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A REVIEW ON MESENCHYMAL STEM CELLS BASED ANTI PARKINSONS TREATMENT

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ABSTRACT

Parkinson's disease (PD) is a degenerative neurological disorder characterized by the cardinal motor features of tremor, bradykinesia and rigidity. It is associated with the extended loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) resulting in a severe deficiency of DA in the striatum required for motor control. There is currently no cure for PD and the majority of treatments available aim to reverse dopamine deficiency and the relief of the symptoms. Based on promising findings from early trials, the transplantation of stem cells or stem cell derived progenitors has raised the possibility of using cell-based therapy to replace lost cells in the diseased brain. Embryonic stem cells (ESCs) are highly expandable and pluripotent cells that have the ability to differentiate into all cell types of the human body, including nervous system tissues, meaning that they have the potential to offer a lasting treatment for PD and other neurological diseases. However, possible issues with safety and ethics associated with the use of undifferentiated ESCs in humans have meant that alternative sources of transplantable cells has to be considered. Additionally, another approach is the stimulation of brain repair by endogenous stem cells via external manipulation. In this review, recent advances in stem cell research in PD will be discussed, giving an overview of the various strategies including the use of different stem cell populations for cell replacement and the possible modulation of endogenous stem cells that have the potential to provide effective cell-based therapy in future.

KEYWORDS: Dopaminergic neurons, neural transplantation, Parkinson's disease, stem cells.

INTRODUCTION

Parkinson's disease (PD) is characterized by motor symptoms, which include prominent akinesia, rigidity, tremor and postural instability. The degeneration of dopamine (DA) neurons in the substantia nigra pars compacta, with consequent reduction of DA in the striatum, plays a central role for these motor symptoms. In later stages of the disease some patients also develop dementia, depression, disturbed sleep and signs of autonomic nervous system impairment. These symptoms, known as 'non-dopaminergic' symptoms are caused by the degeneration of other neuronal systems, such as noradrenergic, serotonergic and cholinergic. Thus, today the neuropathology of PD is viewed as being more complex than previously thought, involving not merely the nigrostriatal DA pathway, but also several other brain systems. The DA precursor L-3,4-dihydroxyphenylalanine (L-DOPA) remains the key treatment for PD, providing excellent symptomatic relief during the first years after start of the therapy. Invariably, the vast majority of patients develop motor fluctuations, known as the 'on–off' phenomenon, after about 5 to 10 years of treatment. Thus, despite careful administration of multiple daily doses of L-DOPA the patients oscillate between a severely akinetic state and a condition when they exhibit disabling abnormal involuntary movement, called dyskinesia .Besides this, with the progression of the disease the benefits of L-DOPA treatment gradually diminish and the time spent in 'on' phase gradually declines. Recently, several DA agonists, inhibitors of DA breakdown and novel surgical approaches (e.g. deep brain stimulation) have been shown to partially ameliorate these problems. These therapies do not, however, prevent disease progression and, furthermore, existing pharmacological treatments do not really improve symptoms believed to be due to nondopaminergic pathology. There is little doubt that other therapeutic strategies are still needed for advanced PD. The shortcomings of pharmacological therapies have led to the search for alternative treatments. Open-label clinical trials applying mesencephalic DA neurons dissected from human embryos have shown that when successful cell replacement therapy is clearly beneficial. Other sources of DA neurons have also been examined in clinical transplantation trials. For example, autografts of sympathetic neurons and carotid body transplants have been tested, but a lack of positive clinical results and evidence for graft survival have been discouraging. Embryonic pig neural cells were shown to survive transplantation into a PD patient. However, the number of surviving cells was very low suggesting that graft rejection had been taking place and there was not significant improvement of function in the patients. Most probably, intracerebral xenografts require aggressive immunosuppression and, additionally, they are associated with the potential risk of animal virus transmission to

