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α-Synuclein: Multiple system atrophy prions

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Running title: α-Synuclein prions in multiple system atrophy

ABSTRACT

Multiple system atrophy (MSA) is a rapidly progressive neurodegenerative disease arising from the misfolding and accumulation of the protein α-synuclein in oligodendrocytes, where it forms glial cytoplasmic inclusions (GCIs). Several years of studying synthetic α-synuclein fibrils has provided critical insight into the ability of α-synuclein to template endogenous protein misfolding, giving rise to fibrillar structures capable of propagating from cell to cell. However, more recent studies with MSA-derived α-synuclein aggregates have shown that they have a similar ability to undergo template-directed propagation, like prions. Almost 20 years after α-synuclein was discovered as the primary component of GCIs, α-synuclein aggregates isolated from MSA patient samples were shown to infect cultured mammalian cells and also to transmit neurological disease to transgenic mice. These findings argue that α-synuclein becomes a prion in MSA patients. In this review, we discuss the in vitro and in vivo data supporting the recent classification of MSA as a prion disease.

INTRODUCTION

Multiple system atrophy (MSA) is a sporadic neurodegenerative disease affecting ~3 per 100,000 individuals annually (Bower et al. 1997; Schrag et al. 1999). The disease typically affects patients from 50 to 75 years of age and is characterized by a combination of autonomic dysfunction with motor abnormalities. MSA is a rapidly deteriorating disease, with a mean survival of 6–10 years (Wenning et al. 2013). The main types of motor abnormalities are parkinsonian features with a poor response to levodopa, particularly in the early disease stages, and cerebellar ataxia. Autonomic manifestations may include a wide range of symptoms, such as cardiovascular, genitourinary, and thermoregulatory, but the defining autonomic features are orthostatic hypotension or autonomic urinary abnormalities.

The term multiple system atrophy was first introduced by Graham and Oppenheimer in 1969 to describe a disorder characterized by autonomic dysfunction (Graham et al. 1969), but the disease itself was first described as olivopontocerebellar atrophy (OPCA) in 1900 (Dejerine et al. 1900). After postmortem assessment of brains from two ataxia patients, Dejerine and Thomas defined OPCA by neurodegeneration in the cerebellum, pons, and inferior olives in the brainstem. In 1960, Shy and Drager described degeneration of the intermediolateral column in the spinal cord, basal ganglia, substantia nigra (SN), cerebellum, and brainstem in patients presenting with parkinsonism associated with autonomic failure and pronounced orthostatic hypotension (Shy et al. 1960); this clinical syndrome was subsequently termed Shy–Drager syndrome (SDS). In the same year, Van der Eecken et al. reported patients with

parkinsonism presenting with pathological findings of neuronal loss in the SN and striatum, providing the basis for what they designated striatonigral degeneration (SND) (van der Eecken et al. 1960).

After reviewing brain tissue from OPCA, SDS, and SND patients, Graham and Oppenheimer suggested in 1969 that the three disorders be grouped together into one disease termed MSA, positing that each was a slightly different manifestation of the same neurodegenerative disease (Graham et al. 1969). This observation was subsequently confirmed in 1989 by Papp et al.; the authors examined the brains from 11 patients who had been diagnosed with OPCA, SDS, or SND, and reported the presence of inclusions in oligodendrocytes, which they termed glial cytoplasmic inclusions (GCIs), in all 11 patients (Fig. 1) (Papp et al. 1989). The presence of GCIs, or Papp–Lantos bodies, in oligodendrocytes along with a decrease in white matter volume led the authors to conclude that the three originally distinct disorders were, in fact, the same disease.

In 2007, a consensus meeting established a new, simplified definition of MSA, dividing the disease into two categories: MSA-P and MSA-C (Gilman et al. 2008). MSA-P denotes patients predominantly exhibiting parkinsonian symptoms, including postural rigidity and instability, bradykinesia, and tremor. This definition includes patients traditionally diagnosed with SND. MSA-C, however, encompasses patients with more prominent cerebellar symptoms, including gait and limb ataxia with cerebellar dysarthria associated with occulomotor dysfunction. This subgroup of MSA patients typically includes individuals previously classified as classic OPCA patients. Importantly, these delineations are made based on the predominant features at diagnosis and can change

throughout a patient's life. In addition to the parkinsonian and cerebellar manifestations, patients with MSA may also present with other neurological abnormalities, such as pyramidal signs and stupor.

