Stem cells in Parkinson's disease: an update

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Stem cells in Parkinson's disease: an update

Seema Gollamudi

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world after Alzheimer's disease (AD). About one percent of people over 60 years of age suffer from PD. Although most cases of PD are sporadic, about 5–10 percent of patients have familial PD (fPD) following an autosomal-recessive or -dominant inheritance pattern[1]. These two inherited forms appear to have different pathologies. Patients with autosomal-dominant fPD typically show extensive formation of Lewy bodies in different parts of the brain unlike patients with autosomal-recessive fPD [1]. The symptoms of PD arise due to loss of dopamine (DA) producing neurons in the substantia nigra (SN). Although ten different subtypes of DA-ergic neurons have been identified in the whole brain, only three of them (A8, A9, and A10) reside in the midbrain [2]. The loss of the pigmented A9 DA-ergic neurons of the ventrolateral pars compacta which control movement give rise to the symptoms of PD [3].

The most common symptoms of PD include bradykinesia, rigidity, tremor and postural instability [4]. At advanced stages of the disease, cognitive defects and behavioral problems may arise including dementia. The neurochemical changes are the loss of DA in the striatum due to loss of nigrostriatal axon terminals. The current treatments include a combination of carbidopa/levodopa, use of dopamine agonists, surgical lesion and deep brain stimulation. The prolonged use of levodopa increases the risk of developing dyskinesias. Some of the other treatments of PD being developed in clinical trials are use of plant derived substance PYM50028 cogane to promote expression of endogenous neural growth factors, gene therapy by viral vector mediated delivery of enzymatic machinery for DA synthesis to striatum, continuous drug delivery and greater physiological stimulation of DA receptors for better delivery systems for levodopa and the adenoassociated virus (AAV) delivery of neuturin/trophic factors [5]. Current pharmacological drugs only provide symptomatic relief but do not retard the disease

progression.

The clinical symptoms of PD do not appear until 60-80% of the neurons have degenerated. Attempts to replace the lost DA neurons in SN using fetal ventral mesencephalic grafts have proved efficacious in some PD trials [6-8]. There is evidence that placement of graft tissue in the striatum is beneficial while grafting such cells to the SN was of no benefit[9]. It has been shown that α-synuclein (SNCA) pathology can even be found in the graft, although this does not negate the motor benefits that they offer some individuals with PD over many years [10, 11]. There are several scientific and ethical considerations to be considered by the PD patients before their participation in any stem cell related therapy [12]. The first cell therapy studies in animal models of PD were performed in 1970s in rats using fetal rat DA containing neurons as donors with the aim of restoring striatal DA levels [13, 14]. After the initial transplantation of mesencephalic tissue from rat embryo to rats, further steps included transplantation of mesencephalic DA-ergic neurons, taken from mouse embryos or human fetuses, into DA-ergically denervated striatum of recipient rats or MPTP-monkeys[15]. The transplants survive, reinnervate the striatum, and generate adequate symptomatic relief lasting as long as sixteen years following transplantation in some patients [16-19].

Stem cells have the benefit of being able to grow indefinitely and providing an unending supply of cells which can be differentiated into numerous cell types for the study of disease pathophysiology and mechanism, genetic correction and transplantation into humans and animal models. Somatic cell reprogramming to produce neurons for neurological disorders has gained significant attention due to its tremendous potential [20]. Transplantation of DA producing neurons into the striatum of PD patients can provide symptomatic relief given that the striatum is sufficiently re-innervated. Various cell sources have been tested in cell replacement studies in PD, including fetal ventral midbrain tissue, embryonic stem (ES) cells, fetal and adult neural stem (NS) cells and, induced pluripotent stem (iPS) cells [21]. Fibroblasts or NS cells from patients can be efficiently reprogrammed into iPS and subsequently differentiated into DA neurons [22]. Mouse and human fibroblasts can be not only be directly reprogrammed into DA-ergic neurons using appropriate neural factors [23], but NS cells or fibroblasts can also be converted into iPS cells in culture via viral transduction of four transcription factors: Oct4, Sox2, Klf4, and c-Myc [24-27]. These iPS cells make it possible to bypass human ES cells, to treat patients with their own somatic cell-derived stem cells, and thereby avoiding immune rejection caused by patient-donor cell incompatibility [28]. In a study comparing iPS cells with human ES cells it was proved that the two had similar genomic stability, transcription profiles, pluripotency, and DA-ergic neuron differentiation capacity [29].

