

SPOTLIGHT

Towards stem cell based therapies for Parkinson's disease

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ABSTRACT

Treating neurodegenerative diseases with cell transplantation has been within reach since the first pioneering clinical trials in which dopamine neuron progenitors from the fetal brain were transplanted to individuals with Parkinson's disease. However, the use of fetal tissue is problematic in terms of low availability and high variability, and it is also associated with ethical concerns that vary between countries. For decades, the field has therefore investigated new scalable source of therapeutic cells from stem cells or via reprogramming. Now it is possible to generate authentic midbrain dopaminergic neurons from pluripotent stem cells and clinical trials using such cells are rapidly approaching.

KEY WORDS: Parkinson's disease, Dopamine neuron, Reprogramming, Stem cell, Translation

Introduction

Parkinson's disease (PD) is a debilitating neurodegenerative movement disorder resulting from progressive loss of dopaminergic (DA) neurons in the midbrain. The relatively focal degeneration makes it a good candidate for cell-based therapies. Since the first clinical trials using fetal midbrain tissue to replace the DA neurons lost in the disease were initiated in the late 1980s, hundreds of patients have been grafted with fetal tissue worldwide, and a number of them have shown long-term graft survival with good clinical outcome, coupled to physiological release of dopamine over decades (reviewed by Barker et al., 2015). With the derivation of human embryonic stem cells (hESCs) (Thomson et al., 1998), a new scalable cell source that could potentially replace fetal tissue became available (Fig. 1A). However, the road to clinical application of such cells is long and entails a number of key steps, relating to the control of differentiation into defined subtypes of cells and assuring that the produced cells are safe and efficacious, as well as more practical concerns relating to Good Manufacturing Practice (GMP), scaling up and regulatory approval of the final cell derivative. Here, I describe the developments towards using DA neurons derived from hESCs in clinical trials, and how new insights from developmental biology have guided several important steps in this process. I will also briefly describe alternative sources of cells that, thanks to rapid developments in cell reprogramming technology, have arisen as potential candidates for patient-specific treatments.

From hESCs to therapeutic DA neurons

The initial strategies for generation of DA neurons from hESCs were based on previous experience with mouse ESCs, which commonly used the developmental cues known at the time (Kawasaki et al.,

2000; Kim et al., 2002). Several of these early differentiation protocols did indeed produce a relatively high number of cells expressing tyrosine hydroxylase (TH, the rate-limiting enzyme in dopamine synthesis and most commonly used marker for DA neurons), yet the midbrain properties of these neurons were not clear and their *in vivo* performance after grafting in standard animal models of PD was modest. A breakthrough in optimization of the differentiation protocols came when our understanding of how midbrain DA neurons are formed during normal development radically changed. In 2007 and 2008, two ground-breaking studies were published, both reporting that midbrain DA neurons were not derived from neuroepithelial cells (like all other neurons) but were in fact derived from floor-plate cells expressing markers such as *Corin*, *FoxA2* and *Lmx1a* (Bonilla et al., 2008; Ono et al., 2007). This insight into the unique cellular origin of midbrain DA neurons inspired new hESC differentiation protocols to recapitulate this developmental process by first generating floor-plate cells (Fasano et al., 2010) and subsequently midbrain DA neurons (Kirkeby et al., 2012; Kriks et al., 2011; Cooper et al., 2010). In contrast to DA neurons that had been generated via developmentally erroneous neuroepithelial intermediates, these floor-plate-derived cells expressed specific markers of midbrain DA neurons, released dopamine and demonstrated efficacious functional properties after transplantation into rodent models of PD (Kirkeby et al., 2012; Kriks et al., 2011; Steinbeck et al., 2015). Importantly, their ability to restore motor deficits and innervate correct target structures was found to be on par with that achieved by human fetal DA neurons (Grealish et al., 2014).

With this realization, the field entered a new era with a focus on generating floor-plate-derived DA neurons under GMP-compliant conditions – necessary for allowing their use in patients (Kirkeby et al., 2017b; Studer, 2017; Barker et al., 2017). Developing GMP-compliant cell-manufacturing processes not only entails the use of appropriate starting cell lines and media components, but also requires monitoring and reduction of variability in cell fate at each step – so that a uniform cell product can be produced with each differentiation round. In such efforts in my own laboratory, we observed significant batch-to-batch variation in how many DA neurons are ultimately produced from FOXA2/LMX1A-expressing progenitors after transplantation and functional maturation *in vivo* (Kirkeby et al., 2017a). This observation was puzzling as, based on what was known about development at the time, these progenitors should all form mature midbrain DA neurons. However, a parallel study based on single cell sequencing of the midbrain *Lmx1a*-expressing progenitors during mouse development revealed that *FoxA2* and *Lmx1a* (as well as most other ventral midbrain markers commonly used to detect the DA progenitor population prior to grafting) are also co-expressed in early diencephalic progenitors that form the subthalamic nucleus (STN), and therefore do not exclusively identify the midbrain DA neuron domain (Kee et al., 2017). Thus, cells expressing FOXA2/LMX1A can give rise to two types of neurons, and the relative proportion of these can now be tracked and controlled using the predictive markers identified that