Similar to other neurodegenerative diseases, a definite diagnosis of MSA can only be made upon autopsy by the presence of GCIs, the pathological landmark of the disease, along with neurodegenerative changes in the striatonigral or olivopontocerebellar structures in an individual's brain (Gilman et al. 2008). The diagnosis of possible MSA is based on the presence of either parkinsonian or cerebellar symptoms in patients, with at least one feature of autonomic and/or urogenital dysfunction, plus one other clinical feature (such as a Babinski sign with hyperreflexia) in patients over the age of 30 presenting with progressive disease. Probable MSA patients, also over the age of 30, exhibit rapidly deteriorating autonomic activity with urinary dysfunction and either poor levodopa-responsive parkinsonism or cerebellar dysfunction. Patient diagnosis may also include neuroimaging to visualize atrophy of the putamen, middle cerebellar peduncle, pons, and/or cerebellum via magnetic resonance imaging or hypometabolism in the putamen, brainstem, or cerebellum via positron emission tomography with fluorodeoxyglucose. (For a complete review of imaging in neurodegenerative disease patients, please see Seeley 2016.) There are no therapies available for MSA patients that address the root cause of the disease. Current treatments are focused on symptom alleviation, but these treatments typically offer only partial and transient relief for patients (reviewed in Fanciulli et al. 2015).

α-SYNUCLEIN AGGREGATES INTO GLIAL CYTOPLASMIC INCLUSIONS

The discovery that the protein α-synuclein is a primary component of GCIs in MSA patients stems from a series of findings originating from research on Parkinson's disease (PD). Friedrich Heinrich Lewy identified Lewy bodies (LBs) as the neuropathological hallmark of PD in 1912 (Forster et al. 1912), but an additional 65 years passed before α-synuclein was discovered as the primary protein component of LBs (Spillantini et al. 1997). (A detailed review of progressive α-synuclein accumulation in PD patients can be found in Braak et al. 2016.)

Lawrence Golbe, a neurologist at the Robert Wood Johnson Medical Center, identified two brothers and a third female patient (later found to be a seventh cousin of the brothers) with PD, each of whom had immigrated to the United States from Contursi, Italy, suggesting a possible familial form of the disease. Recognizing this possibility, Golbe worked with Roger Duvoisin and Italian neurologist Giuseppe Di Iorio to identify other relatives with PD, confirming their original hypothesis and discovering what is now known as the Contursi kindred (Golbe et al. 1990). The Contursi kindred, comprising 574 descendants from a couple married in 1700, 61% of whom were diagnosed with PD, is an Italian family with an autosomal dominant inheritance pattern of PD, including Golbe's three initial patients (Palfreman 2015). Golbe and Di Iorio collected blood samples from several members of the kindred for DNA analysis, and in 1996, the team began collaborating with Robert Nussbaum and Mihael Polymeropoulos at the National Institutes of Health to identify mutated genes that may be responsible for PD.

Nussbaum and Polymeropoulos used linkage analysis to determine that the responsible gene was located on the long arm of 4q21 (Polymeropoulos et al. 1996). After working to sequence the mutated gene, they identified the A53T mutation in the gene *SNCA*, which encodes the protein α-synuclein (Polymeropoulos et al. 1997). The identification of α-synuclein was in part made possible by the addition of the α-synuclein sequence in the GenBank database by Tsunao Saitoh, who had earlier reported the presence of α-synuclein in the β-amyloid plaques found in Alzheimer's disease patients (Uéda et al. 1993). While Nussbaum and Polymeropoulos were working to identify the gene responsible for PD in the Contursi kindred, Maria Grazia Spillantini, who was studying Alzheimer's patient samples and was familiar with Saitoh's work, developed methods for immunostaining α-synuclein. At the same time the A53T mutation in *SNCA* was identified, Spillantini found α-synuclein staining in LBs in PD patient samples (Spillantini et al. 1997). Notably, these patients did not have the A53T mutation, further linking α-synuclein to PD.