Several groups have succeeded in generating DA-ergic neurons from iPS cells [28-31]. In PD models, iPS cell derived DA-ergic neurons were shown to integrate into the striatum with behavioral improvements comparable to those observed using ES cell-derived DA neurons in both rat [28-30] and mice [32]. The advantages of stem cell technology outweigh the disadvantages. Consequently, cell replacement by using human fetal mesencephalic tissue[33, 34] or ES/iPS cell derived DA-ergic neurons [35-37] remains an important therapeutic strategy; however, several practical limitations exist, such as shortage of cell sources, variations in outcomes, adverse effects, and socio-ethical issues [16]. Therefore, stem cell technologies have recently arisen as highly promising tools that can provide an abundant source of cells that can be used for experimental transplantation studies in PD. We provide here an update on the current status in the stem cell research for PD disease modelling and therapeutics.

Criteria of stem cell selection

A stringent panel of assays can determine that putative human ES*i*PS cells are pluripotent [38]. These assays include teratoma formation, embryoid body formation, changes in DNA methylation patterns, and expression of pluripotent markers. A subset of phenotypic markers specific to the development of midbrain DA neurons includes the transcription factors pitx3, lmx1a, ngn2, msx1, girk2, and nurr1 [39]. These markers can be used to track cellular development and isolate specific populations as multipotent progenitor cells mature into postmitotic A9 subtype-specific DA-ergic neurons that express DA transporter and vesicular monoamine transporter as well as the enzymes aromatic amino acid decarboxylase and tyrosine hydroxylase (TH) [39].

Some of the other criteria for assuring pluripotent stem cell quality for cell therapy are normal chromosomal structure, minimal *de novo* mutations associated with neural cell transformation or DA-ergic neuron function and a high yield of A9 DA-ergic neurons [40]. In addition for successful stem cell based therapy in PD, the grafts should (a) exhibit a regulated release of DA and molecular, electrophysiological, and morphological properties similar to those of SN neurons [41, 42]; (b) enable survival of more than 100,000 DA neurons per human putamen [43]; (c) reestablish the DA network within the striatum and restore the functional connectivity with host extra-striatal neural circuitries[44]; (d) reverse the motor deficits resembling human symptoms in animal models of PD and induce long lasting and major symptomatic relief in PD patients; and (e) produce no adverse-effects such as tumor formation, immune reactions and graft-induced dyskinesias (GIDs).

Studies of fetal midbrain graft have suggested that better outcomes could be obtained if the graft consisted of well-differentiated A9 DA-ergic neurons [42, 45, 46], the most severely damaged neuronal type in PD [47]. The A9 DA-ergic neurons are a determinant factor for achieving synaptic formation with host tissues and better behavioral recovery [46]. Both ES cells and iPS cells, can provide an enriched population of therapeutically relevant A9 DA-ergic neurons needed for treating PD patients [28, 31, 48-53]. Non-A9 DA-ergic neurons survived transplantation but provided modest behavioral improvements when grafted in animal models of PD [54-56]. Standardized differentiation protocols yield consistent numbers of DA-ergic neurons across high-quality human iPS cell lines [25, 30]. Recent differentiation protocols use developmental patterning via a midbrain regionalized floor plate neural progenitor cell stage to differentiate authentic A9 and A10 DA-ergic neurons [52, 57]. Both studies report efficient DA release *in vitro* and *in vivo* after transplantation by a large fraction of human A9 DA-ergic neurons and exceptional functional integration leading to improved motor function without uncontrolled cell proliferation after grafting into animal models of PD [52, 57].