humans. Stem cells of human origin are currently considered as the most promising future source of DA neurons for a cell-based therapy for PD. There are at least two major different types of stem cells that have been studied for this purpose: multipotent region-specific stem cells, isolated from embryonic/fetal or adult brain, and pluripotent embryonic stem cells (ESCs), which constitute the inner cell mass of blastocysts. Regardless of the source of cells, the phenotype of the DA neurons used for transplantation ought to fulfill certain criteria before they should be considered relevant to a clinical therapy. Their ability to meet these criteria can be tested by grafting in animal models of PD. For example, they must be capable of synthesizing and releasing DA in a controlled fashion; they need to extend axons that re-innervate the striatum; and they should ameliorate motor symptoms. Recent studies indicate that the DA neurons also ought to express the G-protein-coupled inward rectifying K+ channel subunit (Girk2). The Girk2 protein is predominantly expressed in substantia nigra pars compacta neurons, and not in the DA neurons located in the adjacent ventral tegmental area (VTA).[19,20] A recent study has shown that DA neurons innervating the host striatum in mouse mesencephalic grafts also express Girk2 and are located around the periphery of the grafts.[20] In contrast, the DA neurons located in the core of the implants typically do not express Girk2. Instead, they contain the calcium-binding protein calbindin (normally found in most DA neurons of the VTA) and do not extend axons into the surrounding host striatum. In post-mortem analysis of the brains from two PD patients who received transplants of embryonic mesencephalic tissue it was also observed that the Girk2 positive neurons were preferentially located around the perimeter of the graft tissue. Taken together, it appears that Girk2 is a novel and useful marker to label cells that are true substantia nigra pars compacta DA neurons and those are most likely to efficiently innervate the host striatum. In this review we briefly overview the clinical trials using embryonic mesencephalic tissue, briefly describe different types of stem cells considered relevant to PD and provide a more detailed discussion on hESCs as a source of DA neurons in PD therapy. In Figure 1 are represented the alternative sources of stem cells for a cell-based therapy for Parkinson's disease discussed in this review.

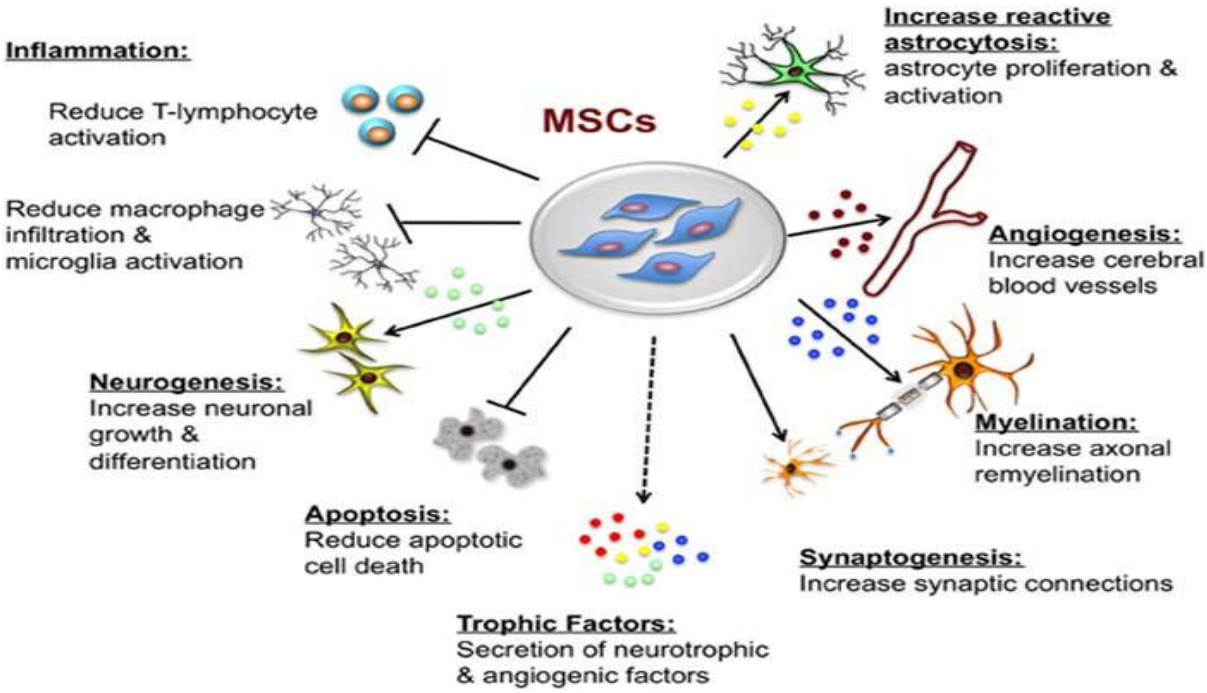
Mesenchymal Stem Cells (MSCs)

