Invited review

Parkinson's disease in a dish – Using stem cells as a molecular tool

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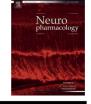
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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disease, with a strong genetic component to both the familial and sporadic forms. The cardinal motor symptoms of the disease result from the loss of dopamine (DA) neurons in the midbrain. There is currently no cure for PD and improved methods for modelling the disease are required in order to develop more effective therapeutic interventions. Patient-derived induced pluripotent stem cells (iPSCs) carry the genetic background of the donor, enabling accurate modelling of genetic diseases in vitro. Various human iPSCs from patients suffering different genetic forms of PD have been differentiated into DA neurons and demonstrated signs of the pathophysiology of PD in vitro. The examination of key cellular pathways such as calcium regulation and autophagy indicate that disease-associated genetic variants may have important implications for cellular function. This review examines and critiques how DA neurons from patient iPSCs have been used to model PD in vitro, and what iPSCs might hold for the future of PD research.
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1. Introduction

1.1. Parkinson's disease

Originally described in 1817 by James Parkinson (Parkinson, 1817), Parkinson's disease (PD) is a neurodegenerative disease affecting over 10 million people worldwide. Disease onset is variable, affecting young, middle-aged and elderly patients, but most commonly those in the later stages of life. PD can be caused by an inherited mutation, or be idiopathic with no known cause. There is also likely a strong contribution of environmental factors in PD. For example, the pesticide rotenone can be used to induce Parkinsonism in animal models, and some links have been shown between rural living or agricultural work and PD (Costello et al., 2009; Sherer et al., 2003). Despite differences in disease onset, much of PD pathology is common and symptoms result from of the degeneration and loss of the A9 subtype of dopamine (DA) neurons in the substantia nigra of the midbrain (Damier et al., 1999). These neurons are involved in the control of motor function in the basal ganglia, and their loss results in disruption to this fine-tuned control, causing the typical motor symptoms of tremor, bradykinesia and gait dysfunction (Gonzalo and Olanow, 2000). As PD progresses, neuronal loss becomes more widespread, affecting neurons of the neocortex, locus coeruleus, pedunculopontine nucleus, including cholinergic and serotonergic neurons in addition to DA neurons (Braak et al., 2003; Mavridis et al., 1991; Zweig et al., 1987). With greater neuronal loss and the occurrence of protein inclusions known as Lewy bodies beyond the nigra and the cortex, additional symptoms of the disease become apparent with time, including psychological symptoms such as hallucinations and dementia (Aarsland et al., 2001; Lees et al., 2009).

The majority of PD cases have unknown aetiology, but the remaining 5–10% have a strong genetic component. There are currently 16 monogenic genes and loci known to cause PD through familial inheritance, known as the PARK genes (Lesage and Brice, 2009), of which the principal ones will be discussed here. The first gene found to cause dominantly inherited familial PD was alpha synuclein (SNCA), and four mutations are now described

Table 1
Summary of the publications using iPSCs for the examination of mutations found in 5 key genes associated with PD.