Stem cell based disease modelling of PD

The purpose of modelling PD using stem cells enables us to get a mechanistic insight into the disease mechanism due to the different PD gene mutations, disease development and progression and gene function. Differentiation and maturation of the ES cells and iPS cells enable the study of disease phenotypes during development and aging for PD modelling [58]. Several iPS cell lines have been generated from fibroblasts of patients carrying several known PD-related mutations, namely SNCA [59, 60], PTEN induced putative kinase 1 (PINK1) [61], parkinson protein 2, E3 ubiquitin protein ligase (parkin) (PARK2) [62] and leucine-rich repeat kinase 2 (LRRK2) [63, 64].

Neurons induced from iPS cell lines from a SNCA triplication PD patient had higher levels of SNCA and lower levels of ß-synuclein (SNCB) and γ-synuclein (SNCG) as compared to neurons differentiated from healthy control iPS cells [59]. In order to accurately model PD, a panel of isogenic disease and control cell lines from human ES cells and human iPS cells were developed by genetically modifying single base pairs in the SNCA gene by combining zinc-finger nuclease (ZFN)-mediated genome editing and iPS cell technology [26]. Patient derived human iPS cells carrying the A53T (G209) SNCA mutation followed by the correction of this mutation or, alternatively, by generating either the A53T (G209A) or E46K (G188A) mutation in the genome of wild-type human ES cells comprised the panel of cell lines [26].

Patient-derived iPS cell lines carrying G2019S mutation in LRRK2 were generated by independent groups. One mutant cell line, had dysregulated expression of several genes, which were under control of extracellular-signal-regulated kinase 1/2 (ERK). When the mutation was genetically corrected, the mutant phenotype was rescued in differentiated neurons [65]. In the iPS cells carrying the G2019S mutation, there was an increase in ERK phosphorylation and the multiple PD-associated phenotypes were ameliorated by inhibition of ERK. Neurons differentiated from iPS cell line carrying a G2019S mutation in LRRK2 gene were also found to be more susceptible to oxidative stress [63]. The neurons had increased levels of SNCA and oxidative stress response proteins MAO-B and HSPB1 and were more sensitive to caspase-3 activation caused by exposure to hydrogen peroxide, MG-132 and 6-OHDA [63].

In mutant NS cells derived from PD LRRK2 (G2019S) patients derivediPS cells, an increased susceptibility to proteasomal stress as well as passage-dependent deficiencies in clonal expansion and neuronal differentiation were observed [66]. Progressive deterioration of nuclear architecture in mutant iPS cells (NSCs-LRRK2 G2019S) but not in wild-type NSCs (NSCs-wt) was observed, which compromised clonal expansion, impaired neural differentiation and increased susceptibility to proteasomal stress. Disease phenotypes could be rescued by targeted correction of the LRRK2 (G2019S) mutation with its wild-type counterpart in PD-iPS cells and recapitulated upon targeted knock-in of LRRK2 (G2019S) in human hES cells. Knock-in ES cells highlighted a role for LRRK2 in the nuclear architecture and as a potential novel organelle affected in PD [66].

In one study, DA-ergic neurons were generated from 7 idiopathic PD patients, 4 familial PD patients carrying the G2019S mutation in the LRRK2 gene, and 4 healthy controls [64]. All had a similar ability to give rise to DA-ergic neurons. When cultured for over 2.5 months to mimic aging *in vitro*, only the DA-ergic neurons differentiated from sporadic PD or G2019S mutant LRRK2 PD developed fewer and shorter neurites and a significant increase in apoptotic cells which are signs of neurodegeneration. Further evidence pointed to a compromised authophagy in the DA-ergic neurons derived from PD patients [64]. In a different study, LRRK2 mutant iPS neurons derived from familial PD patients have been associated with increased sensitivity to oxidative stressors, such as 6-hydroxydopamine or 1-methyl-4-phenylpyridinium (MPP+), hydrogen peroxide or rotenone [63, 65].