Stem cells have the capacity to proliferate and differentiate into multiple cellular lineages. There are different classifications of stem cells that reflect the range of possible cell types they can produce and the ways in which the stem cells are derived. These stem cells include mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), embryonic stem (ES)

cells, progenitor cells, and induced pluripotent stem cells (iPS). To appreciate the potential applications of stem cell technology in neurodegenerative diseases, it is important to understand the characteristics of the various available stem cell types and the potential impact of cellular therapies on disease mechanisms. Each stem cell type possesses certain qualities and advantages, and the rationale for utilizing each type depends on the desired applications and outcomes. Briefly, ES cells are undifferentiated pluripotent cells derived from the inner cell mass of blastocyst stage embryos, which introduced a series of ethical problems in clinical application. To avoid such ethical problems are create histocompatibility, new technologies have enabled tissue cells to become iPS cells. One characteristic of ES and iPS cells is their ability to form teratomas, which, in turn, is a major concern for future clinical application. MSCs are an alternative source of multipotent self-renewing cells. MSCs are derived from various adult and neonatal tissues, such as bone marrow, dental pulp, adipose tissue, amnion, placenta, umbilical cord and cord blood. There are several evidences that MSCs can transdifferentiate into epithelial, endothelial, and neural cells. Therefore, MSCs provide an accessible alternative to ES cells and potentially circumvent the need for immunosuppression in cellular therapies because they are derived from an autologous source. Unlike ES or iPS cells, MSCs have no ethical problems and have a low risk of forming teratoma, however, they are not completely free from malignancy potentials. For cell transplantation therapy, MSCs have two major beneficial effects for PD: (1) differentiation to generate a broad spectrum of cells for the replacement of lost DA neurons and (2) a trophic effect that is mediated by the various types of trophic factors. This review is focused on the potential of MSCs as a therapeutic cell source for PD. Therapeutic potential of MSCs replacement therapies for PD The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plasticadherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro. MSCs can be retrieved from various adult tissues such as and neonatal tissues, such as bone marrow, dental pulp, adipose tissue, amnion, placenta, umbilical cord, and cord blood. MSCs isolated from different tissues are quite versatile and can adopt morphological and phenotypic properties of neuronal cells under various culture conditions. MSCs are characterized by being able to differentiate along several lineages. The majority of the protocols for MSCs neuronal induction utilize different combinations of chemicals, growth factors and signal molecules. MSCs differentiate into DA

neurons can be achieved through different protocols based on chemical induction, gene transfection, co-culturing and use of conditioned mediu. For instance, a system to specifically induce DA neurons from bone marrow MSCs (BMSCs) was reported, although undifferentiated BMSCs natively co-express several neuronal and glial markers, such as βIII-tubulin and glial fibrillary acidic protein (GFAP), respectively. This system first generates postmitotic functional neuronal cells with a high efficiency without contamination by glial cells. The resulting neuronal cells are then further induced into DA neurons. The induction is achieved by lipofection method of plasmid vector containing a Notch1 intracellular domain (NICD) and G418 selection, followed by an administration of trophic factors such as basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), forskolin and glial cell-line derived neurotrophic factor (GDNF). The induced cells express the markers for DA neurons, such as tyrosine hydroxylase (TH), Nurr-1, Lmx1b, En1 and Pax3. In addition, the induced cells released DA into the culture media in response to high K+ depolarizing stimuli. These findings suggest that functional DA neurons can be efficiently induced from BMSCs

Some functions of MSCs



For cell-based therapy, MSCs have two major effects: a trophic effect that is mediated by the various types of trophic factors and cytokines produced by MSCs[13] and differentiation to generate a broad spectrum of cells for the replenishment of lost cells.[14] MSCs normally provide trophic factors to support hematopoietic stem cells in the bone marrow, thus their

trophic effect is part of their normal function. MSCs are multipotent stem cells that are known to differentiate into osteocytes, chondrocytes, and adipocytes.[11] These differentiations are within the same mesodermal lineage, but recent reports demonstrated that MSCs show unorthodox differentiation into ectodermal and endodermal cells.[15–19] These findings stimulated the advancement of regenerative medicine aimed at the generation of desired cells from MSCs. To date, various cell types, such as mesodermal lineage cells (e.g., bone, cartilage, adipocytes, skeletal muscles, and cardiomyocytes), as well as endodermal lineage cells (e.g., airway epithelial cells, hepatocytes, and insulin-producing cells) and ectodermal lineage cells (e.g., neuronal cells and epidermal cells) have been induced from MSCs in vitro by the use of cytokines, trophic factors or gene introduction.

