Development of stem cell-based therapies for Parkinson’s disease

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I. Abstract

Parkinson’s disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) in the brain with an unknown cause. Current pharmacological treatments for PD are only symptomatic and there is still no cure for this disease nowadays. In fact, transplantation of human fetal ventral midbrain cells into PD brains have provided a proof of concept that cell replacement therapy can be used for some PD patients, beneficial for improving their symptoms. However, the ethical and practical issues of human fetal tissue will inevitably limit its widespread clinical use. Therefore, it is essential to find alternative cell sources for the future cell transplantation for PD patients. With recent development in stem cell technology, here we review the different types of stem cells and their main properties currently explored, which could be developed as a possible cell therapy for PD treatment.

II. Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disorder, which affects about 1% of the population over the age of 60 [1]. One of the central pathological features of PD is the progressive loss of nigrostriatal dopamine (DA), which is accompanied by the presence of α-synuclein containing cytoplasmic inclusions known as Lewy bodies. The loss of DA neurons results in the characteristic motor symptoms of PD such as muscle rigidity, tremors and bradykinesia along with some of the cognitive deficits. Although the familial forms of the disorder are associated with some genetic defects, the aetiology of PD remains unknown in the vast majority of sporadic cases.

To date, there is no cure for this debilitating, neurological condition. Currently, the main clinical treatment for PD is dopamine replacement therapy using L-dihydroxyphenylalanine (L-DOPA) and/or dopamine receptor agonists. Pharmacotherapy can improve parkinsonian symptoms during the initial stage of PD, but the efficacy of pharmacotherapy is gradually lost and long-term treatment with L-DOPA consequently produces grave side effects, such as on–off fluctuations and drug-induced dyskinesias [2,3]. In addition, pharmacotherapy cannot delay the progression of the loss of DA neurons, and also cannot recover the lost DA neurons. As a result, there is a need for the development of regenerative therapies for PD.

Parkinson’s disease (PD) is one of the candidate diseases suitable for cell transplantation therapy since successful clinical experiments have accumulated using human fetal tissue grafting for PD patients.

In pioneering work about three decades ago, it was shown that DA neuroblasts from the fetal ventral mesencephalon (VM) could survive long term in the adult brain and re-innervate the striatum of 6-hydroxydopamine (6-OHDA)-induced parkinsonian animal models, with a consequent improvement in most but not all of their behavioral responses [4–6]. Later the first clinical trial of cell transplantation therapy for PD patients was reported in 1987 using aborted human fetal VM tissue [7].

So far, several hundreds of PD patients have been treated as part of this type clinical trial. Some grafted PD patients have exhibited drastic improvements in their symptoms, but only
modest changes in others. These initial studies led on to bigger studies which then reported side effects in some patients in receipt of such grafts in the form of involuntary graft induced movements [8].

In addition, not enough fetal tissue is available to treat the large numbers of people with Parkinson’s, and the use of fetuses also raises ethical concerns, which along with logistic and technical issues will significantly restrict extensive use of fetal tissue for the treatment of Parkinson’s disease. Therefore, it is necessary to develop efficient methods to generate midbrain DA neurons from other possible sources such as stem cells instead of human VM tissue for the treatment of Parkinson’s patients.

In this review, we highlight the recent advances in different DA induction protocols and characterization of various types of stem cells such as neural stem cells (NSCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), and will also discuss their prospects to replace lost midbrain DA neurons for the cell transplantation therapy for PD (Figure 1).

![Schematic diagram of developing stem cells-derived Dopamine (DA) neurons for the treatment of Parkinson’s disease (PD). ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; NSCs: neural stem/progenitor cells; MSCs: mesenchymal stem cells; DPSCs: dental pulp stem cells.](image)

### III. Stem cell sources for PD therapy

In order to move to clinical applications, a readily available and renewable source of cells with the potential to differentiate into fully functional DA neurons after transplantation is essential. If DA neurons can be generated from non-dopaminergic cell sources in vitro through the differentiation of stem cells, and subsequently transplanted into the brain with the
primary aim of providing appropriate, regulated levels of DA release in the striatum, then there is promise of constricting the disease process and recovering motor function in PD patients. Research has demonstrated that transplanted DA neurons generated from stem cells are capable of interconnecting with the host and these transplanted neurons can form neuronal circuitry, enabling directed DA release and the restoration of normal movements.

