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Review

Stem cells and the treatment of Parkinson's disease

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ABSTRACT

Progress in Parkinson's disease (PD) research has been hampered by the lack of an appropriate model which exhibits the core pathology seen in the human brain. Recent advances in deriving cells with neuronal phenotypes from patients with neurodegenerative disorders through cellular reprogramming offer a unique tool for disease modelling and may help shed light on the molecular pathogenesis that drives the progression of the disease. This technology may also help in establishing platforms for drug screening and open up exciting new prospects for cell grafting. In this review, we will discuss progress made in differentiating stem cells into authentic dopamine neurons and where we stand with respect to clinical trials with these cells in patients with PD. We will also examine the various approaches used in cellular reprogramming and their differentiation into patient-specific midbrain dopamine neurons, with an emphasis particularly on modelling familial cases of PD to recapitulate disease phenotypes. This review will highlight some of the challenges that need to be addressed for this technology to have any potential clinical application in cell therapy and personalised medicine.

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Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder characterised by the loss of specific populations of neurons and the accumulation of protein aggregates in the brain. The disease affects more than 1% of the population over the age of 60, and as the population ages this frequency is likely to increase (Lang and Lozano, 1998; Obeso et al., 2010). The aetiology of PD remains unknown in the vast majority of cases, and although Mendelian forms of the disorder are now known, most patients have sporadic disease for which susceptibility loci are only now been identified using genome-wide association studies (GWAS) (IPDGC, 2011; Nalls et al., 2011). However, whilst this has helped define the common high-risk variants for susceptibility, what underlies PD in terms of the triggering event and subsequent spread of the disease is not known.

One of the central pathological features of PD is the progressive loss of nigrostriatal dopamine (DA) neurons, which is accompanied by the presence of α-synuclein containing cytoplasmic inclusions known as Lewy bodies. The loss of these neurons causes some of the debilitating motor deficits associated with the disease, such as the rigidity and bradykinesia along with some of the dysexecutive cognitive deficits. However, the neuronal cell loss is not restricted to the nigral DA neurons, nor are the clinical features which embrace a range of non-motor problems, including the occurrence of dementia in significant numbers of patients (Aarsland et al., 2005; Evans et al., 2011). Furthermore, it is now well recognised that PD is a heterogeneous disorder

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with different evolving pathological processes across networks of cells and which is not just confined to neurons (Williams-Gray et al., 2009).

Whilst there are no disease-modifying treatments for PD; dopamine replacement therapies can help alleviate some of the motor deficits, but do not slow or halt the progression of the disease nor do they affect many of the non-motor symptoms. Furthermore with time they produce their own side-effects such as drug induced dyskinesias and behavioural and neuropsychiatric problems. There has therefore been a keen interest in developing strategies that actually slow down or repair the underlying disease process including the use of neural grafts of DA cells.

Since the early 1980's, efforts have been made to replace the lost DA neurons uisng a cell transplantion approach. Initially this work used a variety of different catecholaminergic cells (reviewed in Barker and Dunnett, 1999) but the most successful work involved the use of tissue dissected from the midbrain of the developing foetus. However whilst this approach proved successful experimentally it translated less well to the clinic for 3 major reasons:

1) The ethical problems inherent in the use of human foetal tissue derived from terminations of pregnancies.
2) The practical problems of having enough foetal tissue to graft patients, as each PD patient requires between 8–12 foetuses for bilateral grafting.
3) The inconsistent results seen in the open label versus double blind placebo controlled trials and the development of graft induced dyskinesias in some patients, which in part relates to the fact that each patient is in receipt of a unique transplant that contains a range of non-DA cells, including serotoninergic neurons (Barker and Kuan, 2010; Galpern et al., 2012; Lindvall and Bjorklund, 2004; Politis et al., 2011).

Thus from a very early stage, much effort was put into finding a more readily available source of nigral DA cells for grafting. This work involved looking at DA neurons from different species such as pigs (Barker, 2000) as well as various different types of stem cells. Of late this latter field has evolved to the point that it is now possible to make large number of nigral DA cells from a stem cell source in a safe and robust fashion. This has not only rekindled the possibility of using stem cells as a source for grafting into patients with PD, but also has opened up the possibility of using these cells for disease modelling through the development of novel reprogramming strategies.

In this review we will discuss these aspects to show how stem cells are currently being used in research directed towards the treatment of PD. We will also consider the major challenges facing the clinical use of stem cells in the treatment of PD, focusing on a number of key requirements derived from foetal midbrain transplantation studies in rodents, non-human primates and humans. These include:

- The long term survival of at least 100,000 DA neurons in the human putamen in order to see a beneficial effect.
- Controlled release of DA and electrophysiological activity similar to that observed in substantia nigra dopamine neurons.
- Functional connectivity to and from the host brain with extensive fibre outgrowth from the grafted cells.
- The reversal of the motor deficits in animal models of PD.
- The absence of side effects, such as tumour formation.

