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Strengths and Limitations of Genetic Mouse Models of Parkinson's Disease

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Abstract

Genetic mouse models based on α-synuclein overexpression are particularly compelling because abnormal accumulation of α-synuclein occurs in sporadic Parkinson's disease (PD). Our laboratory has characterized a mouse overexpressing wild-type human α-synuclein under the Thy1 promoter, which confers broad expression of the transgene in neurons. These mice show progressive sensorimotor anomalies starting at 2 months of age, as well as olfactory and digestive deficits similar to those observed in patients at early stages of PD. Patterns of gene expression examined in nigrostriatal neurons isolated by single-cell laser capture microdissection in these mice at 6 months of age show an upregulation of defence mechanisms including increased levels of genes involved in proteasome and mitochondrial function, as well as cholesterol biosynthesis. At the same time, numerous alterations in genes encoding ion channels suggest that changes in the cellular function of these neurons occur independently of cell death. These data provide information on the early effects – in a mammalian brain – of a mutation known to cause PD, and they identify a number of useful end-points for evaluating potential neuroprotective therapies that could interfere with the pathophysiological mechanisms of PD upstream of neuronal cell death.

Keywords

α-synuclein; genetic models; Parkinson's disease; Thy1 promoter

Introduction

Currently, one of the most contentious issues in the field of Parkinson's disease (PD) is the definition of the "right" animal model with which to study the disease. This is an important question not only for investigators but also for patients. Indeed, what model is used for preclinical testing of new therapies will influence the success of these therapies in the clinic. Furthermore, uncertainties about model adequacy slow progress towards the development of new treatments, as the biotech and pharmaceutical industry are weary of investing in a field that does not have an established, well-characterized, "predictive" animal model for drug testing.

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This problem has only recently become an issue. Since the 1960s, investigators have performed selective lesions of the nigrostriatal dopaminergic system, the neurons most strikingly affected in PD, by locally injecting 6-hydroxydopamine into the substantia nigra, the median forebrain bundle or the striatum [1]. More recently, peripheral injections of MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), paraquat, rotenone and a handful of other toxins have been used to lesion the nigrostriatal dopaminergic neurons in rats and mice [2–6]. All these methods have been extremely useful in examining the consequences of the loss of striatal dopamine and in assessing dopaminergic therapies that have joined the arsenal of symptomatic treatments now offered to patients [7,8]. They have also been widely used to test neuroprotective therapies. Unfortunately, the validity of these models to predict a disease-modifying effect in patients is questionable. Indeed, none of the strategies that protect neurons from the effects of 6-hydroxydopamine or MPTP have yet been successful in the clinic [9].

From toxins to genes

A major problem with the classic toxin-induced models is that they reproduce parkinsonism, which is an ensemble of symptoms related to a loss of nigrostriatal dopamine of various origins, but not PD, which is a neurodegenerative disorder that affects many regions of the nervous system. One defining characteristic of PD is the presence of cytoplasmic proteinaceous aggregates called “Lewy bodies” in distinct populations of central and peripheral neurons. Recent pathological studies have shown that Lewy bodies and other forms of α-synuclein aggregation are almost invariably present in certain brain areas, including the olfactory bulb, dorsal nucleus of the vagus and locus coeruleus, in patients with Lewy bodies and neuronal loss in the substantia nigra pars compacta [10,11]. Furthermore, investigators have found pathology in these regions in “control” cases without loss of dopaminergic neurons or typical parkinsonian symptoms [12]. Together with increasing evidence that a host of non-motor symptoms can precede by many years the onset of the characteristic triad of tremor, akinesia and rigidity, these data strongly suggest that PD affects multiple neuronal systems [13,14]. Non-motor symptoms of PD are varied and include olfactory loss, sleep and affective disorders, and even autonomic and digestive dysfunction [14]. In addition, patients often experience additional motor symptoms such as freezing and balance problems that are not responsive to dopaminergic therapies, suggesting that, in common with non-motor symptoms, they are not primarily due to dopaminergic loss [13]. One implication of these findings is that animal models that only reproduce the loss of nigrostriatal dopaminergic neurons give a very incomplete picture of the disease.

Another major problem with classic toxin-induced models is that they do not reproduce mechanisms that are known to be involved in the disease. It has been argued that neurotoxicity in these models is due to oxidative stress, which may also be involved in the pathophysiology of PD [5], but there is no direct evidence linking 6-hydroxydopamine, rotenone or MPTP with sporadic forms of PD. One exception may be the pesticide paraquat, as long-term exposure to paraquat has been linked to an increased risk of PD [15]. However, paraquat is unlikely to be a single causal factor for the disease and, when administered to rodents, it does not reproduce all the hallmark characteristics of PD [3].

A more compelling approach would be to use genetic findings linking specific mutations to familial forms of PD to design more accurate models of the disease [16]. This approach has been widely used in the last several years but has met with some disappointment because few of these models reproduce all the features of the disease. Most strikingly and with few exceptions, the majority of mice genetically altered to express PD-causing mutations do not show extensive and progressive loss of nigrostriatal dopaminergic neurons [17,18]. The question then becomes: can a mouse without dopaminergic cell loss tell us something about

PD and serve as a useful preclinical model for testing neuroprotective drugs? The answer depends on the mutation expressed and the promoter used.