1. Neural stem cells (NSCs)

Neural stem cells (NSCs) or progenitor cells (NPCs) can be derived from embryonic stem cell sources, and they have also been shown to exist in the adult brain [9]. Transplantation of NSCs in the brain attenuates physical or functional deficits associated with injury or disease in the CNS via cell replacement, the release of specific neurotransmitters, and the production of neurotrophic factors that protect injured neurons and promote neuronal growth. Adult neural stem cells are mainly in the hippocampus and subventricular zone (SVZ) near the lateral. Madhavan and colleagues reported stimulation of endogenous cells after transplantation and hypothesized that there exists a synergism between the actions of endogenous and grafted NPCs after transplantation that would lead to neuroprotection in a 6-OHDA rat model of PD [10]. However, controversy regarding neurogenesis in the SVZ in PD models persists [11].

Neural stem cells (NSCs) obtained from human fetal tissue were the pioneered cell type studied for PD therapy following the isolation and propagation of NSCs [12]. Since then, researchers have employed varying methods to generate DA neurons from NSCs, including treating the cells with cytokines and neurotrophic factors such as GDNF in vitro [13]. It has also been demonstrated the transplanted NPCs derived DA cells illustrated high survival rates which improved the symptoms of PD [14], and human fetal neural stem cells survive long term in the midbrain of dopamine-depleted monkeys after GDNF overexpression and project neurites toward an appropriate target [15]. Consistently, intrastriatal GDNF gene transfer by inducible lentivirus vectors can protect dopaminergic neurons in a rat model of parkinsonism and intrastriatal transplantation of adult human neural crest-derived stem cells improves functional recovery in parkinsonian rats [16, 17].

The important role of Bcl-xL has been revealed in the generation of DA neurons from NSCs. Transcription factors associated with the production of DA neurons such as Nurr1, Pitx3, Lmx1b, and DAT are induced by Bcl-xL establishing a high yield of DA neurons characteristic of the lost cells in the SNpc [18]. We have demonstrated when Pitx3 was overexpressed in NSCs, there was an increase in the differential potential of the generated DA neurons and a substantial improvement in motor function was observed following the transplantation of the grafts containing VM and NSCs overexpressed with Pitx3 in a 6-OHDA animal model [19]. Whereas co-expression of Nurr1 and Brn4 promoted NSCs to differentiate into dopaminergic neurons in vivo, increased the level of DA neurotransmitter in the striatum, resulting in behavioral improvement of PD rats. Brn4 and tyrosine hydroxylase (TH) synergistically promote the differentiation of neural stem cells into dopaminergic neurons and increase cell survival and DA release of dopaminergic neurons [20,21]. Recently, it has been found that induced neural stem cells (iNSCs) that over-expressed Lmx1a (iNSC-Lmx1a) gave rise to an increased yield of dopaminergic neurons and secreted a higher level of dopamine in vitro and mice that received iNSC-Lmx1a vs. iNSC-GFP exhibited better recovery [22].

However, major hurdles in relation to the use of NSCs for the generation of DA neurons need to be overcome before they can be clinically applied for the treatment of PD. It is essential that transplants of NSC/progenitor cells justify their capability to improve the refined motor problems of PD, and long-term culturing of NSC/progenitors and their stable differentiation potential can be guaranteed [13].
Therefore, it has been proposed that the governed differentiation of induced pluripotent stem cells (iPSSs) or ESC-human derived cells may be better employed for the future generation of DA neuronal cell types, although one advantage of NSCs is that they have yet to be shown to form the same type of tumours after transplantation.