One year later, in 1998, Spillantini et al. and Wakabayashi et al. independently identified α-synuclein in the GCIs of MSA patient samples (Spillantini et al. 1998; Wakabayashi et al. 1998). In addition to MSA, Spillantini et al. demonstrated α-synuclein accumulation in the LBs present in Parkinson's disease with dementia (PDD) and dementia with Lewy bodies (DLB) patient samples. These discoveries resulted in the classification of PD, PDD, DLB, and MSA as synucleinopathies, or progressive neurodegenerative diseases characterized by the accumulation of α-synuclein aggregates in the brain (Hardy et al. 1998).

NEUROPATHOLOGY AND GENETICS OF MSA

At the microscopic level, the neuropathological features of MSA include neuronal loss and axonal degeneration within the striatonigral and olivopontocerebellar systems, moderate gliosis, and myelin pallor (deficient maintenance of myelin; reviewed in Ahmed et al. 2012). Although GCIs are the defining hallmark of MSA, sparse α-synuclein inclusions can also be found within the nuclei of oligodendrocytes as well as within the cytoplasm and nuclei of neurons (Papp et al. 1992; Nishie et al. 2004b; Jellinger et al. 2010). In recent years, there have been reports of MSA patients with LBs in multiple brain structures, including the brainstem (Ozawa et al. 2004; Jellinger 2007). During autopsy, these inclusions present in the brains of synucleinopathy patients are typically identified using antibodies that recognize α-synuclein phosphorylated at serine residue 129 (Nishie et al. 2004a).

Although MSA is usually considered to be a sporadic disease, there are case reports of potential familial versions with either autosomal dominant or recessive modes of inheritance (Soma et al. 2006; Hara et al. 2007; Wüllner et al. 2009; Itoh et al. 2014). Single nucleotide polymorphisms either within or surrounding *SNCA* are associated with an increased risk for MSA (Al-Chalabi et al. 2009; Scholz et al. 2009), suggesting that the disease could have a genetic component. Moreover, mutations in the COQ2 gene have recently been found in patients with sporadic or familial MSA (Multiple-System Atrophy Research Collaboration 2013). Interestingly, two mutations in α-synuclein (G51D and A53E) have been identified in cases of mixed PD and MSA pathologies (Kiely et al. 2013; Pasanen et al. 2014). Genome-wide association and sequencing studies for MSA are currently ongoing.

MOUSE MODELS OF MSA

Transgenic mice that overexpress wild-type human α-synuclein specifically in oligodendrocytes have been generated as potential models of MSA. Three different promoters have been used to drive α-synuclein overexpression in oligodendrocytes: proteolipid protein (Kahle et al. 2002), myelin basic protein (MBP) (Shults et al. 2005), and cyclic nucleotide phosphodiesterase (CNP) (Yazawa et al. 2005). Each of these lines develops GCI-like α-synuclein inclusions within oligodendrocytes and displays detergent-insoluble α-synuclein species. Motor deficits are present in the MBP and CNP lines, and there is some evidence for associated neurodegenerative pathology, as well as myelin abnormalities in the brain. The MBP line, with the highest level of α-synuclein expression, exhibits overt signs of neurological illness and has a reduced lifespan. Collectively, these models reveal that increased levels of α-synuclein in oligodendrocytes and the subsequent formation of inclusions are sufficient to drive neurological dysfunction, suggesting that the formation of GCIs may be the primary pathogenic event in MSA.

MODELING α-SYNUCLEIN AGGREGATION IN VITRO

The discovery that α-synuclein, a presynaptic protein composed of 140 amino acids, is the main constituent of LBs and GCIs led to a number of studies investigating the molecular mechanisms underlying the pathogenesis of PD and MSA (see a review of the cell biology of α-synuclein in Burré et al. 2016). In vitro cellular studies have examined α-synuclein-mediated aggregate formation and spreading using a variety of approaches, including overexpression of the protein (Desplats et al. 2009), infection

with synthetic α-synuclein fibrils (Luk et al. 2009; Volpicelli-Daley et al. 2011), and α-synuclein uptake by oligodendrocytes (Kisos et al. 2012; Konno et al. 2012).

