):382–393.
65. Sanberg PR. Neural stem cells for Parkinson's disease: to protect and repair. *Proc Natl Acad Sci U S A*. 2007;104(29):11869–11870.
66. Redmond DE Jr, Bjugstad KB, Teng YD, et al. Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. *Proc Natl Acad Sci U S A*. 2007;104(29):12175–12180.
67. Yasuhara T, Shingo T, Date I. Glial cell line-derived neurotrophic factor (GDNF) therapy for Parkinson's disease. *Acta Med Okayama*. 2007;61(2):51–56.
68. Cooper O, Isacson O. Intrastriatal transforming growth factor alpha delivery to a model of Parkinson's disease induces proliferation and migration of endogenous adult neural progenitor cells without differentiation into dopaminergic neurons. *J Neurosci*. 2004;24(41):8924–8931.

69. Mohapel P, Frielingsdorf H, Haggblad J, Zachrisson O, Brundin P. Platelet-derived growth factor (PDGF-BB) and brain-derived neurotrophic factor (BDNF) induce striatal neurogenesis in adult rats with 6-hydroxydopamine lesions. *Neuroscience*. 2005;132(3):767–776.
70. Jiao J, Chen DF. Induction of neurogenesis in nonconventional neurogenic regions of the adult central nervous system by niche astrocyte-produced signals. *Stem cells*. 2008;26(5):1221–1230.
71. Madhavan L, Daley BF, Paumier KL, Collier TJ. Transplantation of subventricular zone neural precursors induces an endogenous precursor cell response in a rat model of Parkinson's disease. *J Comp Neurol*. 2009;515(1):102–115.
72. Yasuhara T, Matsukawa N, Hara K, et al. Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson's disease. *J Neurosci*. 2006;26(48):12497–12511.
73. Ryu JK, Kim J, Cho SJ, et al. Proactive transplantation of human neural stem cells prevents degeneration of striatal neurons in a rat model of Huntington disease. *Neurobiol Dis*. 2004;16(1):68–77.
74. Kobayashi T, Ahlenius H, Thored P, Kobayashi R, Kokaia Z, Lindvall O. Intracerebral infusion of glial cell line-derived neurotrophic factor promotes striatal neurogenesis after stroke in adult rats. *Stroke*. 2006;37(9):2361–2367.
75. Robin AM, Zhang ZG, Wang L, et al. Stromal cell-derived factor 1 alpha mediates neural progenitor cell motility after focal cerebral ischemia. *J Cereb Blood Flow Metab*. 2006;26(1):125–134.
76. Ericson C, Georgievska B, Lundberg C. Ex vivo gene delivery of GDNF using primary astrocytes transduced with a lentiviral vector provides neuroprotection in a rat model of Parkinson's disease. *Eur J Neurosci*. 2005;22(11):2755–2764.
77. Palma V, Lim DA, Dahmane N, et al. Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. *Development*. 2005;132(2):335–344.
78. Shyu WC, Lin SZ, Yen PS, et al. Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. *J Pharmacol Exp Ther*. 2008;324(2):834–849.
79. Rolls A, Shechter R, London A, et al. Toll-like receptors modulate adult hippocampal neurogenesis. *Nat Cell Biol*. 2007;9(9):1081–1088.
80. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131(5):861–872.
81. Park IH, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. *Cell*. 2008;134(5):877–886.
82. Wernig M, Zhao JP, Pruszak J, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci U S A*. 2008;105(15):5856–5861.
83. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318(5858):1917–1920.
84. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008;321(5893):1218–1221.
85. Ebert AD, Yu J, Rose FF Jr, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*. 2009;457(7227):277–280.
86. Okita K, Nakagawa M, Hyunjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science*. 2008;322(5903):949–953.
87. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science*. 2008;322(5903):945–949.
88. Soldner F, Hockemeyer D, Beard C, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell*. 2009;136(5):964–977.
89. Markoulaki S, Hanna J, Beard C, et al. Transgenic mice with defined combinations of drug-inducible reprogramming factors. *Nat Biotechnol*. 2009;27(2):169–171.
90. Jiang C. Stem cell research: from molecular physiology to therapeutic applications. *Sci China C Life Sci*. 2009;52(7):597–598.
91. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res*. 2000;61(4):364–370.
92. Blondheim NR, Levy YS, Ben-Zur T, et al. Human mesenchymal stem cells express neural genes, suggesting a neural predisposition. *Stem Cells Dev*. 2006;15(2):141–164.
93. Mahmood A, Lu D, Chopp M. Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. *Neurosurgery*. 2004;55(5):1185–1193.
94. Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. *Int J Biochem Cell Biol*. 2004;36(4):568–584.
95. Lee PH, Park HJ. Bone marrow-derived mesenchymal stem cell therapy as a candidate disease-modifying strategy in Parkinson's disease and multiple system atrophy. *J Clin Neurol*. 2009;5(1):1–10.
96. Karussis D, Kassis I, Kurkalli BG, Slavin S. Immunomodulation and neuroprotection with mesenchymal bone marrow stem cells (MSCs): a proposed treatment for multiple sclerosis and other neuroimmunological/neurodegenerative diseases. *J Neurol Sci*. 2008;265(1–2):131–135.
97. Li Y, Chen J, Chen XG, et al. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology*. 2002;59(4):514–523.
98. Zhang J, Li Y, Chen J, et al. Human bone marrow stromal cell treatment improves neurological functional recovery in EAE mice. *Exp Neurol*. 2005;195(1):16–26.

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