α-synuclein overexpression

Familial forms of PD are very rare and each mutation only causes a very small number of cases compared with the frequency of sporadic cases [16]. To date, a direct link between a PD-causing mutation and sporadic forms of the disease has only been established for α-synuclein overexpression. α-synuclein can cause PD either when mutated or when the wild-type form is increased due to gene duplication or triplication [16]. Furthermore, polymorphisms in the α-synuclein promoter that increase the level of protein are associated with an earlier age of onset of sporadic cases of PD. Therefore, mice overexpressing wild-type human α-synuclein have excellent construct validity as models of sporadic PD [17]. It is important to remember, however, that α-synuclein is widely expressed in human neurons and that α-synuclein pathology is widespread in PD as described earlier [11,12]. Therefore, an accurate model of PD must reflect this broad distribution. This would not be accomplished by driving α-synuclein overexpression only in catecholaminergic neurons, for example, with a tyrosine hydroxylase promoter. One caveat is that some broad promoters have particularly high levels of expression in neurons that are typically not affected in PD, resulting in patterns of cell death that do not reproduce those seen in patients [19]. This is particularly the case with the prion promoter that drives high levels of transgene expression in motoneurons, with subsequent motoneuron degeneration. Background strain can also modify the pattern of transgene expression as shown by high levels of motoneuron pathology in mice overexpressing α-synuclein under the Thy1 promoter in the FVB background but not in the C57Bl6 background (T. Sudhof, personal communication).

One expectation for mice that broadly overexpress α-synuclein in neurons normally affected in PD is that they should reproduce some of the non-motor symptoms observed in patients with PD [14]. Interestingly, these symptoms often precede classic motor symptoms, thus suggesting that they could be present even if the mice do not show dopamine cell loss. Indeed, our laboratory has extensively characterized a mouse model overexpressing wild-type human α-synuclein under the Thy1 promoter in a mixed C57Bl6/DBA background [20]. Similarly to PD patients, these mice exhibit reduced olfaction as manifested by reduced time sniffing novel odors in different tests or a longer time to detect a buried scented food pellet [21]. The deficits can be observed as early as 3 months of age and are still present at 9 and 11 months. Control experiments show that the deficits are not due to impairments in the motor skills necessary for the test. These mice show motor deficits as early as 2 months of age only when tested in challenging tasks especially designed to reveal deficit in the nigrostriatal system and basal ganglia function [22]. These include a challenging beam test where a narrowing beam is overlaid with a metal grid, the pole test where time to turn and descend a centimetre-wide wooden pole is measured, a cylinder test used to measure hindlimb and forelimb movements, and the cotton bin test in which the ability of the animal to grasp cotton in a feeding bin is measured [23].

Unlike what is observed in PD, deficiency in these motor tasks is not reversed by L-dopa or apomorphine, a dopaminergic agonist in these mice [24]. This is probably due to the fact that the animals have a full complement of dopaminergic neurons and terminals. In support of this hypothesis, the motor deficits are even worsened by the dopaminergic agonists, an observation reminiscent of the inverted U curve of dopamine effects on motor and cognitive function [24]. Thus, the early motor deficits observed in mice overexpressing α-synuclein under the Thy1 promoter are clearly distinct from those observed in patients with manifest PD. They are indicative, however, of neuronal dysfunction caused by a mutation known to cause PD in humans. Moreover, these mice reproduce pathological findings in early PD (broad-based but

regionally selective accumulation of insoluble α-synuclein) and exhibit multiple deficits observed in early PD, including olfactory deficits and autonomic dysfunction [21,25]. Therefore, they may represent very early stages of the disease and provide information on mechanisms that initiate the pathophysiological cascades in PD.

Of note, these mice show a decreased level of noradrenaline in the cerebral cortex, profound synaptic dysfunction in the striatum and anomalies in ion channel gene expression in nigrostriatal dopaminergic neurons (N. Maidment, personal communication) [26,27]. Most importantly, transcriptome analysis of nigrostriatal dopaminergic neurons isolated by laser capture microdissection in these mice reveals an upregulation of numerous genes that presumably could contribute to an increased resilience of these neurons [27]. This may explain why nigrostriatal neurons do not die at early ages in these mice and points to efficient defence mechanisms against the effects of α-synuclein overexpression and abnormal accumulation. This is probably one of the most promising contributions provided by genetic mouse models of PD that do not lose their nigrostriatal dopaminergic neurons for the discovery of effective neuroprotective strategies for the disease.

In summary, available genetic mouse models of PD should be considered as a valid alternative to the clearly flawed classically used toxin-based models. Although the lack of nigrostriatal dopaminergic cell loss is often interpreted as the absence of the “hallmark of PD”, one should remember that these new models strikingly reproduce early characteristics of the disease [10, 11]. Accordingly, they should not be discounted but used judiciously to bring new information on PD and its treatment.

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