In 2009, Desplats et al. (2009) used SH-SY5Y cells differentiated toward dopaminergic neurons to study the propagation of α-synuclein in vitro. Using a co-culture approach, the group overexpressed myc-tagged α-synuclein in one group of cells (the donor group) while fluorescently tagging the second group with Qtracker (the acceptor group). Critically, the acceptor cells did not overexpress α-synuclein. Within 24 hours of co-culturing the two cell lines, the authors detected α-synuclein aggregates in the Qtracker-labeled acceptor cells, demonstrating cell-to-cell propagation of α-synuclein. Furthermore, the aggregates in the acceptor cells were ubiquitinated and positive for thioflavin S (ThioS) staining, similar to GCIs in MSA patients. In a similar co-culture approach, Hansen et al. (2011) developed both human embryonic kidney (HEK) cell lines and SH-SY5Y neuroblastoma cell lines expressing α-synuclein fused to either DsRed or green fluorescent protein (GFP). When the two α-synuclein fusion proteins were expressed in co-culture, regardless of cell type used, the authors found that GFP-positive α-synuclein had propagated to cells expressing α-synuclein fused to DsRed, and vice versa. Together, these findings provided important insight into α-synuclein propagation in the central nervous system, suggesting a mechanism by which the aggregated protein could progressively spread and cause disease.

This hypothesis was bolstered by subsequent studies published by Luk et al. (2009) and Volpicelli-Daley et al. (2011). Using HEK cells overexpressing wild-type α-synuclein, Luk et al. tested the ability of exogenous α-synuclein preformed fibrils (PFFs) to induce intracellular aggregation (Luk et al. 2009). Myc-tagged PFFs were used to

infect HEK cells, and 48 hours later, α-synuclein aggregates were detected in the cultured cells. These aggregates were hyperphosphorylated, detergent-insoluble, and ubiquitinated, similar to aggregates isolated from human samples. Interestingly, co-staining for myc and phosphorylated α-synuclein revealed that the exogenous PFFs formed the core of the aggregates, whereas endogenous α-synuclein formed the exterior. Volpicelli-Daley et al. (2011) found that α-synuclein PFFs could also induce endogenous α-synuclein aggregation in primary neuron cultures. After 4 days of incubation, α-synuclein aggregates were seen in the neurites, which spread to the soma of the neurons by day 10. Interestingly, hippocampal neurons grown in microfluidic chambers and infected with PFFs demonstrated retrograde spreading of α-synuclein aggregates starting in neurites and moving up to the soma, as well as anterograde propagation from the soma down to the neurites. All together, these findings suggest that cell-to-cell spreading of α-synuclein may initiate new aggregate formation as the disease propagates in the brain of an MSA patient. (The transcellular propagation of α-synuclein is also reviewed in Tofaris et al. 2016.)

Although the experiments from Volpicelli-Daley et al. demonstrated that exogenous α-synuclein can induce protein aggregation in neurons, the predominant protein inclusions in MSA are found in oligodendrocytes, which initially were not thought to express α-synuclein (Solano et al. 2000; Ozawa et al. 2001; Miller et al. 2005). Recent findings suggest that the protein may be expressed in oligodendrocytes, albeit at lower levels than neurons (Asi et al. 2014; Djelloul et al. 2015); however, a number of studies indicate that α-synuclein must be somehow secreted from neurons and taken up by surrounding oligodendrocytes to form GCIs (Reyes et al. 2014). This idea has gained

support from two studies revealing α-synuclein uptake by oligodendrocytes in cell culture. First, using two immortalized oligodendrocyte cell lines and rat primary oligodendrocytes, Kisos et al. (2012) exposed cells to recombinant α-synuclein monomer or conditioned media from neuronal cells that were either wild-type or engineered to overexpress α-synuclein. Following incubation for 16 hours, α-synuclein was detected throughout the cell bodies of all three oligodendrocyte lines incubated with recombinant α-synuclein or conditioned media from the neurons overexpressing α-synuclein. However, oligodendrocytes incubated with conditioned media used to grow wild-type neurons did not show α-synuclein accumulation. Similarly, Konno et al. (2012) reported clathrin-dependent internalization of recombinant α-synuclein after incubation for 24 hours with the KG1C oligodendrocyte cell line. The resulting α-synuclein aggregates were ThioS-positive, ubiquitinated, and immunoreactive for the phosphorylated α-synuclein antibody (pSer129), which is commonly used for the pathological confirmation of synucleinopathy postmortem (Rey et al. 2016), demonstrating the ability of oligodendrocytes to take up and accumulate α-synuclein into GCI-like structures.

