CELL & GENE THERAPY INSIGHTS

ADVANCED CELL THERAPIES – PROGRESS TOWARDS THE CLINIC

SPOTLIGHT

EXPERT INSIGHT

Development of iPSC technology in Parkinson's Disease

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The central nervous system has very little potential for regeneration which is why cell replacement therapy offers great potential for functional recovery in neurodegenerative diseases. Following the discovery of human embryonic stem cells in 1998 and induced pluripotent stem cells in 2007, we are now able to manipulate the quantity and quality of donor cells utilized in stem cell-based therapies. For Parkinson's disease in particular, which will be the focus of this article, precise protocols to establish induced pluripotent stem cells and to derive dopaminergic neurons have been developed to clinical grade, and preclinical data concerning the efficacy and safety of these cells now exists for rodent and monkey models. Based on these efforts, clinical trials for a number of neurodegenerative disease are expected to commence in the near term.

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Due to the poor regenerative ability of the CNS, neurodegenerative diseases have become the focus of efforts to develop cell replacement strategies. In Parkinson's disease (PD), midbrain dopaminergic (DA) neurons degenerate, and therefore cell transplantation represents a plausible approach to replace these lost neurons. Among neurodegenerative diseases, PD has seen a large number of clinical cases of cell transplantation since the late 1980's [1]. Accordingly, the focus of this article is to describe the development of a relatively new cell source – induced pluripotent stem cells (iPSCs) – for cell therapies to treat PD, with other neurodegenerative diseases described briefly.

PD is caused by the progressive loss of nigrostriatal DA neurons, and the main symptoms are motor dysfunctions such as tremor, rigidity and akinesia. In 1987, early clinical research utilized fetal cells from the ventral mesencephalon which were transplanted into PD patients, with the results of clinical trials demonstrating that the grafted cells survived and functioned as DA neurons over 20 years in some patients [2]. However, ethical issues regarding the use of fetal tissues and limited amounts of accessible donor tissues prevented fetal cell transplantation from becoming a standard therapy to treat PD patients. Stem cells, in particular pluripotent

stem cells such as iPSCs and embryonic stem cells (ESCs), therefore offered a promising alternative donor cell source.

METHODS TO ESTABLISH IPSCS

ESCs were reported as the first human pluripotent stem cells in 1998 [3] and are derived from the inner cell mass of human blastocysts. Importantly, they are able to proliferate indefinitely and maintain their pluripotency, which makes them potentially ideal candidates for cell-based therapies. However, the inclusion of blastocysts in the ESC-generating process has raised a number of ethical questions thus making it difficult to use or even establish human ESCs. In addition, any cell therapies generated from ESCs will be allogeneic and thus life-long immunosuppression would be required, with immune rejection remaining a strong possibility in patients.

The first report of murine iPSCs was published in 2006 [4] and that of human iPSCs in 2007 [5,6]. Since then, iPSCs have been widely investigated as they hold the potential to produce numerous different cell types and tissues both *in vitro* and *in vivo*. They too are pluripotent and self-renewing and can be generated as autologous therapies, thus avoid both of the aforementioned issues with ESC use. Nevertheless, other issues have limited their clinical application thus far. In the original iPSC-generating method, four transcriptional factors (c-Myc, Oct4, Sox2, Klf4) were introduced into mouse embryonic or adult fibroblasts via retroviral vectors [4]. However, concerns arose regarding the use of the proto-oncogene c-Myc as this could increase the risk of tumorigenesis, so its expression in the method was replaced by Nanog and LIN28 [6], Glis1 [7], or L-Myc [8] or by inhibiting p53 [9,10]. Another problem concerned retroviral integration, which may cause genomic mutations [11]. This risk can be circumvented by integration-free methods using alternative vectors such as adenovirus [12], Sendai virus [13] or plasmids [14].

Additionally, the use of fibroblasts requires skin biopsies, which is an invasive procedure for the patient or donor volunteer. It was since determined that iPSCs can be established from a variety of somatic cells, and efficient iPSC induction from cord blood cells and peripheral blood cells was reported [15], the acquisition of which is less invasive.

Finally, the original human iPSCs were established on feeder cells, namely mouse embryonic fibroblasts. However, for clinical applications xeno-derived cells should be avoided. Accordingly, a xeno-free matrix has been developed to achieve xeno-free, feeder-free culture of iPSCs [16], in addition to further improvements such as development of laminin fragment (LM511-E8), which supports not only iPSC culture [17,18], but also neural induction [19]. Overall, the methods to establish and expand human iPSCs have been steadily progressing towards clinical application (Table 1).

PROTOCOLS TO INDUCE NEURAL CELLS

Leading on from the discovery and establishment of ESCs and iPSCs, protocols were developed to induce neural cells. Lineage specification from pluripotent stem cells is determined by several signals such as BMP, TGF/Activin/Nodal, and Wnt [20]. For efficient neural induction, the inhibition of both BMP and TGF/Activin/Nodal signaling is essential, and can be achieved by the inhibition of SMAD1/5/8 and SMAD2/3 (i.e., dual SMAD inhibition) [21]. By using this method, almost 100% of human ES/iPSCs can be differentiated into a neural lineage.

