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In vivo gene delivery for development of mammalian models for Parkinson's disease

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Abstract

During the last decade, identification of the genes involved in familial forms of Parkinson's disease (PD) has advanced our understanding of the mechanisms underlying the development of different aspects of PD. However the available animal models still remain as the main limiting factor for the development of neuroprotective therapies that can halt the progression of the disease, through which we wish to provide a better quality of life for the PD patients. Here, we review the recently developed animal models based on overexpression of PD-associated genes using recombinant viral vectors. Recombinant adeno-associated viral vectors, in particular, have been very useful in targeting the nigral dopamine neurons both in the rodent and the primate brain. In order to provide insights into the establishment of these models in the laboratory, we will not only give an overview of the results from these studies but also cover practical issues related to the production and handling of the viral vectors, which are critical for the successful application of this approach.

Keywords: Parkinson's disease; Recombinant viral vectors; Gene transfer; Alpha-synuclein; Disease modeling

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that typically presents with motor symptoms characterized by tremor, rigidity bradykinesia/akinesia and impaired balance. These symptoms are secondary to striatal deficiency of dopamine as a consequence of neurodegeneration of midbrain dopamine neurons, which is an invariable feature of PD. On the other hand, it is now widely accepted that PD pathology is beyond the nigrostriatal projection neurons and may involve also other neurotransmitter systems as well. As a consequence, the behavioral presentation of the patients extends to involve non-motor symptoms. Importantly, not only the phenotypes but also the etiology of the disease is variable between subjects. Increasing evidence indicates that several cellular events such as protein aggregation, dysfunction in the ubiquitin proteasome system or the mitochondria, or oxidative stress might be involved in the development of PD (Chiba et al., 1984; Jenner, 2003; Lansbury and Lashuel, 2006; McNaught et al., 2003; McNaught and Jenner, 2001; Yao et al., 2004).

For several decades, the familial component of PD was not recognized. The idea that there is no or minimal genetic contribution to the etiology of PD was suggested by the early twin studies performed during 1980s (Marttila et al., 1988; Ward et al., 1983). More recently, large family studies and linkage analysis indicated that genetic factors do play a substantial etiologic role, which led to the mapping of a gene to several chromosomes (Gasser et al., 1998; Matsumine et al., 1997; Polymeropoulos et al., 1996). Up to date, 11 different loci have been linked to familial forms of PD and thus far six genes have been identified (Table 1; see also Hardy et al., 2006 for a recent review).

Abbreviations: 6-OHDA, 6-hydroxydopamine; AAV, adeno-associated virus; Ad, adenovirus; CsCl, cesium chloride; DA, dopamine; DAT, dopamine transporter; LRRK2, leucine-rich repeat kinase-2; LV, lentivirus; MFB, medial forebrain bundle; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Pael-R, parkin associated endothelin-receptor like receptor; PD, Parkinson's disease; PDGF-β, platelet derived growth factor; P:I, particle-to-infectious units; PINK-1, PTEN-induced kinase 1; Prp, prion protein; PSI, Z-lle-Glu(OtBu)-Ala-Leu-al; qPCR, quantitative polymerase chain reaction; RCA, replication center assay; SN, substantia nigra; TU, transducing units; UCH-L1, ubiquitin carboxy-terminal hydrolase L1; VG, vector genomes; wt, Wild type.

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Table 1
Genes involved in familial Parkinson's disease

Gene	Locus	MOI	Function	Neuropathology	Reference
α-syn	PARK1 (PARK4)	AD	Unknown function related to membrane trafficking and synaptic function	Duplications: idiopathic PD; some postural tremor; slow progression Triplications: PD; PD with dementia; diffuse LBs disease; aggressive course Mutations: A53T, A30P, E46K, idiopathic PD; parkinsonism and diffuse LBs	(Chartier-Harlin et al., 2004; Kruger et al., 1998; Polymeropoulos et al., 1997; Singleton et al., 2003; Zarranz et al., 2004)
parkin	PARK2	AR	E3 ubiquitin ligase	Juvenile parkinsonism with or without LBs	(Kitada et al., 1998)
UCH-L1	PARK5	AD	Ubiquitin C-terminal ligase	NA	(Leroy et al., 1998)
PINK1	PARK6	AR	Mitochondrial serine–threonine kinase	NA	(Valente et al., 2004)
DJ-1	PARK7	AR	Oxidative stress response	NA	(Bonifati et al., 2003) (Clements et al., 2006)
LRRK2	PARK8	AD	Protein kinase	PD with diffuse LBs, tau inclusions and amyloid plaques	(Paisan-Ruiz et al., 2004; Zimprich et al., 2004)

MOI: mode of inheritance, AD: autosomal dominant, AR: autosomal recessive, PD: Parkinson's disease, LB: Lewy bodies.