PROPAGATING MSA PRIONS IN CULTURED CELLS

Studying α-synuclein aggregation and propagation in cells using PFFs provided important evidence supporting the hypothesis that α-synuclein becomes a prion during disease. Prions, or misfolded proteins capable of templating additional protein misfolding (i.e., self-propagation), were originally described as the disease-causing agent in scrapie and Creutzfeldt–Jakob disease (Prusiner 1982), but are now known to feature in a large number of neurodegenerative diseases (Prusiner 2012; Goedert

2015). A key in vitro experiment to demonstrate that α-synuclein misfolds into a prion will be to isolate and propagate α-synuclein aggregates from synucleinopathy patient samples in cultured cells.

Progress toward addressing this objective was achieved by experiments in which both wild-type and mutated α-synuclein were overexpressed in HEK293T cells to identify spontaneous aggregation-promoting regions of the protein (Burré et al. 2012). Myc-tagged α-synuclein mutants were transiently expressed in HEK293T cells for 2 days, after which cells were analyzed for the formation of spontaneous α-synuclein aggregates. Using these approaches, the Südhof group found that three familial PD point mutations (A30P, E46K, and A53T) independently promoted α-synuclein aggregation, as did several C-terminal truncations, compared with the wild-type-expressing cells. Of note, the three point mutations studied were the three known familial PD α-synuclein mutations identified at the time (Hardy et al. 2006); since then, the A53E (Pasanen et al. 2014) and G51D mutations (Kiely et al. 2013; Lesage et al. 2013) have been identified in atypical synucleinopathy patients presenting with mixed PD and MSA pathology.

Building on this work, we engineered HEK293T cells to stably express α-synuclein fused to yellow fluorescent protein (YFP) (Woerman et al. 2015). This approach, first developed by Marc Diamond's laboratory to characterize tau prions in HEK293 cells expressing a tau fragment fused to YFP (Sanders et al. 2014 and reviewed in Holmes et al. 2016), facilitates live-cell imaging of YFP-positive intracellular α-synuclein aggregates and thus rapid detection of induced α-synuclein accumulation. As expected based on the systematic mutagenesis studies, we found that cells

expressing mutated α-synuclein (α-syn140*A53T–YFP) were more susceptible to infection with PFFs compared with cells expressing wild-type α-synuclein (α-syn140–YFP), although neither cell line exhibited spontaneous aggregate formation (Woerman et al. 2015). After isolating protein aggregates from 6 MSA patient samples (Lee et al. 2005), we incubated the brain extracts with the α-syn140*A53T–YFP cells for 4 days and found that all 6 samples induced α-synuclein–YFP accumulation, as defined by the appearance of bright foci within the cells. This discovery was specific to MSA; none of the 17 control patient samples nor the 3 PD patient samples infected the α-syn140*A53T–YFP cells, demonstrating selectivity for α-synuclein prions from MSA patient samples.

Importantly, we also tested the ability of MSA prions to serially propagate in cultured cells (Fig. 2). Following infection of α-syn140*A53T–YFP cells with α-synuclein prions isolated from patient MSA14, we established two clones that stably exhibited patient-derived aggregates, MSA14-11 and MSA14-M1 (Fig. 2*A*). Lysate harvested from both stable clones and uninfected α-syn140*A53T–YFP cells was incubated with naïve HEK293T cells for 3 days. Both clones robustly infected the cells, whereas lysate from the uninfected cells had no effect (Fig. 2*B*). Serial propagation or templating of protein misfolding, as described herein with α-synuclein, is a hallmark of prion diseases. Significantly, the ability to continuously propagate a prion strain in vitro provides an opportunity to rapidly investigate the disease process and potentially identify compounds that interfere with disease progression.

NONTRANSGENIC ANIMAL MODELS OF SYNUCLEINOPATHY

Like PrP prions, α-synuclein prions can be propagated in vivo via intracerebral inoculation of wild-type animals. In 2012, Luk et al. demonstrated that one injection of PFFs into the striatum of either C57BL/6SJL or CD1 mice induced widespread pSer129 α-synuclein neuropathology and dopaminergic neuron loss in the SN pars compacta (SNpc) and ventral tegmental area at 180 days post-inoculation (dpi) (Luk et al. 2012a). Importantly, PFFs inoculated into α-synuclein knockout mice (*Snca*$^{-/-}$) did not elicit similar results, indicating that the PD-like pathology arose specifically from α-synuclein prion propagation.