2. Embryonic stem cells (ESCs)

Embryonic stem cells (ESCs) have the ability to develop into any cell type of the body. Research has revealed that ESCs may generate DA neurons at a specific developmental stage; that is prior to or during the cells’ fate to a neural phenotype, therefore influencing the application of sonic hedgehog (Shh) and fibroblast growth factor 8 (FGF8) at the height of the production of neural Nestin+ and Sox1+ progenitors. At the ESC differentiation stage, factors such as Shh and FGF8 can be induced to provide a ~30% TH+ neuronal population observed in culture [23]. Another study has shown that differentiation of human ES cells into VM dopaminergic neurons requires a high activity form of Shh, FGF8a and specific regionalization by retinoic acid (RA) [24].

In addition, when NPCs derived from ESCs are exposed in vitro to both FGF8 and Shh, they are capable of differentiating into Lmx1a-DA neurons [25]. Another study illustrated the usefulness of FGF20 and FGF2 involved in the differentiation of DA neurons from human ESCs. These findings will aid the efficient generation of DA neurons from ESC required for cell replacement therapies for PD [26]. Hong and associates have established that overexpression of each factors, Nurr1, Pitx3, and Lmx1a robustly promoted the dopaminergic differentiation of ESC-NP cells exposed to SHH and FGF8 and demonstrated that key midbrain DA (mDA) regulators (Nurr1, Pitx3, and Lmx1a) play overlapping as well as distinct roles during neurogenesis and neurotransmitter phenotype determination of mDA neurons. This further proved the link of generation of DA neurons from ESCs with the overexpression of genes and transcription factors, such as Nurr1, Pitx3 and Lmx1a [27,28]. Human ESCs also display electrophysiological features of DA neurons and most importantly are capable of DA release in vitro whilst demonstrating gene expression. Several studies have illustrated methods to enhance the generation of DA neurons from human ESCs [28,29]. These findings highlight the necessity for the identification of differentiation and survival mechanisms and the eradication of factors responsible for excessive proliferation, required for protocols for cell replacement therapies today. Scientists have also illustrated that a high percentage of ESCs do not undergo differentiation into the desired cell type when those differentiation protocols are applied [8,29].

Using protocols entirely based on extrinsic patterning cues that mimic fetal midbrain development, it is now possible to generate DA neurons with a midbrain phenotype from human embryonic stem cells that survive transplantation and that can restore motor deficits in animal models of PD [30-33]. More remarkably, Grealish and colleagues have shown long-term survival and functionality using clinically relevant MRI and PET imaging techniques and demonstrate efficacy in restoration of motor function with a potency comparable to that seen with human fetal dopamine neurons. Furthermore, hESC-derived dopamine neurons can project sufficiently long distances for use in humans, fully regenerate midbrain-to-forebrain projections, and innervate correct target structures. This provides strong preclinical support for clinical translation of hESC-derived dopamine neurons using approaches similar to those established with fetal cells for the treatment of Parkinson’s disease [34].

Among the different stem cell sources available, human ES cells have advanced a great deal. However, a number of crucial issues still need to be addressed in preclinical studies before these cells can be considered for clinical use: it is important to verify that their functional efficacy is robust and stable over significantly long time periods; The incomplete differentiation of all ESCs is a major challenge for the development of an effective PD therapy and the risk associated with tumourigenesis may hinder the applications of ESCs [8].
3. Induced pluripotent stem cells (iPSCs)
Induced pluripotent stem cells (iPSCs) are an advantageous cell replacement source for PD as these autologous stem cells can be derived from the patient eliminating the risk of immune rejection and the requirement of immunosuppressive therapy. Unlike human ESCs, iPS cells are not hindered by ethical issues [35].

Research studies carried out using animal models displayed promising results for iPS cells in the generation of DA neurons. An experimental study was carried out using mouse fibroblasts to produce iPS cells. It was observed that the generated iPS cells could be differentiated into DA neurons of the midbrain which subsequently diminished motor asymmetry in 6-OHDA rodent models induced with lesions [36]. Shh, FGF8, FGF2 and retinoic acid (RA) together successfully pre-differentiated iPS cells into functional midbrain DA neurons. This led to the integration of the DA neurons into the host striatum of rodent models induced with PD, resulting in an enhancement of behavior [24,37]. However, like ESC grafts, the development of neural outgrowths was observed as a result of the transplanted cells [38].