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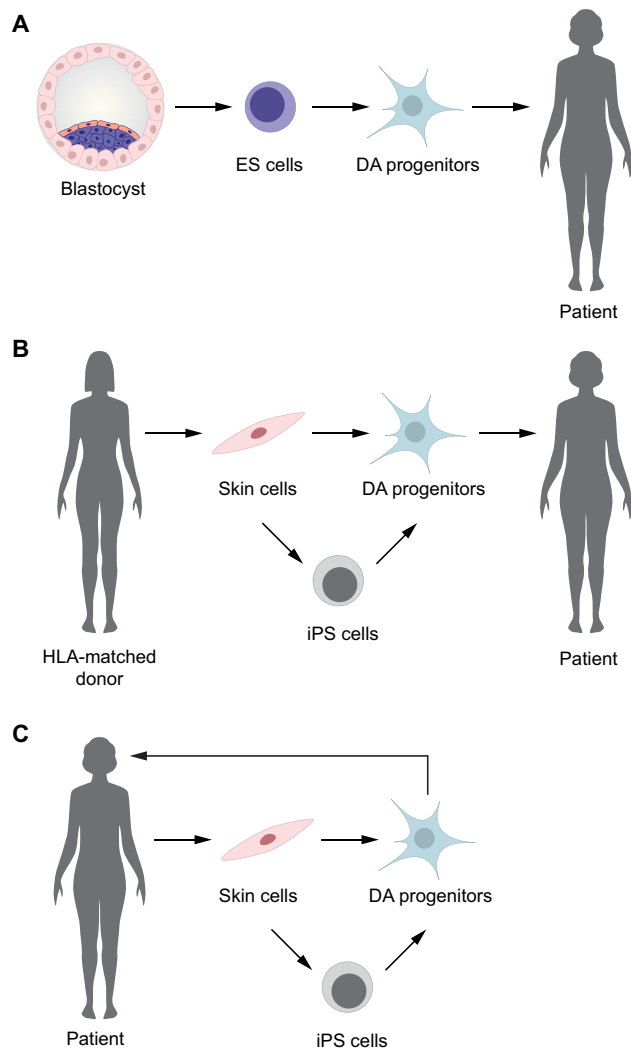


Fig. 1. Therapeutic cells for cell-based therapy. (A) hESCs can be differentiated into midbrain dopaminergic (DA) neuron progenitors that undergo their last cell division after transplantation and mature into functional DA neurons in the brain. (B,C) Somatic cells such as fibroblasts or blood cells can be reprogrammed, either via iPS cells that are subsequently differentiated into DA neuron progenitors (lower path) or directly converted into neurons (upper path) for transplantation. This allows for HLA-matched donors (B) or personalized treatments using patient's own cells (C).

are specifically expressed in the DA domain (Kee et al., 2017; Kirkeby et al., 2017a). This allowed us to better control stem cell differentiation *in vitro* by adding FGF8 to sufficiently caudalize the cells such that DA (and not STN cells) are formed with a high yield and purity (Kirkeby et al., 2017a). Thus, similar to the studies elucidating the role of the floor plate during development (Bonilla et al., 2008; Fasano et al., 2010; Kawasaki et al., 2000), refined understanding of developmental biology, this time achieved by the high resolution of cell diversity achieved via single cell sequencing, was essential for precise control of stem cell differentiation *in vitro* (Kee et al., 2017; Kirkeby et al., 2017a).

Considerations for clinical use

The first use of an hESC-derived cellular product took place in a spinal cord injury trial in 2010 (Lebkowski, 2011) and since then a small number of hESC-derived RPE transplant trials have been performed (reviewed by Tang et al., 2017). Although efficacy from

these trials still needs to be documented, none has so far reported adverse events relating to the pluripotent origin of the cells – which is a major concern of using cells derived from hESCs. Nevertheless, the pluripotent origin of the cells requires caution, especially as proliferating DA neuron progenitors derived from pluripotent cells are directly transplanted to the brain because post-mitotic cells do not survive intracerebral grafting procedure (Fig. 1A). Therefore, the strategies for the first clinical trials are being designed to produce a large number of cryopreserved progenitors (~300 doses), which allows for extensive preclinical assessments of cell quality, safety and potency (as summarized in Box 1) of the exact same batch of progenitors that will later be used in the patients in clinical trials (Barker et al., 2017; Kirkeby et al., 2017b; Studer, 2017).

Other cell types of interest on the horizon

With the discovery of induced pluripotent stem cells (iPSCs) in 2007, the past decade has flourished with new developments towards using these cells in medical applications (Shi et al., 2017). In terms of generating DA neurons, human iPSCs are very similar to hESCs in that they respond to the same differentiation cues and generate mature cells with very similar functional properties (Doi et al., 2014; Kikuchi et al., 2017; Kriks et al., 2011). Also in these studies, markers that predict a good outcome have been identified and shown to overlap with the predictive markers identified using hESCs (Kikuchi et al., 2017).