These findings were followed one year later by studies from Masuda-Suzukake et al. (2013) that demonstrated the first transmission of α-synuclein misfolding from a human synucleinopathy sample. After inoculating insoluble PFFs prepared from either mouse or human recombinant α-synuclein into the SN of C57BL/6J mice, the authors found widespread pSer129 α-synuclein deposition that co-localized with ubiquitin and p62 immunostaining 15 months post-inoculation. After their studies with PFFs, which induced α-synuclein pathology in ~94% of the mice, the authors inoculated sarkosyl-insoluble extracts prepared from DLB patients into the SN of wild-type mice. Half of the inoculated mice developed ipsilateral α-synuclein pathology (only 7% showed spreading to the contralateral hemisphere 15 months after inoculation).

In 2014, Recasens et al. isolated Lewy bodies from the SNs of three PD patients and inoculated the aggregated protein directly adjacent to the SN in C57BL/6 mice (Recasens et al. 2014). Seventeen months post-injection, the mice showed a

substantial decrease in the number of tyrosine hydroxylase (TH) positive, or dopaminergic, fibers and an increase in pSer129 α-synuclein immunostaining in the striatum and SNpc. Moreover, 12 months after the authors inoculated the same PD patient samples into the striatum or SNpc of macaque monkeys, they also found a reduction in TH-positive neurons by ~40% and ~15% in the the striatum and SNpc, respectively. This loss of dopaminergic neurons was accompanied by an increase in phosphorylated α-synuclein deposition, suggesting that human synucleinopathies are transmissible to both rodents and primates. (Additional information about experimental α-synuclein pathology can be found in Hasegawa et al. 2016.)

TRANSMISSION OF α-SYNUCLEIN PRIONS TO TRANSGENIC MICE

The discovery that α-synuclein PFFs and human LB samples induced α-synuclein neuropathology in wild-type animals supported the hypothesis that α-synuclein misfolds to become a prion. However, these studies were hampered by the lack of concomitant motor deficits that typically accompany disease progression in synucleinopathy patients. In 2002, Giasson et al. developed a transgenic mouse model expressing human α-synuclein with the A53T mutation, which causes familial PD, expressed under the mouse prion protein, *Prnp*, promoter (Giasson et al. 2002). The homozygous mice, termed M83$^{+/+}$ mice, spontaneously developed motor deficits around 1 year of age, on average, along with substantial pSer129 α-synuclein pathology in the spinal cord, brainstem, and cerebellum. Using brain homogenate prepared from aged M83$^{+/+}$ mice with motor signs (12 and 18 months old), Mougenot et al. (2012) inoculated young asymptomatic M83$^{+/+}$ mice, decreasing the onset of disease from >1 year to <6.5 months. However, when the authors performed inoculations with brain homogenate

prepared from 2-month-old asymptomatic M83$^{+/+}$ mice, the mice remained free of motor deficits for ~1 year.

Similar to these results, Luk et al. (2012b) inoculated brain homogenate from symptomatic M83$^{+/+}$ mice into the striatum and overlying cortex of young M83$^{+/+}$ mice and also found that the inoculations induced progressive motor abnormalities along with an increase in pSer129 α-synuclein, ubiquitin, and ThioS immunostaining in the brain. To confirm that the findings were caused by α-synuclein prion transmission, the authors inoculated the mice with PFFs prepared from recombinant human α-synuclein. The M83$^{+/+}$ mice developed analogous motor deficits and neuropathology findings, indicating that acceleration of the disease observed by both groups of investigators arose from transmitting a spontaneous synucleinopathy that develops in the M83$^{+/+}$ mice.

In contrast to homozygous animals, hemizygous M83$^{+/–}$ mice do not develop spontaneous disease, living the full lifespan of a wild-type animal, but they do develop motor deficits and pathological α-synuclein accumulation following inoculation with aged M83$^{+/+}$ brain homogenate (Watts et al. 2013). We inoculated M83$^{+/–}$ mice with brain homogenate prepared from two MSA patient samples and found that the mice developed signs of neurological dysfunction ~125 dpi. Remarkably, transmission of MSA to the M83$^{+/–}$ mice was faster than the incubation period following inoculation with aged M83$^{+/+}$ homogenate (~217 dpi). Both inoculations induced robust pSer129 α-synuclein deposition in the hindbrain and in some areas in the mesencephalon (Watts et al. 2013). Sarkosyl-insoluble fractions from MSA brain extracts also induced cerebral pSer129 α-synuclein deposition 6–9 months after intracerebral inoculation of transgenic mice expressing wild-type human α-synuclein (Bernis et al. 2015). However, no signs of

neurological illness were observed in these mice, which overexpress α-synuclein under the control of its endogenous promoter but do not express mouse α-synuclein, following inoculation with the MSA samples.

