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The mitochondrial permeability transition pore regulates Parkinson's disease development in mutant α-synuclein transgenic mice

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ABSTRACT

Parkinson's disease (PD) is a movement disorder caused by neurodegeneration in neocortex, substantia nigra and brainstem, and synucleinopathy. Some inherited PD is caused by mutations in α-synuclein (αSyn), and inherited and idiopathic PD is associated with mitochondrial perturbations. However, the mechanisms of pathogenesis are unresolved. We characterized a human αSyn transgenic mouse model and tested the hypothesis that the mitochondrial permeability transition pore (mPTP) is involved in the disease mechanisms. C57BL/6 mice expressing human A53T-mutant αSyn driven by a *thymic antigen-1* promoter develop a severe, age-related, fatal movement disorder involving ataxia, rigidity, and postural instability. These mice develop synucleinopathy and neocortical, substantia nigra, and cerebello-rubro-thalamic degeneration involving mitochondriopathy and apoptotic and non-apoptotic neurodegeneration. Interneurons undergo apoptotic degeneration in young mice. Mutant αSyn associated with dysmorphic neuronal mitochondria and bound voltage-dependent anion channels. Genetic ablation of cyclophilin D, an mPTP modulator, delayed disease onset, and extended lifespans of mutant αSyn mice. Thus, mutant αSyn transgenic mice on a C57BL/6 background develop PD-like phenotypes, and the mPTP is involved in their disease mechanisms.

1. Introduction

Parkinson's disease (PD) is a chronically progressive, age-related, fatally incapacitating movement disorder in humans. Estimates indicate that 4–6 million people are diagnosed with PD, and this disease affects about 2% of the population at some time in life (Dorsey et al., 2007; Van Den Eeden et al., 2003). The greatest prevalence of PD occurs in the USA, with 100–250 cases per 100,000 (Van Den Eeden et al., 2003), placing PD as the second most common neurodegenerative disease with an adult onset (after Alzheimer's disease). Progressive resting tremor, rigidity, bradykinesia/akinesia, gait disturbance, and postural instability characterize PD clinically (Jankovic, 2008; Olanow and Tatton, 1999). Cardinal neuropathological features of PD are degeneration and elimination of dopamine neurons in substantia nigra (SN) and in other brainstem regions, dopamine depletion in striatum, and α-synuclein (αSyn) pathology (Dickson, 2012; Giasson et al., 2000a; Jellinger, 2012; Lowe et al., 1997; Olanow and Tatton, 1999). The molecular pathogenesis of PD is not understood. At least 2 forms of PD exist: idiopathic (sporadic) and heritable (familial) (Klein and Schlossmacher, 2007; Olanow and Tatton, 1999; Schapira, 2006a). Most PD cases are sporadic with no known genetic component. Epidemiologic studies reveal several risk factors for developing idiopathic PD. Aging and αSyn are the most common risk factors for idiopathic PD (Klein and Schlossmacher, 2007). Pesticides are also linked to the origin of PD (Ascherio et al., 2006; Tanner et al., 2011). Herbicides, well water (contaminated with pesticides), and industrial chemicals are possible environmental agents related to the development of PD (Schapira, 2006b).

Mitochondrial mechanisms are believed to be involved in PD pathogenesis (Coskun et al., 2012; Olanow and Tatton, 1999; Pilsl and Winklhofer, 2012). Mitochondria became suspects in PD etiology when heroine abusers presented with PD after exposure to 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (Langston and Ballard, 1983) which is converted by monoamine oxidase to a complex I inhibitor and free radical generator (Cleeter et al., 1992; Nicklas et al., 1985; Ramsay et al., 1986). Mitochondrial

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involvement in idiopathic PD etiology became more compelling by the discovery that complex I activity (NAPH dehydrogenase) is reduced in the SN (Schapira et al., 1989) and skeletal muscle (Bindoff et al., 1989) of PD cases. Complex I inhibitors, notably 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine and the pesticide rotenone, cause damage to dopaminergic neurons and are the basis of several animal and cell models of PD (Shimohama et al., 2003). Although mitochondrial mechanisms have been implicated in PD pathogenesis for over 2 decades, direct cause-effect relationships between mitochondrial damage and disease initiation and progression are still unclear. Mutations in nuclear genes encoding definite mitochondrial proteins such as complex I, mitofusin 2, frataxin, and optic atrophy protein 1, as well as DNA polymerase δ, adenine nucleotide translocase 1, and twinkle, have been linked to Leigh's syndrome, Charcot-Marie-Tooth disease type 2, Friedreich's ataxia, autosomal dominant optic atrophy, and chronic progressive external opthalmoplegia, respectively (Schapira, 2012), but linkage of these mutations to PD has not been seen. Furthermore, mutations in the mitochondrial DNA encoded complex I subunits cause Leber's hereditary optic neuropathy (Vilkki et al., 1989) but not PD (Olanow and Tatton, 1999). About 5%–10% of people with PD have familial inheritance (Schapira, 2006a, 2006b). Most identified mutations occur in genes encoding proteins that have both non-mitochondrial and putative mitochondrial functions, including αSyn (Polymeropoulos et al., 1997; Singleton et al., 2003), DJ-1 (Bonifati et al., 2003), parkin (Kitada et al., 1998; Leroy et al., 1998), and leucine-rich repeat kinase-2 (Paisán-Ruíz et al., 2004; Zimprich et al., 2004), confounding the interpretation of the intracellular mechanisms leading to PD. However, mutations in phosphatase and tensin homolog-induced putative kinase-1 (PINK1) do directly link mitochondrial dysfunction to the etiology of some early-onset recessive forms of PD (Hatano et al., 2004; Pilsl and Winklhofer, 2012; Valente et al., 2004), but PINK1 null mice do not develop neurodegeneration, despite mitochondrial abnormalities (Gispert et al., 2009). Thus, further interrogation of mitochondrial dysfunction as a general cause of PD is still needed.