Adult stem cells typically generate the cell types of the tissue in which they reside, and thus the range of their differentiation capabilities is considered limited. For example, hematopoietic stem cells generate blood cells, and NSCs generate neurons and glial cells MSCs differ from these typical somatic stem cells because, as stated previously, they differentiate not only into the same mesodermal-lineage cells of bone, cartilage, and adipocytes, but also into other lineages of ectodermal and endodermal cells. As MSCs can generate cells representative of all three germ layers, it has been debated whether MSCs are pluripotent cells. Recently, pluripotent stem cells named multilineage-differentiating stress enduring (Muse) cells were found among adult human mesenchymal stem cells (BMSCs and skin fibroblasts) as well as in mesenchymal tissues (bone marrow and dermis). Muse cells are capable of self-renewal and of differentiating into cells representative of all three germ layers from a single cell, which may partly explain the broad spectrum of differentiation observed in MSCs.

MSCs and Their Differentiation Ability

The possibility of MSC plasticity and “transdifferentiation” was initially described following in vivoexperiments in which transplanted donor bone marrow-derived cells differentiated into glial cells in the recipient brain. While some studies suggested that MSCs are plastic based on their expression of cell-specific markers, the functions of the transdifferentiated cells were not clearly demonstrated in other cases. Moreover, questions have been raised regarding the interpretation of “transdifferentiation” of infused cells into neuronal lineage cells because some investigators have suggested that the transdifferentiation observed was rather a result of fusion between infused bone marrow cells and the host brain

cells Despite this uncertainty, accumulating evidence supports the broad differentiation of MSCs both in vivo and in vitro. Based on the frequency and ratio of MSCs integrated and differentiated into the host tissue, fusion alone cannot explain all of the phenomena observed after MSC infusion. Furthermore, experiments using a Cre-lox system clearly demonstrated that MSCs can transdifferentiate into epithelial cells in vivo without fusion. In vitro differentiation of MSCs provides further evidence for MSC transdifferentiation because there are no preexisting differentiated cells to be fused at the beginning of induction under culture conditions BMSCs(Bone marrow Mesenchymal Stem Cells).

There have been many attempts to infuse BMSCs into a PD model aimed at ameliorating PD symptoms. As mentioned previously, BMSCs have trophic effects that are mediated by the various types of trophic factors and cytokines they produce. Therefore, naive adult BMSCs engrafted to the striatum induce partial but not drastic recovery of the dopamine pathway in a rat model of PD. Findings from a human pilot study of autologous naive BMSC transplantation performed in PD patients and followed for up to 36 months indicated a certain degree of amelioration of symptoms with no tumor formation. While BMSCs have advantages over some other stem cells regarding their safety, easy accessibility, and trophic effects, naive BMSC transplantation has limitations for definitive care because most of the transplanted cells do not survive in vivo for a long time, and thus the trophic effects gradually decrease.

In addition to naive BMSC transplantation, genetically modified BMSCs have been applied to the PD model. Cells genetically modified to produce L-DOPA or neurotrophic factors such as neurotrophins and glial cell line-derived neurotrophic factor (GDNF) are reported to be somewhat effective for the amelioration of PD symptoms.

While naive BMSC transplantation is indeed a simple and accessible method for providing trophic effects, dopamine neurons would be a rational ultimate solution to PD. Naive BMSCs, in general, do not differentiate spontaneously in vivo after transplantation. Even if they did differentiate, the ratio of differentiated cells would be extremely low. For practical use, it would be more desirable to establish a specific system for inducing BMSCs to produce dopamine neurons prior to transplantation.