Following our initial study with two patient samples, we collected samples from an additional 12 patients from 3 continents, for a total of 19 brain regions from 14 different patients, and inoculated the additional samples into M83$^{+/-}$ mice (Prusiner et al. 2015). Consistent with our original findings, all 19 samples transmitted MSA to the mice, causing CNS dysfunction in 134 of the 135 inoculated animals. To confirm that we had infected the mice with MSA, we tested brain samples from the terminal mice in the α-syn140*A53T–YFP cell assay described above and found that each mouse brain tested contained α-synuclein prions that infected the cells. However, mice that had been inoculated with brain homogenate prepared from a control patient did not infect the cells.

To demonstrate that transmission of neurological disease arises from aggregated protein alone, we digested brain homogenate from an MSA patient sample in benzonase to degrade the nucleic acids and precipitated the remaining sarkosyl-insoluble protein aggregates using sodium phosphotungstic acid (Woerman et al. 2015). After inoculating the M83$^{+/-}$ mice with the resulting PTA extract, the mice developed neurological disease with similar pSer129 α-synuclein deposits in the brain, indicating that the misfolded protein is, indeed, responsible for disease transmission. Notably, inoculation with brain homogenate prepared from PD patient samples did not transmit neurological disease to the M83$^{+/-}$ mice, suggesting that the two synucleinopathies arise from distinct conformations of misfolded α-synuclein (Prusiner et al. 2015).

CONCLUDING REMARKS

The discovery that LBs and GCIs, the key neuropathological hallmarks of PD and MSA, respectively, are composed of aggregated α-synuclein initiated further research into the underlying molecular mechanism(s) of these diseases. Following this discovery, research over the last 20 years using synthetic α-synuclein PFFs and synucleinopathy patient samples has accumulated substantial evidence that α-synuclein misfolds and becomes a prion in MSA patients. Important in vitro and in vivo models for studying α-synuclein prion formation, transmission, and propagation have been recently developed, and future research utilizing these tools will be invaluable in developing successful therapeutics that can halt the progression of MSA.

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FIGURE LEGENDS

Figure 1. Glial cytoplasmic inclusion (GCI) neuropathology in a multiple system atrophy (MSA) patient sample. GCIs in the basal ganglia from an MSA patient sample were immunostained using the α-synuclein antibody clone 42 (BD Biosciences). (*A*) Microscopic examination of the patient sample shows dense α-synuclein neuropathology throughout the basal ganglia. (*B*) Magnification of inset from (*A*) shows α-synuclein accumulation into GCIs, indicated by arrows. Scale bar, 100 μm.

Figure 2. Stable propagation of multiple system atrophy (MSA) prions in cultured cells. HEK293T cells expressing α-synuclein with the A53T mutation fused to yellow fluorescent protein (α-syn140*A53T–YFP cells) were infected with α-synuclein prions isolated from patient MSA14. (*A*) Two monoclonal cell lines stably propagating the MSA prions were established using fluorescence-activated cell sorting (FACS). Lysate from two clones, MSA14-11 and MSA14-M1, as well as from uninfected α-syn140*A53T–YFP cells, was collected. (*B*) MSA14-11, MSA14-M1, and α-syn140*A53T–YFP lysates were incubated with naïve α-syn140*A53T–YFP cells at a final protein concentration of 0.1 μg for 3 days. The cells were imaged using the GE IN Cell Analyzer 6000, and the total fluorescence per cell was measured for each condition. MSA14-11 and MSA14-M1 lysate both induced a robust infection in the α-syn140*A53T–YFP cells, compared to lysate from uninfected cells (*$p < 0.001$).