The first gene mutation linked to familial PD was the missense mutation in the *α-synuclein* (α-syn) gene leading to an alanine-to-threonine change in position 53 (A53T) (Polymeropoulos et al., 1997). The same year α-syn was identified as a major component of Lewy bodies, which have long been recognized as the pathological hallmark of PD (Spillantini et al., 1997). Besides two other mutations (A30P and E46K) identified in the *α-syn* gene, duplications and triplications were also found to cause familial parkinsonism (Chartier-Harlin et al., 2004; Kruger et al., 1998; Singleton et al., 2003; Zarranz et al., 2004). The latter findings provided important clues to the toxicity mediated by α-syn as mere overexpression of the wild-type protein was sufficient to cause disease.

The association of ubiquitin proteasome system to PD was identified with the discovery of two genes involved in familial PD, *parkin* and *ubiquitin carboxy-terminal hydrolase L1* (*UCH-L1*) (Kitada et al., 1998; Leroy et al., 1998). These discoveries were followed by the identification of familial forms of PD caused by mutations in *DJ-1* and *PTEN-induced kinase 1 (PINK-1)* (Bonifati et al., 2003; Valente et al., 2004). Although the functions of these genes are poorly understood, both DJ-1 and PINK1 are localized to the mitochondria. Recently, six different mutations in the *Leucine-rich repeat kinase-2 (LRRK2)* gene have been linked to familial PD (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). Most of the patients carrying *LRRK2* mutations show typical symptoms of PD with variable age of onset but demonstrated a range of α-syn and tau pathology in neuronal and glial cells (Zimprich et al., 2004).

The incidence of PD is very low in young subjects remaining less than 1 in 10,000 up to the age of 60. Between 60 and 80 years of age, however, the risk increases exponentially corresponding to 3- to 4-fold higher numbers of patients diagnosed per decade (Van Den Eeden et al., 2003). Therefore, age is considered as one of the strongest risk factors for developing PD. Variations have been observed in the prevalence of parkinsonism in different populations suggesting that there might be different genetic susceptibilities or environmental risk factors (Barbosa et al., 2006; Chan et al., 2005; Okubadejo et al., 2006; Zhang et al., 2005). Epidemiologic studies suggest a relatively consistent association between exposure to pesticides and an increased risk of developing PD (Brown et al., 2006). Another epidemiological study demonstrated abnormally high frequency of atypical parkinsonism in Guadeloupe in French West Indies (Caparros-Lefebvre and Elbaz, 1999). They found out that these patients consumed significantly more fruit and herbal tea of a tropical plant, *Annona muricata*, and that these plants contained acetogenins inhibiting mitochondrial complex I, a property shared with several other dopaminergic neurotoxins.

Limitations of PD models based on specific neurotoxins

Until recently, most commonly used animal models for PD were based on systemic or localized injection of specific neurotoxins and thereby inducing degeneration of dopaminergic (DAergic) cells in the brain. 6-Hydroxydopamine (6-OHDA) has been widely used to create parkinsonian animals for almost 50 years (Ungerstedt, 1968). 6-OHDA is selectively taken up by the monoaminergic neurons, where it induces neurodegeneration by oxidative stress through formation of reactive oxygen species and quinones (Cohen, 1984). Today, 6-OHDA lesion models typically use injections into the medial forebrain bundle (MFB) or striatum, which induces a selective lesion of the ascending DAergic cells. In the latter case, the lesion is even more subscribed to the nigrostriatal projection and preserves the ventral tegmental area. In addition, by using different doses and injection sites it is possible to generate different levels of lesions corresponding to the different stages of PD (Hefti et al., 1980; Kirik et al., 1998; Sauer and Oertel, 1994). Another advantage of the 6-OHDA lesion model is that the toxin can be injected unilaterally to produce hemiparkinsonian animals where the intact side can be used as an internal control. The animals display overt motor impairments that are maintained long term. Nevertheless, this model does not mimic the neuropathological process, in particular the protein dysfunction/misfolding and aggregation, seen in PD patients. The 6-OHDA lesion model also fails to provide a true protracted insult but is a single hit