There are several reports of the induction of dopamine neurons from BMSCs, but in these reports the effectiveness of the induced cells in vivo was not evaluated by transplanting them

into a PD model. Another study reported that MSCs induced into immature neurons using basic fibroblast growth factor (bFGF), epidermal growth factor, platelet-derived growth factor, sonic hedgehog, FGF-8, GDNF, or the reagents butylated hydroxyanisole and dibutyryl cAMP were transplanted into a PD model, but these immature neurons did not effectively ameliorate the PD symptoms. In this manner, growth factor-based methods allow MSC differentiation toward immature neuronal-like cells, but are not efficient in PD models. On the other hand, when MSCs were induced into fully functional dopamine neurons and then transplanted into a PD model, they were clearly effective, as described in the next section

Transplantation of BMSC-Derived Dopamine Neurons into PD Models

Induced dopamine neurons (1×10^5 cells) from either rodent or human (under the control of immunosuppressant) BMSCs were transplanted into the striatum of a PD model rat induced by 6-hydroxydopamine (6-OHDA). Unilateral administration of 6-OHDA into the medial forebrain bundle selectively destroys dopamine neurons in the substantia nigra, leading to quantifiable changes in rotational behavior and providing a useful and commonly used model of PD. Model rats receiving a transplantation demonstrated a substantial decrease in apomorphine-induced rotation behavior, and nonpharmacologic behavior tests, such as adjusting step and paw-reaching tests, also demonstrated significant improvements in both rodent and human induced cell transplantation. Grafted dopamine neurons migrated and extended beyond the injected site, and approximately 30% of the cells remained in the striatum 10 weeks after transplantation. The grafted striatum showed the migration of GFP-positive transplanted cells that expressed neurofilament, TH, and DAT. Brain slice culture experiments demonstrated the production of dopamine in the transplanted brains. No tumor formation was observed in the brain, demonstrating that dopamine neurons induced from BMSCs do not have the ability to form tumors.

In summary, introduction of NICD followed by bFGF, CNTF, forskolin, and GDNF administration can efficiently induce functional dopamine neurons that lead to functional recovery after transplantation in a rodent model of PD.

Notch signaling inhibits neuronal differentiation and promotes glial differentiation during development. Although the above discussed induction system seems inconsistent with the well-known actions of Notch signaling, it is presumed that cell susceptibility to Notch signaling in MSCs is different from that of cells in the process of normal neuronal

development. Distinct cellular responses to Notch signals; for example, the protein repertoire and active factors, might be quite different between conventional NPCs and BMSCs. In fact, neuronal basic helix-loop-helix factors (Mash1, Math1, and neurogenin1), together with the glial factors Hes1, Hes5, STAT1, and STAT3, are detected in naive BMSCs in reverse transcription-polymerase chain reaction analyses, while after NICD transfection, expression of STAT1 and STAT3 is downregulated and expression of Mash1, Math1, and neurogenin1, as well as Hes1 and Hes, is retained in the BMSCs . Although it is believed that the major intracellular effect of NICD introduction is the activation of Hes1 and Hes5, the introduction of either Hes1 or Hes to BMSCs, instead of NICD, does not induce NPC marker-positive cells. In contrast, administration of the Janus kinase (JAK)/STAT inhibitor WHI-P131, instead of NICD transfection, successfully produces NPC-like cells, which are partially induced to be MAP2-antibody-positive cells with neurite-like processes after additional trophic factor induction. These facts suggest that the downregulation of STAT expression by NICD-transfection is closely related to the transformation of MSCs to NPC-like cells and that Hes activity is not involved in this process.

OTHER KINDS OF MSCS AND PD

The umbilical cord and adipose tissues are other realistic sources of MSCs. Mesenchymal tissues of the umbilical cord, so-called Wharton's jelly, as well as fat tissues, contain an abundance of MSCs. These cells have an advantage over BMSCs in that the umbilical cord derives from postnatal tissue that is discarded after birth, and thus cell collection is not an invasive procedure for donors. Adipose tissue, which is easily obtained from liposuction, also contains large amounts of MSCs called adipose-derived stem cells (ADSCs). Because of the ability of umbilical cord mesenchymal stem cells (UC-MSCs) and ADSCs to differentiate into other cell types and to proliferate, these cells are considered to be a practical source for cell-based therapies.

In ADSCs, Tuj-1-positive cells, but not fully differentiated dopamine neurons, induced, and transplanted into a PD model, demonstrated that these neuron-like cells are effective for treating PD to a certain degree after transplantation.

