Journal Club

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Mice Expressing Mutant Parkin Exhibit Hallmark Features of Parkinson's Disease

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Review of Lu et al. (http://www.jneurosci.org/cgi/content/full/29/7/1962)

Parkinson's disease (PD) is the second most prevalent late-onset neurodegenerative disorder and affects ~1% of the population older than 65 years. PD manifests as a movement disorder clinically characterized by resting tremor, bradykinesia, and postural rigidity. Hypokinetic movements, along with the progressive loss of dopaminergic (DA) neurons in the substantia nigra (SNc) and accumulation of α-synuclein, are the hallmark characteristics of the disease. Although ~95% of PD cases appear to be sporadic, familial cases have led to the discovery of PD-linked genes, including α-synuclein, parkin, DJ-1, PINK1, LRRK2, and ATP13A2 (Yang et al., 2009). Mutations in the parkin gene, which encodes an E3 ubiquitin ligase involved in proteasomal degradation, are a major cause of hereditary PD (Lücking et al., 2000). Parkin mutations result in early-onset autosomal recessive PD; however, some evidence also indicates that heterozygous mutations in parkin may increase susceptibility to sporadic late-onset disease (Klein et al., 2007).

Current animal models of PD have facilitated the study of parkinsonian mechanisms and disease processes, but almost all fail to accurately recapitulate all of the hallmark pathologies and symptoms. Thus, an animal model of PD that exhibits progressive neurodegeneration of relevant neuronal populations, as well as the appropriate neurochemical, neuropathological, and behavioral alterations, is desperately needed (Moore and Dawson, 2008). Models of PD generated through administration of neurotoxins, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and paraquat, mimic some aspects of PD but are incomplete and often lack progressive onset at older ages, which may be particularly relevant to the pathoetiology of PD (Dauer and Przedborski, 2003). Other animal models using the genetics of familial PD have generated phenotypes that mimic PD, but have been only partly successful in fully modeling the disease (Table 1). While this article focuses on murine models of PD, other genetic model systems have provided useful and equally representative genetic PD models (Auluck et al., 2002; Outeiro and Lindquist, 2003; Hamamichi et al., 2008). Multiple groups have shown that mice lacking parkin expression do not provide a model of disease, but have allowed characterization of the normal function of wild-type parkin (Palacino et al., 2004). Two groups recently demonstrated that expressing forms of mutant parkin protein, including a truncation mutant parkin-Q311X, in *Drosophila* resulted in age-dependent degeneration of DA neurons. While wild-type parkin appears to be neuroprotective and knock-out of parkin results in some toxicity, these mutants showed a dominant gain of function causing additional toxicity (Sang et al., 2007; Wang et al., 2007). These studies prompted the question of whether expressing mutant parkin in a mammalian system *in vivo* would have similar pathological effects.

The recent article by Lu et al. (2009) in *The Journal of Neuroscience* demonstrates that expression of truncated Q311X mutant parkin in mice results in age-dependent disease phenotypes that resemble the hallmark characteristics of PD. To generate the mice, the authors inserted the Q311X mutated human *parkin* into exon 2 of the *Slca3* gene (dopamine transporter) contained within a bacterial artificial chromosome (BAC) [Lu et al. (2009), their Fig. 1]. Analysis of two lines of transgenic mice, one with a single copy (line A) and one with tandem integration of two copies (line D), demonstrated that the transgene was specifically expressed in DA neurons in the SNc and ventral tegmental area (VTA) [Lu et al. (2009), their Fig. 1, Fig. 2]. The authors analyzed expression of the transgene at the mRNA level to ensure that phenotypes were not simply attributable to high levels of mutant protein, and showed that parkin-Q311X mRNA levels were 42% or lower than endogenous mouse parkin.

The authors first assessed the transgenic mice for behavioral motor phenotypes using standard motor function tests, including the challenging beam traversal, the cylinder test, adhesive removal, and open field locomotor activity. Parkin-

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Table 1. Examples of rodent models used to study Parkinson's disease genes

Genetic model	Description	PD phenotype			Reference
		Motor deficits	DA neuron degeneration	α-Synuclein pathology	
α-Synuclein (PARK1, PARK4)	Overexpression of WT α-synuclein using PDGF-β promoter	Yes	Subtle defects	Yes	Masliah et al. (2000)
	Expression of A53T mutant α-synuclein using mouse prion promoter	Yes (lethal)	No	Yes	Giasson et al. (2002); Lee et al. (2002)
	Expression of A30P mutant α-synuclein using Prp promoter	Yes (progressive)	No	Atypical	Gomez-Isla et al. (2003)
	Lentivirus injection of WT or A30P (rat)	No	Yes	Yes	Lo Bianco et al. (2002)
Parkin (PARK2)	Knock-out	Subtle	No	No	Goldberg et al. (2003); Itier et al. (2003)
	Expression of mutant parkin-Q311X using dopamine transporter promoter in a bacterial artificial chromosome	Yes (progressive)	Yes (selective and progressive)	Atypical	Lu et al. (2009)
DJ-1 (PARK7)	Knock-out	Yes	Subtle defects	No	Goldberg et al. (2005); Chen et al. (2005)
PINK1 (PARK6)	Knock-out	Yes	Subtle defects	No	Kitada et al. (2007)
Engrailed (En1 +/−)	Knock-out	Yes	Yes (selective and progressive)	No	Le Pen et al. (2008)
MitoPark (Tfam)	Conditional knock-out, using Cre expressed in dopaminergic neurons (dopamine transporter promoter-Cre)	Yes (progressive)	Yes (selective and progressive)	Atypical	Ekstrand et al. (2007)

Advantages and disadvantages of different models are evaluated using three criteria: (1) motor deficits, (2) DA neuron degeneration, and (3) pathological changes in neurons. All are mouse models except where indicated.