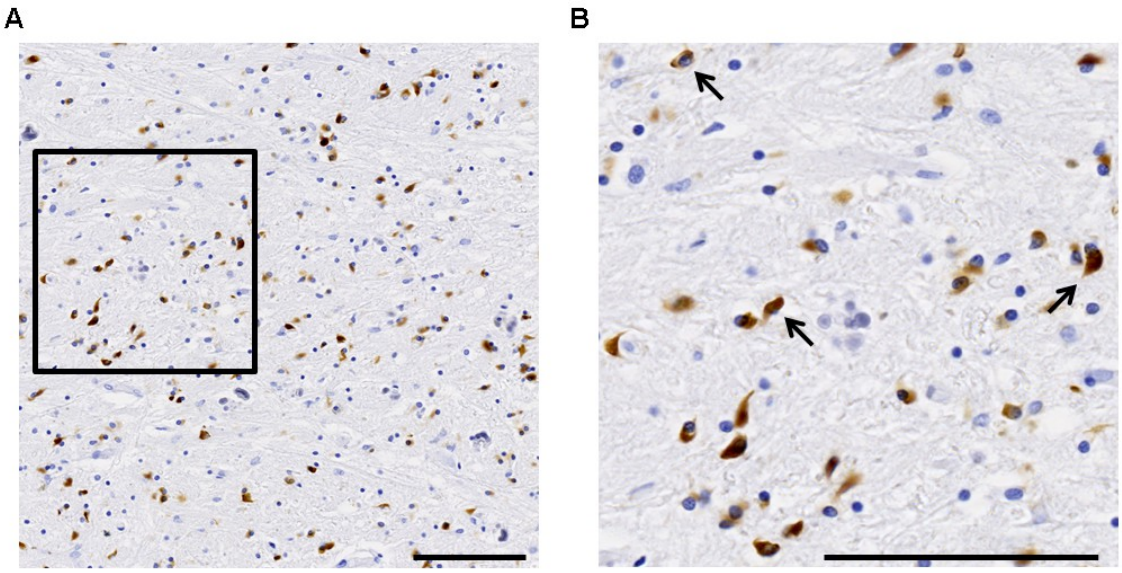

Figure 1

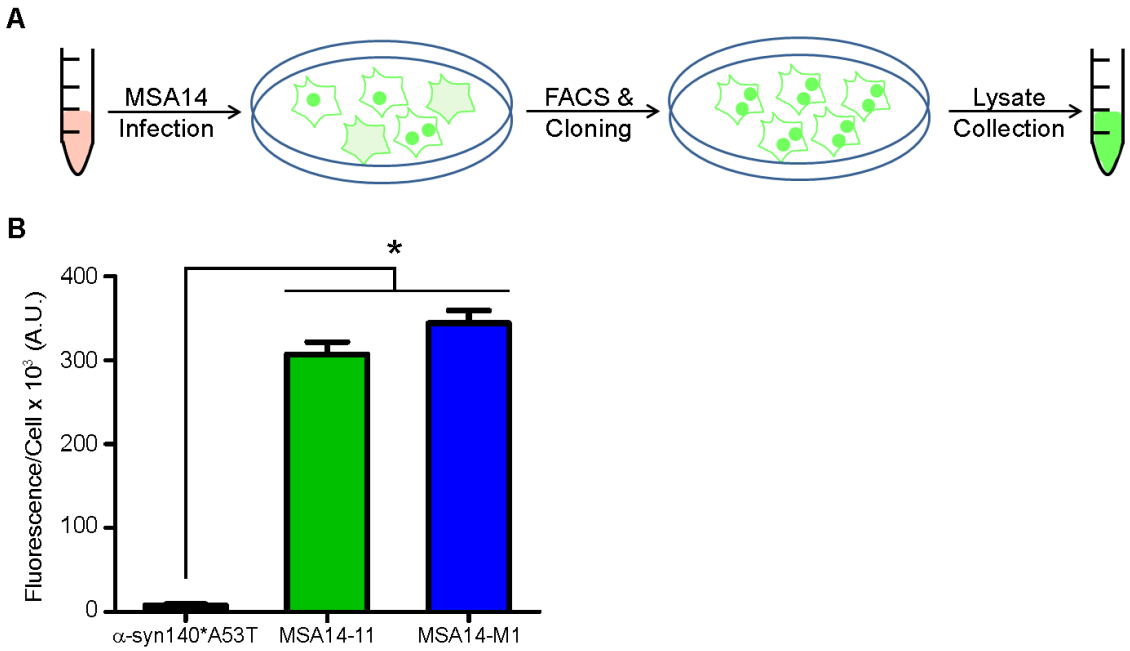
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Figure 2