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Transgenic rodent models of Parkinson's disease

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Summary

In the case of Parkinson's disease (PD), classical animal models have utilized dopaminergic neurotoxins such as 6-hydroxydopamine (6OHDA) and 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP). More recently, human genetic linkage studies have identified several genes in familial forms of PD. Transgenic models have been made that explore the function of PD-linked genes (e.g. α-synuclein, DJ-1, LRRK2, Parkin, UCH-L1, PINK1). Recent evidence suggests mitochondrial dysfunction may play a major role in PD. Manipulation of mitochondrial respiratory genes (e.g. mitochondrial transcription factor A or TFAM) also elicits a PD phenotype in mice. Transgenic mice (MitoPark) were developed that have TFAM selectively knocked out in dopaminergic neurons. The nigral dopamine neurons of MitoPark mice show respiratory chain dysfunction, accompanied by the development of intraneuronal inclusions and eventual cell death. In early adulthood, the MitoPark mice show a slowly progressing loss of motor function that accompanies these cellular changes. The MitoPark mouse enables further study of the role of mitochondrial dysfunction in DA neurons as an important mechanism in the development of PD. Transgenic technology has allowed new insights into mechanisms of neurodegeneration for a number of neurological disorders. This paper will summarize recent studies on several transgenic models of PD.

Keywords

Parkinson's disease; DJ-1; PINK1; Parkin; transgenic; mitopark

Introduction

Parkinson's disease is emerging as a complex interplay of the environment and genetic risk factors. Overall, PD is primarily idiopathic with a subset (<15% of cases) with a family history of PD. In pedigrees with a pattern of inherited PD, genetic linkage studies have identified 13 PARK loci to date (OMIM 168600). Molecular genetics studies have identified genes associated with 7 of 13 PARK loci and we will be describing the current and possible transgenic animals for three of these genes (i.e. Parkin, DJ-1 and PINK1). We will also discuss a recently developed transgenic animal (MitoPark) that focuses on mitochondrial dysfunction as a pathogenic mechanism of PD.

Parkin (PARK2)

Studies of autosomal recessive inheritance pattern of early-onset PD in a group of Japanese families led to the identification of the Parkin gene at the PARK2 locus [24]. Additional studies have confirmed that mutations in Parkin are linked to autosomal recessively inherited PD.

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Unlike α-synculein that has few identified mutations, more than a 100 mutations have been identified in the Parkin gene [1, 18, 19, 26, 28, 29, 34]. Parkin has E3 ubiquitin-protein ligase activity [39] and targets proteins for degredation by the proteosome [8, 21, 46].

Several laboratories have generated Parkin knockout mice by targeting different exons of the Parkin gene [13, 22, 30, 33, 37, 45]. In mice missing exon 3, striatal dopamine levels are increased; synaptic excitability in striatal spiny neurons and DAT levels are decreased. However, the number of nigral dopaminergic neurons remains normal for up to 2 years. The mice exhibit behavioral deficits that are associated with the basal ganglia function and have decreased DA release in response to amphetamine [13, 22]. The exon 3 knockout mice show reduced mitochondrial respiration and increased oxidative damage [30]. Similar to exon 3 deletion, exon 7 deletion did not affect the nigral neuron numbers, but decreased TH-producing cells in the locus coeruleus [45]. In contrast, mice without exon 2 of Parkin exhibited no alterations in behavior, catecholamine levels or altered sensitivity to methamphetamine or 6-OHDA [32, 33]. Sato *et al*. [37] generated mice with a knockout of exon 2 and identified age-related declines in striatal dopamine and increase in D1/D2 receptor binding using ex-vivo PET imaging. Behavioral testing and immuno-labeling of dopaminergic nigral neurons revealed no abnormalities compared to wild-type mice [37]. Overall, Parkin knockout mice fail to develop a Parkinsonian phenotype, but the different knockout models may provide a means to examine the role of Parkin in protein turnover, oxidative stress and mitochondrial dysfunction.

DJ-1 (PARK7)

The DJ-1 gene was identified at the PARK7 locus [44] with a point mutation (L166P) that cosegregated with the disease allele in an Italian family [5]. PARK7, like PARK2 is inherited in an autosomal recessive manner. Many mutations in the DJ-1 gene have been associated with early onset PD [2,3,5,15, 17]. DJ-1 is involved in multiple cellular processes including oxidative stress and cellular transformation [27].