Q311X mice showed normal motor function at 3 months in six of seven analyses of the motor tests. However, deficits in motor function were demonstrated at 16–19 months with significant decreases in motor function in six of seven analyses [Lu et al. (2009), their Fig. 3]. Although the transgenic mice showed progressive age-onset motor deficits, the robustness of these phenotypes was not overwhelmingly strong.

Lu et al. (2009) further demonstrated that DA neurons were lost progressively and specifically in the SNc, and to a lesser extent in the VTA, and indicated that there is a slight reduction in overall tyrosine hydroxylase (TH) gene expression. Design-based stereology quantification revealed that parkin-Q311X mutants have normal numbers of DA neurons at 3 months in the SNc, as shown by immunoreactivity to TH. However, these mice showed a significant ~40% reduction in TH-positive cells and a corresponding ~30% reduction in total neuron number as shown by Nissl-positive cells at 16 months [Lu et al. (2009), their Fig. 5]. These results were further validated using TH-GFP-expressing mice as an independent control of TH-expressing neurons. Furthermore, the loss of DA neurons appears to be progressive and age dependent. In addition to the loss of DA neurons in the SNc, the authors demonstrate a loss of striatal DA terminals in the parkin-Q311X mutants, thus representing another clinically relevant characteristic of human PD [Lu et al. (2009), their Fig. 7].

The authors next analyzed midbrain sections of the parkin-Q311X mice for immunoreactivity to proteinase K (PK)-resistant α-synuclein [PK(R)-Syn], a form of misfolded and relatively insoluble α-synuclein. This analysis revealed comparable levels of PK(R)-Syn in wild-type and mutant mice at 3 months. At 16 months, wild-type mice had little or no PK(R)-Syn immunoreactivity, while parkin-Q311X mice showed high levels of immunoreactivity [Lu et al. (2009), their Fig. 8]. The authors noted that the PK(R)-Syn was cytoplasmic, unlike that observed in traditional Lewy bodies, suggesting that the α-synuclein pathology observed with parkin-Q311X may differ from classical pathology. The authors do not explicitly distinguish whether PK(R)-Syn was localized in DA neurons. To address whether oxidative stress played a role in the observed toxicity, the PK(R)-Syn immunoreactivity was analyzed for colocalization with the marker 3-nitrotyrosine. The results demonstrate that 3-nitrotyrosine is colocalized with the PK(R)-Syn [Lu et al. (2009), their Fig. 9]. The results from this article demonstrate that in addition to progressive age-onset motor deficits and DA neuron loss, the parkin-Q311X mice also model some aspects of PD pathology of age-onset synucleinopathy, and hint that this may be because of changes in oxidative stress.

The construction of a wide range of animal models of PD has allowed extensive research into the molecular mechanisms and genetic susceptibilities that may cause or be a part of PD. However, an accurate model that captures all of the pathologies of PD has yet to be created. The Q311X mice created in the paper by Lu et al. (2009) demonstrate age-onset progression of the major hallmark pathologies of PD. The authors demonstrate progressive motor deficits using motor tests commonly used in PD animal models (Meredith and Kang, 2006), as well as DA neuron loss in brain regions relevant to PD. While pan-neuronal expression of parkin-Q311X in *Drosophila* indicated DA neuron-specific degeneration (Sang et al., 2007), it would be interesting to replicate in a mammalian system. Although α-synuclein pathology and the presence of Lewy bodies in parkin related cases remains to be fully elucidated, evidence suggests that parkin plays a role in formation of α-synuclein inclusion bodies (von Coelln et al., 2006). α-Synuclein pathology in parkin cases may vary in response to a loss of function versus a toxic gain of function as observed with parkin-Q311X. However, inconsistencies between parkin-Q311X mouse α-synuclein pathology with parkin clinical manifestations remain to be resolved. The paper confirms that parkin-Q311X toxicity is likely due to a dominant gain of toxic function, albeit probably in addition to the consequences of a loss of function.

Following publication of this article, a

study demonstrated that parkin is part of a ubiquitin ligase complex with two other PD genes, DJ-1 and PINK1 (Xiong et al., 2009). These results are supported by the similarity of phenotypes observed in knock-out animals of parkin, DJ-1, or PINK1 (Moore and Dawson, 2008). The finding that three different PD-linked genes converge on a single pathway/complex sheds light on susceptibility factors for PD and corroborates that the parkin-Q311X mice could provide a useful model to further study this complex and the interactions of these proteins. Study and characterization of the Q311X mice will give insight into the mechanisms of parkin toxicity, both normal and mutant, and may give clues to similar pathobiologies in development of familial and sporadic PD. Age-related changes in oxidative stress pathways may play a significant role in the etiology of PD, demonstrating the necessity for a model of age-onset progressive degeneration, which the parkin-Q311X mice may fulfill.

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