Gene	Publication	Mutation	DA differentiation	Findings
SNCA	Devine et al., 2011	Triplication	Chambers et al., 2009, Fasano et al., 2010 28–37% TH yield 21–30 days of differentiation	Increased α-SYN expression in neurons but not in fibroblasts
	Byers et al., 2011	Triplication	Perrier et al., 2004 No comments on yield 50 days of differentiation	Increased α-SYN expression and increased expression of genes associated with OS
	Soldner et al., 2011	A53T, E46K	Cells were not differentiated towards DA neurons	Correction of A53T mutation by ZFN and generation of A53T and E46K mutants from hESCs
GBA1	Mazzulli et al., 2011	N370S	Seibler et al., 2011 80% TUJ1, 10% TH/TUJ1 30 days of differentiation	Reduced GCase activity in neurons, causing α-SYN accumulation
	Panicker et al., 2012	L444P, N370S	Cells were not differentiated towards DA neurons	Neurons had low GCase activity and Accumulation of glycosylsphingolipids
PINK1	Seibler et al., 2011	C1366T, C509G	Chambers et al., 2009 with modifications 60% TUJ1, 11–16% TH Duration of differentiation not specified	Parkin fails to be recruited to the mitochondrial membrane
	Rakovic et al., 2013	C509G	Seibler et al., 2011 72% TUJ1, 11.3% TH Duration of differentiation not specified	Overexpression of Parkin in fibroblasts induces mitophagy and rescues PINK1 phenotype, but phenotype is not rescued in patient iPSC derived neurons
	Cooper et al., 2012	Q456X	Cooper et al., 2010 with small molecule SAG ≈35% TUJ1 and 10% TH 22 days of differentiation	Disease phenotypes aren't evident in fibroblasts Phenotype rescue using Co enzyme Q, LRRK2 inhibitor (GW0574) and Rapamycin
PARK2	Jiang et al., 2012	Exon 3/5 deletion	Zhang and Zhang, 2010, Chambers et al., 2009 70 days of differentiation	Reduced DAT expression and low dopamine uptake with increased MOA activity and susceptibility to OS
LRRK2	Sánchez-Danés et al., 2012a,b	G2019S	Sánchez-Danés et al., 2012a,b Transduction with LMX1A encoding virus 40% TUJ1, 9–29% TH/TUJ1 30–75 days of differentiation	Increased α-SYN expression and neurite shortening in DA neurons, with accumulation of autophagosomes and reduced autophagic flux
	Nguyen et al., 2011	G2019S	Chambers et al., 2009 3.6–5% TH 30–35 days	Increase in α-SYN expression and elevated mRNA for genes associated with OS. Elevated caspase-3 activation in G2019S DA neurons after exposure to hydrogen peroxide
	Orenstein et al., 2013	G2019S	Sánchez-Danés et al., 2012a,b No comments on yield Duration of differentiation not specified	Failed degradation of mutant LRRK2 by CMA causes accumulation of α-SYN with LAMP2A at the lysosomal membrane
	Reinhardt et al., 2013	G2019S	Chambers et al., 2009, Nguyen et al., 2011 20% TH/TUJ1/DAPI 30–35 days	Reversal of the G2019S phenotype through ZFN gene correction and LRRK2 inhibitors
	Cooper et al., 2012	G2019S, R1441C	Cooper et al., 2010 with small molecule SAG ≈35% TUJ1 and 10% TH 22 days of differentiation	Increased mitochondrial mobility and shorter mitochondrial length in PD iPSC-derived neurons reduced oxygen consumption rate in LRRK2 iPSC derived-neurons. Phenotype rescue with Rapamycin, Co Enzyme Q and GW5074.

(Appel-Cresswell et al., 2013; Krüger et al., 1998; Polymeropoulos et al., 1997; Zarranz et al., 2004). Two other dominant forms of PD, caused by mutations in the leucine rich repeat kinase 2 (LRRK2) and glucocerebrosidase (GBA) genes, have also been well-characterised (Aharon-Peretz et al., 2004; Neumann et al., 2009; Zimprich et al., 2004). There are three recessive forms of PD caused by homozygous mutations in PTEN induced kinase 1 (PINK1), Parkin (PARK2) and DJ1 (PARK7) (Bonifati et al., 2003; Kitada et al., 1998; Valente et al., 2004). More recently, genome wide association studies (GWAS) performed on very large numbers of PD patients have found many more genes in which common variation contributes to susceptibility to PD, for example at the microtubule associated protein tau (MAPT), SNCA, LRRK2, human leukocyte antigen (HLA) loci (International Parkinson Disease Genomics Consortium et al., 2011). However, some genes known to be a risk factor for PD are not detected in GWAS studies due to their low frequency of occurrence in some of the populations studied, such as GBA1.

In order to develop better therapies for PD, it is important to understand the consequences of the various genetic mutations associated with PD. Post-mortem analysis is informative of the end-stage pathology of PD, but understanding the early molecular changes associated with the initiation of the disease is required to help develop improved therapies for halting disease progression. Human induced pluripotent stem cells (iPSCs) represent an excellent tool for this work with many advantages for research into neurological disease than models currently in use.

Previous models of PD have relied on a combination of established cell lines, such as human embryonic kidney (HEK) 293 cells or SH-SY5Y neuroblastoma cells, primary rodent neuronal cultures, and transgenic rodent lines. Whilst cell lines and primary neuronal cultures are tractable and can be genetically modified by gene transfection or siRNA knockdown to provide relatively cost effective models of PD, they have limitations for modelling the susceptible DA neuron subtype. Animal models have the advantage of being able to recapitulate the complex brain circuitry required to study network dysfunction in PD. However, the ability to now study disease processes in human DA neurons from PD patients provides unparalleled opportunity to better understand disease mechanisms and ultimately develop novel therapies.