Increased sensitivity to oxidative toxins have also been reported withiPS cell derived neurons that harbor PD associated homozygous recessive mutations in PINK1 [58], or a familial inherited triplication of the SNCA gene [65]. Studies in human iPS cell derived neuronal models of PD have revealed mechanistic details about PD etiology, such as mitochondrial alterations, and how these may lead to pathological features of the disease [61, 62]. iPS cell derived neurons with mutations in PINK1 have been reported to display mitochondrial function abnormalities, defective mitochondrial quality control, and altered recruitment to mitochondria of

exogenously transduced PARKIN [67]. PARKIN-deficient iPS cell derived neurons from familial PD patients did not appear to show frank mitochondrial defects, suggesting potential redundancy [62], although in another study, PARK2 iPS cell derived neurons showed increased oxidative stress and enhanced activity of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway [68]. iPS cell derived neurons, but not fibroblasts oriPS cells, exhibited abnormal mitochondrial morphology and impaired mitochondrial homeostasis [68].

Areas of interest would be to explore why the DAergic neurons are prone to a higher level of intrinsic oxidative stress, which predisposes the cells to damage in the context of PD familial genetic mutations and to explore how the environmental toxins predispose these cells to degenerate and develop strategies to prevent it.

Stem cell based therapeutics for PD

Neural stem cells have been reported to have a number of properties that might make them useful for brain repair [69]. However, it has proven difficult to differentiate these cells into legitimate A9-subtype midbrain DA-ergic neurons i*n vitro*, and speculation still continues as to their DA-ergic capacity *in vivo* post transplantation, thus limiting their preclinical and clinical application [70]. Numerous cell types have been reprogrammed into DA-ergic neurons for transplantation studies. One of the earliest studies involved transplantation of undifferentiated ES cells which were able to differentiate into DA-ergic neurons with concomitant clinical improvements in parkinsonian rats [48]. Most transplantation studies using mouse ES cells showed significant improvement of parkinsonian rats in the rotational test [71, 72], whereas grafts with human ES cells showed only partial [55, 56] or even no [73] improvement. Rodent and human ES cells derived DA-ergic neurons have been shown to survive transplantation into the striatum of PD rats and generate some degree of functional recovery, however the survival of ES cell derived DA-ergic neurons post-transplantation is relatively low [35, 51, 55, 56, 73]. A major concern with using ES cell-derived DA-ergic neurons for transplantation in PD patients is the risk of adverse effects such as tumor formation which have been reported in rats [55, 73].

In another study iPS cell derived neural cells were derived from 2 asymptomatic individuals and 3 familial PD patients carrying the recessive homozygous Q456X mutation in PINK1, the dominant homozygous G2019S mutation in LRRK2 and the heterozygous R1441C substitution in LRRK2 and 2 healthy subjects not carrying these mutations [74]. A gradual increase in sensitivity to cellular stress was seen as the cell type analyzed became functionally closer to the vulnerable cell types in the PD patient's brain. Cellular vulnerability associated with mitochondrial function in iPS cell derived neural cells from PD patients and at-risk individuals could be rescued with coenzyme Q10, rapamycin or the LRRK2 kinase inhibitor GW5074 [74].

Human retinal pigment epithelial (hRPE) cells have the characteristics of neural progenitor cells and can be induced to differentiate into DA-ergic neurons. In one clinical trial, cells from postmortem human eye tissue were cultured *in vitro* and implanted in PD postcommissural putamen with stereotactic operation in 12 patients with PD[75]. Eleven patients showed improvement in the primary outcome measure at 3 months post-treatment. Positron emission tomography (PET) revealed increased DA release during the first 6 months. It is now therefore suggested that hRPE cells, might serve as a useful source of DA-ergic neurons for neural graft in the treatment for PD.