It has also revealed that DA neurons can be generated from human iPS cells, as well as from mouse iPS cells. Human fibroblasts have been used to generate iPS cells which subsequently resulted in the generation of TH+ neurons [39]. The iPS cell–derived NSCs were also found to survive and integrate into the brain and differentiated into neurons, including DA neurons in vivo and improved functional recovery of PD rats up to 16 weeks after transplantation [40].

Work by Hallett and colleagues showed pre-clinical test of transplantation of autologous iPSC-derived dopamine neurons in a cynomolgus monkey model of Parkinson’s disease provides proof of principle for long-term innervation and functional benefit without a requirement for immunosuppression [41].

It has also been shown that human iPSC-derived DA progenitor cells can be efficiently isolated by cell sorting using a floor plate marker, CORIN. After induction, the sorted CORIN(+) cells expressed the midbrain DA progenitor markers, FOXA2 and LMX1A. When transplanted into 6-OHDA-lesioned rats, the CORIN(+) cells survived and differentiated into midbrain DA neurons in vivo, resulting in significant improvement of the motor behavior, without tumor formation [42].

As we know, a frequent cause of familial PD arises from mutations in LRRK2. Dopamine neurons derived from mutated LRRK2 iPSCs showed increased expression of α-synuclein, suggesting a connection between these two risk genes in a pathogenic pathway, as had been previously hypothesized [43]. A subsequent study also indicated phenotypic differences between LRRK2 mutant cells and control cells [44]. PD iPSCs from Parkin patients also showed evidence of increased oxidative stress and enhanced activity of the related NRF2 pathway [45].

In the meantime, PD iPSCs differentiated into dopamine neurons could be transplanted into the adult rodent striatum, and developed axons projecting into the striatum. 6-OHDA-lesioned rats transplanted with the neurons showed reduced amphetamine-induced rotational asymmetry [46]. Similarly, transplantation of human protein-based iPSCs into rats with striatal lesions could rescue motor deficits [47].

In this context, the ability to reprogram somatic cells to pluripotency represents a promising new tool in disease modelling and drug discovery for neurodegenerative diseases, such as PD as iPSCs contain the entire genome of the patient and thus have potential utility in studying early disease initiating events and developmental progression of the disease, as well as the disease pathology [48].

However, a number of issues need to be addressed as this cell type derived from PD sufferers are associated with mutations, polymorphisms or epigenetic marks, making them exposed to...
the occurrence of PD-like characteristics [38]. Additionally some reprogramming factors and/or viral vectors integrated into the genome increases the risk of mutations and tumourigenesis [49].

In brief, human induced pluripotent stem cells (iPSCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy for Parkinson's disease. One of the studies has highlighted their ability to generate 20-40% of neurons derived from iPSCs into a DA phenotype [13]. Nevertheless, iPSC-derived donor cells may contain tumorigenic or inappropriate cells.

4. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells (MSCs) possess several attractive properties for use as a novel therapeutic for neurodegenerative disorders, including PD as they can be easily extracted, cultured and expanded. Many studies in PD animal models have verified that bone marrow-derived MSCs (BMSCs) have the capacity to protect and regenerate damaged DA neurons. [50]. It was first demonstrated that behavioral recovery after BMSCs transplantation in MPTP-induced mouse model of PD [51]. Later, increased viability and migration of transplanted BMSCs were observed after 6-OHDA-induced loss of DA neurons [52]. In addition, BMSCs grafted into the striatum [53] intranasally [54] or intravenously [55] delivered BMSCs were also evident to exert neuroprotective effects against nigrostriatal degeneration and to improve motor function in 6-OHDA lesioned rats. Human BMSCs also have a protective effect on the progressive loss of DA neurons in vitro and in vivo in rat [56]. When neuralized BMSCs were transplanted in a 6-OHDA mouse model of PD, most of the transplanted cells survived in striatum, expressed TH and behavioral recovery was observed [57]. In addition, micrografted bone marrow derived neuroprogenitor-like cells were shown to induce rejuvenation of host DA neurons in 6-OHDA partially lesioned rat brain [58]. MSCs isolated from other sources, such as adipose tissue and umbilical cord, have also shown beneficial effects in PD models as well. Furthermore, the naive and neurally-induced adipose derived MSCs have neuroprotective effects against 6-OHDA-induced DA neuron death through secretion of neurotrophic factors [59]. Interestingly, MSCs isolated from umbilical cord exhibit neuroprotective and neuroregenerative effects in 6-OHDA [60,61] and in vitro-generated first trimester placental MSCs-derived neural progenitors are capable of terminal differentiation in vivo and can attenuate motor defects associated with PD [62]. MSCs are unique compared to other stem cells in that they could theoretically be utilized for personalized medicine in which MSCs for brain engraftment would be collected from the individual to receive grafted cells in order to avoid immune responses and graft rejection [50].