Based on a mutation observed in human DJ-1 by Bonifati *et al*. [5], a transgenic mouse missing the first 5 exons and part of the promoter of DJ-1 was created [7]. No observable expression of DJ-1 was observed in the homozygous null mice which did show a progressive decline in selected motor tests. There was increased striatal dopamine and evoked dopamine overflow in the striatum. There was no change in the number of nigral dopaminergic neurons or markers of these neurons [7]. Similarly, by disrupting exon 2 of DJ-1, Goldberg *et al*. [14] generated mice with decreased evoked dopamine overflow in the striatum and lower locomotor activity compared to wild-type mice. No change in the number of dopaminergic neurons of the substantia nigra was observed [14]. A third study by Kim *et al*. [23] generated a DJ-1 knockout by also disrupting exon 2, the first coding exon of DJ-1, and found no change in striatal dopamine levels or nigral dopaminergic neuron numbers. These mice did exhibit decreased locomotion in response to amphetamine and increased sensitivity to MPTP which could be restored by viral vector delivery of DJ-1 to the striatum. Cortical neurons derived from embryonic brain of DJ-1 knockout mice were more sensitive to oxidative stress [23]. Overall, these studies demonstrate that absence of DJ-1 expression 1) decreases motor functions and 2) alters dopamine function in the nigrostriatal pathway. The DJ-1 knockout mice may provide a useful platform for testing gene therapeutic strategies for patients carrying deletion mutations and offer opportunities to study the function of DJ-1 in oxidative stress.

PINK1 (PARK6)

Analysis of the PARK6 locus on chromosome 1 [43] led to the identification of point mutations in the PINK1 gene, a putative mitochondrial kinase [42]. Mutations in PINK1 are the second most frequently occurring cause of autosomal recessively inherited early-onset PD [6,16, 20,

35, 36, 40]. PINK1 is localized throughout the brain and colocalizes to mitochondria where it is thought to prevent mitochondrial dysfunction [12].

Recently, two studies describe knockdown [47] and knockout [25] of PINK1 gene in mice. First, Zhou *et al*. [47] used RNAi and the Cre-loxP system to induce expression of a PINK1 shRNA in the presence of Cre. Using CMV-Cre transgenic animals crossed with inactive PINK1 shRNA espression, they observed widespread silencing of the PINK1 gene in brain and other tissues. Despite decreased PINK1 mRNA and protein, no change in striatal dopamine, nigral dopaminergic neurons numbers and motor activity (rotarod test) was observed in the PINK1 knockdown mice compared to wild-type mice [47]. In the second study, Kitada *et al*. [24] created a PINK1 knockout mouse by deleting exons 4–7 (kinase domain) and introducing a nonsense mutation starting in exon 8. Mice deficient in PINK1 expression had normal levels of striatal dopamine and nigral dopaminergic neurons. Similar to observations of DJ-1 knockout mice, evoked dopamine overflow in the striatum is reduced in PINK1 knockout mice [25]. Mutations in both DJ-1 and PINK1 genes have been identified in subset of patients with early onset PD. Biochemical studies suggest DJ-1 stabilizes PINK1 and works cooperatively to protect cells against oxidative stress [41]. Studies with Drosophilia found that PINK1 may function through a similar pathway as Parkin as well [9, 31] Future studies examining the interactions of PINK1, Parkin and DJ-1 may lead to the development of a mouse model that more closely resembles the pathology of PD.

MitoPark mice

Indirect evidence suggests a role for mitochondrial dysfunction in sporadic PD [10]. In addition, studies of families with rare inherited forms of PD have identified genes involved in regulating mitochondrial function [9, 31, 38]. The hypothesis that mitochondrial dysfunction may be of etiological importance in PD has recently gained renewed attention because it has been shown that PD patients have an increased number of midbrain DA neurons with respiratory chain deficits compared to non-PD patients [4]. To experimentally test whether the respiratory chain deficiency was a primary abnormality leading to inclusion formation and DA neuron death, or whether generalized metabolic abnormalities within the degenerating DA neurons cause secondary damage to mitochondria, a conditional knockout of the mitochondrial transcription factor A (Tfam) in DA neurons was created [11]. When crossed with a DAT-Cre transgenic mouse, these knockout mice (termed MitoPark mice) had reduced mtDNA expression and a respiratory chain deficiency in midbrain DA neurons. The knockout mice exhibited a Parkinsonian phenotype with adult onset of slowly progressive impairment of motor function accompanied by formation of intraneuronal inclusions and dopamine nerve cell death. Confocal and electron microscopy show that the inclusions contain both mitochondrial protein and membrane components. Overall, the MitoPark mice are a transgenic model of PD based on an underlying deficiency of the repiratiory chain in dopaminergic neurons of the midbrain. The MitoPark mice display several essential features of PD including 1) adult onset of neurodegeneration, 2) slowly progressive behavioral changes, 3) presence of intraneuronal inclusion (possibly a Lewy body equivalent), 4) preferential death of dopaminergic neurons in the SN compared to the VTA and 5) responsiveness to leva-dopa. The MitoPark mice may provide a useful model for testing potential therapies for the treatment of Parkinson’s disease.

Concluding remarks

As mutations in human genes are identified and associated with neurodegenerative disease, transgenic models offer an opportunity to invasively study the mutant gene or combination of mutant genes and their role in the pathophysiology of the disease. More importantly, they provide a platform to evaluate potential genetic and pharmacological therapies.

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