A major difference, however, lies in how iPSCs are derived – via reprogramming of easily accessible somatic cells such as fibroblasts or blood cells, rather than from a human embryo. In addition to the potential concerns surrounding the use of pluripotent cells, which apply equally to hiPSCs as to hESCs, there are additional safety issues for hiPSCs that stem from the use of somatic cells and the reprogramming process itself (Liang and Zhang, 2013). This increases the pre-clinical assessments required, but by no means precludes them from clinical use.

iPSCs allow, in principle, for both human leukocyte antigen (HLA)-matched donor (Fig. 1B) and patient-specific treatments (Fig. 1C). The fetal grafts performed to date have all used unmatched tissue from multiple donors per patient (Barker et al., 2015) and short-term standard immunosuppression protocols (≤ 18 months) have resulted in graft survival for >20 years (Li et al., 2016). Immunocompatibility may therefore not be a major limiting factor in the context of cell therapies for PD, but emerging data demonstrate that major histocompatibility complex matching offers some advantages as it reduces the immune response of microglia and lymphocytes, and increases the survival of iPSC-derived DA neurons in non-human primates (Morizane et al., 2017). However, with the current limited repertoire of predictive markers for safety and efficacy, each one of these patient-specific cell products would need to be subjected to the same pre-clinical testing as batch-produced hESC-derived DA neurons (Box 1) in order to ensure safety and efficacy of the DA progenitors prior to clinical delivery. The cost and time needed for this is largely what is hindering the use of patient-specific iPSC-based approaches at present.

An interesting cell type on the horizon is induced neurons (iNs), which are neurons directly reprogrammed from skin cells without passing via a proliferative intermediate. These cells were first described in 2010 using mouse embryonic fibroblasts as starting cells (Vierbuchen et al., 2010). Shortly after, conditions for obtaining human neurons, including human DA neurons, via this type of direct conversion were established (Pfisterer et al., 2011; Caiazzo et al., 2011). Like iPSC-derived DA neurons, iNs would

Box 1. Summary of pre-clinical assessments

Summary of key parameters measured in the three major steps for taking stem cell-derived dopaminergic neurons to clinical trial. These include:

(A) monitoring of the cell product to ensure cells manufactured for clinical use are of sufficient quality and quantity, without any contaminating pluripotent cells and with minimal contamination of forebrain and hindbrain progenitors; (B) *in vivo* testing to ensure that cells used for grafting are safe; and (C) ensuring that cells have the ability to survive and mature *in vivo*. The most-important features are that they can mediate functional recovery in dopamine-depletion models and are able project long distances such that innervation is sufficient for use in humans.

| (A) Manufacturing process | (B) Safety | (C) Efficacy |
|---|---|---|
| <ul style="list-style-type: none"> In process Quality Control (QC) and Release Criteria (RC) to ascertain quality of cells and comparability between batches Eighty percent of cells co-expressing midbrain markers such as FOXA2, LMX1A, EN1 and CORIN Absence of pluripotency markers Low expression of non-neural markers Low numbers of forebrain and hindbrain progenitors (i.e. PAX6⁺, FOXG1⁺, GBX2⁺) (Kirkeby et al., 2017b; Studer, 2017; Takahashi, 2017) | <ul style="list-style-type: none"> Tumorigenicity Rule out pluripotent cells or other contaminants <i>in vitro</i> and ensure no tumor formation <i>in vivo</i> can be done in nude mice or rats at certified contract research organization Biodistribution Standard <i>in vivo</i> testing at certified CRO Toxicology Standard <i>in vivo</i> testing at certified CRO (Kirkeby et al., 2017b; Studer, 2017; Takahashi, 2017) | <ul style="list-style-type: none"> Yield Estimate the number of mature dopaminergic neurons obtained per 100,000 grafted cells Ability to restore motor function Full recovery of amphetamine-induced rotations in 6-hydroxydopamine lesion model Long-range target specific innervation Intranigral grafting in rat model, or intrastriatal grafting in a large animal model (Barker et al., 2017; Grealish et al., 2014; Kikuchi et al., 2017) |

allow for HLA and patient-specific treatments (Fig. 1B,C), but with the advantage of avoiding the pluripotent state. Thus, many of the safety issues could be side-stepped, making cell production and subsequent safety testing less demanding, and the feasibility for patient-specific treatments one step closer. However, this type of reprogramming is new and before such cells can be developed for clinical use, their stability, safety and function needs to be extensively explored in pre-clinical *in vivo* models.

Concluding remarks

The field of stem cell-based therapies for PD is about to enter a new and exciting era, where a number of first-in-human clinical trials using cells derived from both hESCs and iPSCs are on the horizon (Barker et al., 2017). Experience gained from these pioneering clinical trials will guide the design of new and better cell-based treatments for PD, and hopefully also pave the way for developing stem cell based therapies for other neurological disorders.

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Competing interests

M.P. is the owner of Parmar Cells.

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