Because αSyn and mitochondria both seem to have roles in PD, it is possible that interplay between these 2 entities participates in PD pathogenesis (Hsu et al., 2000). αSyn is largely a cytoplasmic and nuclear protein that is enriched in nervous tissue axon terminals (Maroteaux et al., 1988; Murphy et al., 2000; Lesuisse and Martin, 2002) and functions in presynaptic vesicle dynamics during activity-dependent neurotransmitter release (Chandra et al., 2005; Fortin et al., 2005); yet, it also has actions at mitochondria. αSyn associates with mitochondrial membranes in neurons (Nakamura et al., 2011) and has been found to be imported into mitochondria to cause complex I impairment (Devi et al., 2008). Human αSyn (hαSyn) with an alanine-53→threonine mutation (A53T) directly interacts with mitochondria in transgenic (tg) mice, and these mice develop mitochondrial abnormalities in association with a severe movement disorder, synucleinopathy, and a shortened lifespan (Lee et al., 2002; Martin et al., 2006) but no loss of dopaminergic neurons in substantia nigra (Daher et al., 2012). Unexpectedly, the motor abnormality and neuropathology in mice with prion protein promoter-driven hαSyn-A53T transgene expression is more consistent with a motor neuron disease, rather than a PD phenotype, because of the profound loss of spinal motor neurons (Martin et al., 2006). Tg mice expressing hαSyn-A53T driven by the human neuron-specific *thymic antigen-1* (Thy1)-promoter have also been generated, but their neurologic and neuropathological characterization is limited (Chandra et al., 2005) and relevance to PD as a disease model is uncertain. The brain distribution of pathology and possible mitochondrial mechanisms of disease in these mice have not been studied. In this study we characterized the neuropathology in Thy1-hαSyn-A53T tg mice and tested the hypothesis that mitochondrial abnormalities are related causally to the disease process in PD-linked mutant αSyn tg mice through the mitochondrial matrix protein cyclophilin D (CyPD) that modulates the mitochondrial permeability transition pore (Baines et al., 2005; Bernardi et al., 2006; Crompton, 2004; Halestrap, 2009; Nakagawa et al., 2005).

2. Methods

2.1. Transgenic mice

The PD mice studied here were Thy1-hαSyn-A53T tg mice. The original founder mouse of line B6.Cg-Tg[Thy1-SNCA*A53T]M53Sud/J (stock #008135) was purchased from The Jackson Laboratory (Bar Harbor, ME, USA). There is no characterization of the brain neuropathology in these mice. We bred this line, a hybrid of SV129 and C57BL/6 strains, with pure C57BL/6 mice and then progeny were backcrossed at least 7 generations into a pure C57BL/6 strain background with the goal of eliminating the SV129 background. The SV129 genetic background is known to increase susceptibility to excitotoxic and necrotic neurodegeneration (Kofler et al., 2004; Schauwecker and Steward, 1997), and thus we wanted to minimize this prominent strain effect. All mice were genotyped at the age of 1 month to identify individuals with the A53T transgene. The primer pair used for polymerase chain reaction was: 5′-GGCACC TAGAGGATCTCGACTAGTGG-3′ (forward) and 5′-GGACCTCGACGCT TAAGGCTTCAGG-3′ (reverse). The agouti fur coat was eventually eliminated and studies were conducted exclusively on Thy1-A53T tg mice on a C57BL/6 background (black fur coats). We evaluated A53T mice at presymptomatic stages of disease (n = 10), early- to mid-stages of disease (n = 10), defined by presence of bradykinesia and ataxia, and at near endstage disease (n = 20), defined by postural stiffness and immobility (Supplementary Data Videos 1 and 2). Age-matched non-tg littermates served as controls. The CyPD null mice are described elsewhere (Basso et al., 2005; Martin et al., 2011). The GlyT2-eGFP tg mice, with expression of eGFP only in glycinergic interneurons, are described elsewhere (Martin, 2011; Zeilhofer et al., 2005). The institutional Animal Care and Use Committee approved the animal protocols.

2.2. Brain harvesting and processing for histology

Mice were anesthetized with an overdose of sodium pentobarbital and perfused through the heart with ice-cold phosphate buffer-saline (100 mM, pH 7.4) followed by ice-cold 4% paraformaldehyde. After perfusion-fixation, the brain was removed after 2 hours, post-fixed overnight in 4% paraformaldehyde, and cryoprotected 24 hours in 20% glycerol-phosphate buffer-saline. The brains were frozen and serially sectioned from frontal pole to posterior cerebellum in the coronal plane at 40 μm on a sliding microtome with every section being saved individually in 96-well plates containing antifreeze buffer. The sections were stored at −20 °C. For histologic analyses, sections were selected systematically and stained using cresyl violet (CV) for cell morphology and counting, FD-silver for neurodegeneration, the terminal transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) method (Martin, 1999; Martin et al., 2006; Portera-Cailliau et al., 1997), as an assay for cell death based on the detection of DNA double-strand breaks, and immunohistochemistry.

2.3. Immunohistochemistry

We evaluated the localizations of hαSyn, tyrosine hydroxylase (TH), the interneuron marker parvalbumin (Kita et al., 1990), selected cell death proteins, and putative mitochondrial permeability