A highly efficient and specific induction of cells with neuronal characteristics, without glial

differentiation, from both rat and human bone marrow stromal cells was developed using gene transfection with Notch intracellular domain (NICD) and subsequent treatment with bFGF, forskolin, and ciliary neurotrophic factor [76]. Intrastriatal transplantation of these GDNF-treated cells in a 6-OHDA rat model of PD, resulted in integration of TH+ and DAT+ cells and functional recovery in motor behaviors in apomorphine-induced rotational behavior and adjusting step and paw-reaching tests [76]. Long term survival of transplanted cells and restoration of motor function was also found after autologous engraftment of A9 DA-ergic neuron-like cells induced from mesenchymal stem cells (MSCs) in affected portions of striatum in hemiparkinsonian macaques [77]. For seven months DAT expression remained above baseline levels whereas for nine months cells positive for DAT and other A9 DA-ergic neuron markers were consistently demonstrated in the engrafted striatum [77]. Noninvasive intranasal (IN) delivery of MSCs to the brains of unilateral 6-OHDA-lesioned rats resulted in the appearance of cells in the olfactory bulb, cortex, hippocampus, striatum, cerebellum, brainstem, and spinal cord [78]. It was efficacious in increasing % survival of implanted neurons, % of proliferative neurons and improvement of motor functions. The potential advantage of IN delivery of stem cells is that it is a safe and non surgical alternative for stem cell therapy. Human and rat MSC have been effectively transplanted into PD models with improvement of behavioral deficits and survival of grafts and DA-ergic differentiation of the transplanted progenitors [79-82].

When embryonic neural progenitor cells were transplanted in the host striatum, they not only survived for at least three weeks after transplantation but also differentiated into DA (TH+) and medium size spiny (DARPP-32- positive) neuronal phenotypes [37]. They could functionally integrate in the striatum and ameliorate motor deficits as indicated by the statistically significant decrease of contralateral rotations after apomorphine treatment [37]. Human tNSCs (trophoblastic neural stem cells) isolated from preimplantation embryos in women with ectopic pregnancy have been successfully transplanted intracranially into lesioned striatum of acute and chronic PD rats and found to improve behavioral deficits and neuropathology [83]. DA-ergic neurons were also found to be regenerated in these mice in their nigrostriatal pathway at 18-weeks. Neuronal-primed adipose mesenchymal stromal cells (ASCs) derived from rhesus monkey (rASCs) combined with adenovirus containing neurturin (NTN) and tyrosine hydroxylase (TH) (Ad-NTN-TH) were implanted into the striatum and SN of MPTP lesioned hemi-parkinsonian rhesus monkeys and found to integrate and ameliorate behavioral symptoms [84]. The advantage of using ASCs is that they are readily available and can be obtained and used with neither ethical nor immune-reactive considerations, as long as they are of autologous tissue origin. Clinical trials for PD have transplanted cell preparations dissected from the human fetal ventral midbrain; [11, 19, 42, 85-89]. Of note, ESC-derived oligodendrocyte progenitors are already being used for spinal-cord injury in the first FDA-approved clinical trial using a pluripotent derived progenitor cell [88]. In another recent clinical trial fetal-derived neural precursor cells have been used for Batten disease, a rare, fatal pediatric disorder [89].

Conclusions and future directions

Although the advantages of using iPS cells are numerous compared to ES cells, certain standards need to be met for a successful outcome. A successful, clinically competitive stem cell based therapy in PD needs to produce long lasting symptomatic relief without side effects while counteracting PD progression. Prior to entering clinics with stem cell-derived DA-ergic neurons, cell populations must be characterized thoroughly both *in vitro* and *in vivo.* This is necessary to ensure that the A9 midbrain DA-ergic neurons phenotype can functionally integrate, release DA and provide the symptomatic relief needed. Future studies will need to focus on i) the mechanisms of action and integration of the human stem cells in the parkinsonian brain ii)

correction of familial PD gene mutations for autologous transplantation iii) transplantation of iPS cells or DA-ergic neurons several years prior to onset of the disease based positive biomarker results as a preventive measure iv) non surgical alternative strategies to introduce stem cells to the brain v) Developing iPS cells which can target multiple diseases at the same time vi) genetic interaction and complementation studies between genes using mutant iPS cells and/or knockout mouse lines vii) functional studies by generating gene knockouts, knockins and conditional knockout models in iPS/ES cell lines.

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