As for UC-MSCs, transplantation of naive cells and cells genetically modified to produce VEGF were partly effective. The potential of UC-MSCs to differentiate into neuronal cells does not differ from that of BMSCs, and dopamine neurons can be induced from UC-MSCs

using neuron-conditioned medium, sonic hedgehog, and FGF-8. Those cells are also effective in PD models.

The pathological characteristics of PD include selective death of mesencephalic nigral DA neurons and the presence of Lewy bodies in the substantia nigra. We have reported that chronic Central Bringing Excellence in Open Access Kitamura et al. (2016) Email: Ann Neurodegener Dis 1(1): 1002 (2016) 4/8 oral administration of rotenone caused specific nigrostriatal DA neurodegeneration in C57BL/6 mice. Chronic exposure of rotenone produced some TH+ neurons, which induced a high level of cytoplasmic α-synuclein immunoreactivity in the substantia nigra. Genetic studies led to the discovery of a small percentage of familial PD cases linked directly to genetic mutations, as well as gene duplications and triplications. The first gene associated with PD was α-synuclein (PARK1). Furthermore, duplication and triplications of α-synuclein are linked to an early onset familial PD (PARK4). These genetic studies suggest that excess increase of α-synuclein protein levels may represent a gain of toxic function. In our previous study, α-synuclein+ /TH+ cells in the substantia nigra decreased on MSCs injection into the tail vein. Although the exact mechanism remain unclear, neuroprotective effects of stem cells could involve a reduction in intracellular α-synuclein. The results suggest that MSCs transplantation may be a useful therapy for patients with PD as well as for those with other α-synucleinopathies such as multiple system atrophy and dementia with Lewy bodies. Neuroinflammation has been described as an important participant in several neurodegenerative diseases including PD, Alzheimer's disease (AD), amyotrophic lateral sclerosis, and multiple system atrophy (MSA). McGeer et al. reported the presence of activated microglia and inflammatory macrophages as well as proinflammatory cytokines in SN postmorten samples from PD patients Activated microglia are also present in patients with early PD and they are correlated with the degree of DA neuronal loss. Evidence supporting the inflammatory hypothesis of neurodegeneration comes from studies showing the expression of a bunch of inflammatory markers within the brain including specific proteins, pro-inflammatory cytokines and markers of active glial cells. Degenerative DA neurons caused by LPS or MPTP can be prevented by treatment of anti-inflammatry drugs sush as aspirin, dexamethasone, and the selective COX-2 inhibitor rofecoxib. Several lines of evidence also indicated that anti-inflammatory responses by other clinical medicines such as simvastation, minocyclin, and memantine induced to reduce the inflammatory process and neuronal death by LPS. Therefore, the involvement of inflammation and oxidative stress in PD pathophysiology suggests that anti-inflammatory and

anti-oxidative stress effects of MSCs partially underlie their benefical effects. MSCs migrate to sites of inflammation and injured tissue. At these locations, MSCs repair the damaged region under conditions of inflammation and oxidative stress, by paracrine mechanisms where they stimulate endogenous stem cells and/or modulate the functions of immune cells, such as monocytes, macrophages, dendritic cells (DCs), and T and B cells as well as natural killer cells (NK). Although the exact mechanism of MSCs-mediated immunoregulation is not understood, the anti-inflammatory role of MSCs has been demonstrated in vitro and in vivo PD models. Along with differentiational potency and trophic effects, the anti-inflammatory properties of MSCs could have therapeutic implications in the treatment of PD.

CONCLUSION

MSCs have several disadvantages relative to ES cells and iPS cells, such as insufficient numbers of stem cells, reduced proliferation and differentiation capacity with age in vitro and after stem cell transplantation in vivo. However, MSCs can be obtained from patients with PD (for autologous transplantation) as well as from healthy donors (for allogeneic transplantation). MSCs are not burdened with the ethical issues associated with ES cells. Due to the focused loss of DA neurons, PD is particularly suitable for cell transplantation therapy. MSCs can be retrieved from various adult tissues. Functional DA neurons can be efficiently induced from MSCs. Several studies, including our studies, have shown that MSCs can protect and/ or stimulate regeneration in host-damaged DA neurons mainly through secretion of trophic factors and cytokines from MSCs. These results demonstrate the potential of MSCs derived from an autologous source for clinical applications for PD, although further studies are required.

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