5. Other stem cells

The Oral and/or dental stem cells, including dental pulp stem cells (DPSCs) and oral mucosa stem cells, have recently been explored for the use beyond dentistry as they have attractive virtues of easy access and autografting possibility. Some of these oral/dental stem cells have shown the abilities of neural differentiation and neuroprotection, hence have great potential for treating neurodegenerative diseases [63]. For example, Nesti and colleagues have found the co-culture with DPSCs significantly attenuated MPP+ or rotenone-induced toxicity in primary cultures of mesencephalic neurons, presumably due to soluble factors such as BDNF and NGF released by DPSCs [64]. Later, it has been demonstrated that some soluble factors involved in the development of DA neurons induced a DA phenotype in human oral mucosa stem cells (hOMSCs) in vitro that significantly improved the motor function of hemiparkinsonian rats. Immunofluorescence analysis of specific DA marker of naive and differentiated hOMSCs showed that 35% and 82% were positive for TH respectively [65].
More recently, a study showed engrafted DAergic neuron-like SHEDs (stem cells derived from human exfoliated deciduous teeth) survived in the striatum of Parkinsonian rats, improved the DA level more efficiently than engrafted undifferentiated SHEDs, and also promoted the recovery from neurological deficits, which suggests that stem cells derived from dental pulp have therapeutic benefits for PD [66].

Studies have also illustrated that the bone marrow, skin, muscles and adipose tissue can generate cells staining positive for neuronal markers. However, this group of cells is not an advantageous source for the generation of DA neurons for PD [67]. More research is needed for the enhancement of adult stem cell isolation, culture procedures and differentiation protocols if there is any hope for them to be used for PD treatment.

IV. Perspectives

As discussed above many types of stem cells, such as ESCs, iPSCs and NSCs, can be induced into dopaminergic or dopaminergic-like neurons for the treatment of Parkinsonian models. Understandably each type of stem cells has pros and cons in terms of their efficacy, safety and availability. At this point, it looks still an open race which cell type will be the best solution for the future cell replacement treatment of PD although some people may believe that ESCs and iPSCs are the front runners at the moment.

So far human fetal VM cells are the only one clinically proved functional for some Parkinson’s patients, thus can serve as a gold standard for stem cell-derived DA transplantation for the moment at least.

Besides the important focus on cell sources and differentiation protocols, the optimal transplantation strategies should also need to be further studied before any clinical applications of these stem cells.

One of the issues is how to find a better and more reliable delivery system for stem cell transplantation in future. With the advances in biomaterials and nanomaterials, ideal scaffolds could be developed soon for clinical stem cell replacement, particularly when it is necessary to combine with growth factors and/or gene therapy. These materials should be biocompatible as well as biodegradable so that they can accommodate implanted cells with physical support/protection in a 3D environment, and also help the cells access to nutrients and integrate into neural network in vivo.

Another concern is the identity and/or characteristics of the stem cells and the derived progeny, i.e. do they proliferate in a controlled manner and not differentiate into non-dopaminergic or even tumor cells?

Other issues such as transplantation locations and timing also need to be further explored before clinical use of stem cells-derived dopaminergic neurons for the transplantation into Parkinson’s patients.

All in all, although cell replacement therapy for PD is in sight, maybe we still need to wait until the time is right [8].

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VI. References


