Stem Cells and Cloning: Advances and Applications

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REVIEW

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

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Abir Oueida El-Sadik

Department of Anatomy and Embryology, Scientific Research Unit, Female Health Science College, King Saud University, Riyadh, Kingdom of Saudi Arabia

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 dopamine[1] (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 striatum[8] 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

Correspondence: Abir Oueida El-Sadik
Department of Anatomy and Embryology, Scientific Research Unit,
Female Health Science College,
King Saud University,
PO Box 270645, Riyadh 11352,
Kingdom of Saudi Arabia
Email abgh50@yahoo.com

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 immunorejection[8] 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 al[16] 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 al[17] 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 cells[31] and genetic modification[32] 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 al[42] 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-hydroxydopamine (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 al[43] 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 al[12] 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 al[51] 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-glial 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 al[66] 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 al[71] demonstrated that graft-expressed GDNF, SHH, and stromal cell-derived factor 1 alpha (SDF-1α) 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-1α 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-1α 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 al[71] 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 al[82] 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 retrovirus[80] and lentivirus[83] 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 al[86] and Stadtfeld et al[87] 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 al[88] 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, unkown 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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