This review, whilst looking to see the extent to which stem cell derived dopaminergic neurons fulfil the above criteria and thus may be useful for treating PD, will also look at recent developments in the field of disease modelling uisng stem cells.

Turning stem cells into dopamine neurons

Numerous cell types have been used in the search for a readily available supply of expandable stem cells which can be differentiated into authentic nigral A9 DA neurons for the treatment of PD (Lindvall and Kokaia, 2009). Of all of these sources, embryonic stem cells are now viewed as the most reliable for the generation of such cells (Kriks et al., 2011). Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the blastocyst (Evans and Kaufman, 1981; Martin, 1981). They can be maintained in culture for extended periods of time and differentiated into any cell type in the body (Smith, 2001). They present an ideal means of circumventing some of the practical problems associated with the foetal tissue transplantion procedure used to treat PD, such as tissue availability and the heterogeneous nature of the tissue. However these cells do bring with them ethical and tumourigenic issues, as well as questions as to the true identity and capacity of any nigral DA cells so generated.

Mouse ES cells

Early studies involving the transplantion of undifferentiated mouse ES cells demonstrated that they could be successfully grafted to the rodent striatum (Deacon et al., 1998) and had the ability to differentiate into DA neurons and alleviate some of the behavioural deficits in a rat model of PD (Bjorklund et al., 2002). These initial studies, however, also illustrated one of the main problems that can arise from transplanting undifferentiated ES cells — namely the formation of teratoma-like tumours in many of the animals. For the purpose of therapeutically treating PD, it was apparent that ES cells needed not only to be directed to a neuronal DA fate, but also that those precursor cells capable of tumour formation needed to be totally eliminated.

In this first regard, it was demonstrated early on that mouse ES cells could be directed towards a neural fate (or neuralised) with the application of retinoic acid (Bain et al., 1995, 1996; Fraichard et al., 1995; Li et al., 1998; Rohwedel et al., 1999) or bFGF (Lee et al., 2000; Mujtaba et al., 1999; Okabe et al., 1996). These cultures involved growing the ES cells as serum-stimulated, three-dimensional colonies known as embryoid bodies (EB) (Bain et al., 1995), sequential culturing of EBs in serum, and then serum free conditions (Okabe et al., 1996). This was later followed by the differentiation of mouse ES cells either on stromal cells (Kawasaki et al., 2000) or as a monolayer culture in serum-free medium (Tropepe et al., 2001). These different approaches lead to ever increasing percentages of neurons in the cultures.

In order to generate higher numbers of DA neurons, work shifted towards the use of developmentally relevant DA specific factors, such as Sonic hedgehog (SHH), fibroblast growth factor 8 (FGF8), brain derived neurotrophic factor (BDNF) and ascorbic acid (Lee et al., 2000; Park et al., 2004). This approach raised the percentage of DA neurons produced to approximately 20–30% of the total cell population. Co-culture studies demonstrated that ES cells grown with certain stromal feeder cell lines appeared to be pushed towards a DA fate, and some of these ES cell derived DA neurons could then be successfully transplanted into the brain (Kawasaki et al., 2000). Subsequent work demonstrated that these co-cultured ES cell derived neural progenitor cells (NPC) could also be isolated from the feeder layer, re-plated alone and steered towards a DA fate using specific cocktails of factors, such as SHH and FGF8. Again these cells could be successfully transplanted into animal models of PD with behavioural benefits (Barberi et al., 2003; Perrier et al., 2004).

The discovery that cell-intrinsic transcription factors play a role in normal DA neuron development, led to a new approach involving the over-expression of some of these genes in ES cells. This protocol has led to the generation of high numbers of DA neurons in vitro. Specifically, the individual over-expression of the Nuclear receptor related 1 protein (Nurr1) (Chung et al., 2002; Kim et al., 2002; Sonntag et al., 2004), the pituitary homeobox 3 (Pitx3) (Chung et al., 2005; Maxwell et al., 2005), LIM homeobox transcription factor 1 alpha (Lmx1a) and beta (Lmx1b) (Andersson et al., 2006; Tian et al., 2012) have all resulted in large numbers of DA neurons being generated in culture that are also readily transplantable. However the use of viral agents renders these converted ES cells unusable for clinical transplantation therapy, and as such the search for more clinically acceptable approaches has been sought.