Furthermore, differentiation into specific neuronal subtypes from pluripotent stem cells has also been extensively studied. Based on established methods for *in vitro* differentiation of DA neurons, efficient protocols for dopaminergic differentiation of iPSCs were then developed. These protocols are based on the recapitulation of embryonic neural development and are performed by either neurosphere or monolayer culture. It is known that DA neurons of the nigro-striatal pathway are derived from the floor plate in the midbrain [22], thus to derive DA neurons from iPSCs requires a combination of dual SMAD inhibition, midbrain specification by Wnt signaling activation, and ventralization by Sonic hedgehog (Figure 1) [23–25].

TABLE 1

Development of iPSCs towards clinical application

	Original iPSCs	Advanced iPSCs
Tissue sample	Dermal fibroblast	Peripheral blood
Reprograming factors	c-Myc, Oct4, Sox2, Klf4	Without c-Myc, Nanog and LIN28, Glis1, L-Myc, inhibition of p53, instead
Vectors	Retrovirus	Adenovirus, Sendai virus, plasmid
Feeder cells	Mouse embryonic fibroblast	Feeder-free. Laminin or laminin fragment

PRECLINICAL STUDIES USING ANIMAL MODELS

Before moving towards clinical application of iPSC-derived neural cells, cell efficacy and safety must be examined carefully through animal experiments. These studies have indicated donor neurons derived from ESCs or iPSCs show similar properties. Therefore the following descriptions of these studies refers to either or both cell source interchangeably.

As discussed, for PD proof-of-concept of cell replacement therapy has already been established through early clinical trials using fetal ventral midbrain cells [1,2]. Whilst fetal cell transplantation for PD patients is certainly not perfect, there are many patients who have benefited from the therapy for nearly 20 years [32–34], and a new trial is ongoing to optimize the protocol [35]

With efficient protocols established to induce midbrain DA neurons, animal studies have looked at the impact of these induced DA neurons in animal models of PD. When grafted into the striatum of 6-OHDA-lesioned rats [24,26] or MPTP-treated monkeys [27], the neurons showed robust survival and function, which improved behavioral impairments in the animals. In addition, human ESC-derived DA neurons showed equal potency and efficacy to fetal midbrain DA neurons [28]. These results support the idea that ES/iPSCs can be used as a cell-based therapy for PD; however, it is important to note that residual undifferentiated stem cells or proliferating neural progenitor cells may cause tumor formation [27,29,30]. To reduce the tumorigenic potential of the ES/iPSC-based protocol, fluorescence activated cell

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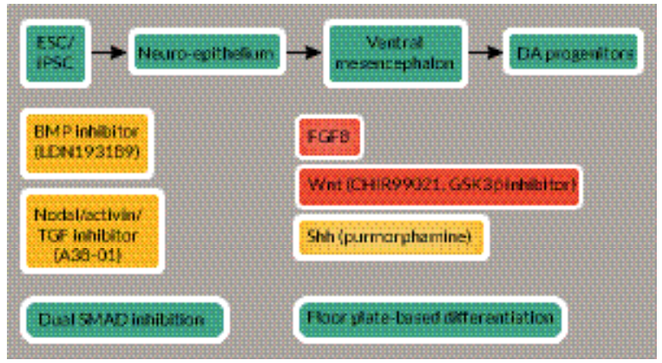
FIGURE 1

Protocols to induce neural cells.



Neural cells are induced from pluripotent stem cells by inhibiting both BMP and TGF/ Activin/Nodal signaling, a strategy called "dual SMAD inhibition". Subsequent midbrain specification is made by Wnt signaling activation and ventralization by Sonic hedgehog, a stragtegy called "floor plate-based differentiation".

sorting using antibodies for CORIN, a floor plate marker [19], or ALCAM, a CNS microvascular endothelium marker [31], have been developed. For example, by sorting CORIN+ cells, researchers can enrich DA progenitor cells as donor cells, which increases the number and density of DA neurons in the graft [19]. In addition, undifferentiated and proliferating cells can be removed, which prevents tumor formation by the grafted cells via this method. The differentiation efficiency differs from culture to culture, but by using this sorting procedure, we are always able to obtain high quality donor cells. These features are important, especially in clinical settings and this cell sorting technology can be utilized across a range of cell therapies for various diseases.

AUTOLOGOUS VS ALLOGENEIC TRANSPLANTATION

One of the advantages of iPSCs is that they enable autologous transplantation, which does not require immunosuppression, thus avoiding the adverse effects associated with long-term immunosuppressant drug use. iPSC-derived DA neurons autologously transplanted in non-human primates have already shown good survival without triggering an immune response by the host brain [32,33], indicating this treatment is ideal from the immunological point of view. Clinical-grade manufacturing of autologous iPSCs for a patient, however, is costly and laborious, and additionally there is concern regarding the vulnerability of iPSCs derived from patients who have disease-specific genetic backgrounds. These problems may be surmountable by harnessing new technologies, such as gene editing and automated cell culture systems, which would potentially allow the widespread use of autologous technologies.

Another strategy to reduce the immune response in cell transplantation is matching human leukocyte antigens (HLA), which in the case of organ transplantations such as kidney and bone marrow, matching HLA-A, -B, and -DR improves graft survival. Major histocompatibility complex (MHC)-matched transplantation of retinal pigment epithelial cells derived from MHC-homozygous iPSCs resulted in no immune response in monkeys [34]. Another advantage of iPSCs is that they can be established from individuals with homozygous HLA haplotypes, which simplifies HLA-matched transplantation, and could further allow clinical-grade manufacturing at affordable prices. It is estimated that a cell bank from HLA-homozygous volunteers of the 10 most frequent haplotypes would match 37.7% of the UK population, and 150 similar volunteers would match 93% [35]. According to another estimate, 50 lines would cover 90.7% [36] or 73% [37] of the Japanese population. Based on these estimates, several projects are underway to establish clinical-grade iPSC stocks from HLA-homozygous volunteers.

GETTING TREATMENTS TO THE CLINIC

As mentioned, clinical trials in PD using fetal ventral midbrain cells have shown varying degrees of benefit [1,2]. Subsequent double-blind, placebo-controlled clinical trials failed to show significant differences between the transplanted and control group [38,39]. Moreover, these studies reported the appearance of graft-induced dyskinesia. These negative results were discouraging, but the studies also revealed that a subpopulation of the cases had significantly restored function and improved quality of life. In addition, several problems in the protocol, such as the absence of immunosuppression and a too short period of observation, were posited as potential reasons for the negative outcomes. Whilst fetal cell transplantation for PD patients is not ideal, recent studies have reported that there are many patients who have benefited from the therapy for nearly 20 years [40–42]. Currently, a new trial is ongoing to optimize the protocol and to study any benefits in a more rigorous and consistent way than previous trials [43]. The optimization of future clinical trials using ES/iPSC-based therapies for PD, including criteria for patient selection, observation time, and assessment of symptoms, will benefit from previous fetal-cell-based trials.

OTHER NEURODEGENERATIVE DISEASES

While ES/iPSC-based therapies for PD is the focus of this Expert Insight, I would also like to give brief attention to neurodegenerative diseases that target other neuronal subtypes. Pluripotent stem cells, for example have been used to generate the cerebral neocortex in a 3D culture that allows a self-organization that mimics human neocorticogenesis [44]. Similar self-organization strategies can be applied to other types of neural cells such as pituitary gland [45], retina [46], and hippocampus [47]. Additionally, patient-derived iPSCs are a powerful tool for analyzing the pathology of specific diseases and drug discovery. In fact, iPSCs have already been established from patients of various neurological diseases including PD [48], AD [49], amyotrophic lateral sclerosis [50], and schizophrenia [51].

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In neurodegenerative diseases such as AD, cell transplantation is more complicated than in PD because the degenerated neurons are widely spread. Mouse AD models have indicated that the transplantation of mesenchymal or neural stem cells could improve cognitive and memory function. More specifically, bone marrow-derived mesenchymal stem cells were shown to ameliorate Aβ-induced neurotoxicity and cognitive decline by inhibiting apoptotic cell death and oxidative stress in the hippocampus [52]. When hippocampal neural stem cells were injected into the bilateral hippocampi of aged triple transgenic mice that express pathogenic forms of amyloid precursor protein, presenilin, and tau, they enhanced hippocampal synaptic density in a manner mediated by brain-derived neurotrophic factor and ameliorated the complex behavioral deficits associated with widespread AD pathology [53]. However, in these cases, the grafted stem cells functioned via cytokine effects rather than by cell replacement. In fact, it remains unknown whether ES/iPSC-derived neural cells function in the brain of animal models of AD or other neurodegenerative diseases. Therefore, more studies about the pathology of the diseases and ES/iPSC technologies are required for the development of ES-iPSC-based therapies against neurodegenerative diseases.

With that said, clinical trials using fetal neural stem cells have been performed not only for PD but also for Huntington's disease [54] and amyotrophic lateral sclerosis [55]. Unfortunately, like PD, the results varied widely between patients, making it premature to judge whether stem cell-based therapies can become the standard for neurodegenerative diseases. Two reasons are the complexity of the CNS and pathology of the diseases.

CONCLUSION

In the case of neuronal cell transplantation, survival of the grafted cells is not sufficient for an effective clinical outcome as the grafted cells must also extend neurites and form synapses with the host neurons for curative effects to be observed. In this context, not only the donor cells but also the host environment is important. Therefore, drugs or gene modifications that promote cell survival, neurite extension, and synapse formation would enhance the therapeutic effect of the grafted cells. Rehabilitation will also contribute to the construction of appropriate neuronal circuits by activity-dependent modifications. Thus, a key to successful regenerative medicine for neurodegenerative diseases is the combination of cell transplantation, medical treatment, and rehabilitation. Thanks to the development of ES/iPSCs, we now have technologies to manipulate the quantity and quality of donor cells to realize a new era of cell-based therapies.

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