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Genetic animal models for evaluating the role of autophagy in etiopathogenesis of Parkinson disease

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Parkinson disease (PD) is the most common neurodegenerative movement disorder and is characterized pathologically by the formation of ubiquitin and SNCA/α-synuclein-containing inclusions (Lewy bodies), dystrophic midbrain dopaminergic (DAergic) terminals, and degeneration of midbrain DAergic neurons. The vast majority of PD occurs sporadically, while approximately 5% of all PD cases are inherited. Genetic mutations of a few genes have been identified as causes of familiar PD, i.e., mutations in *SNCA*, *PARK2/parkin*, *UCHL1*, *PARK7/DJ1*, *PINK1* and *LRRK2*, leading to DAergic cell death, but variable pathological changes. The evidence supports the hypothesis that several pathogenic mechanisms are likely involved at initial stages of the disease, and eventually they merge to cause parkinsonism. The current challenge facing PD research is to unravel the components in these pathways that contribute to the pathogenesis of PD. Accumulating evidence has implicated dysfunctional autophagy, a regulated lysosomal pathway with a capacity for clearing protein aggregates and cellular organelles, as one of the pathogenic systems contributing to the development of idiopathic PD.

Keywords: autophagy, neurodegeneration, Atg7, Parkinson disease, LRRK2, alpha-synuclein, dopamine, motor deficits

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In our recent study we generated and characterized conditional knockout mice with a cell-specific deletion of the essential autophagy gene *Atg7* in midbrain DAergic neurons to test in vivo whether impaired autophagy in DAergic neurons leads to PD-like pathology. The targeted deletion of *Atg7* in DAergic neurons results in early axonal dystrophy and degeneration, striatal dopamine depletion, enlarged dendritic swellings, delayed and progressive DAergic neuron loss, and late-onset locomotor dysfunction in mutant mice. Furthermore, we demonstrate a link between impaired autophagy and augmented protein levels of the SNCA and LRRK2 proteins at presynaptic terminals.

Previous studies indicate that *Atg5* or *Atg7* deletion in the whole mouse brain leads to neurodegeneration with little detail regarding the fate of specific neuron populations. In addition, it is unclear whether the neuronal loss is a cell autonomous event or due to impaired glia-neuron interaction. While in our previous study genetic ablation of *Atg7* in cerebellar Purkinje cells causes rapid cell death, with the majority loss as early as seven weeks, we presently show that disruption of autophagy by deletion of *Atg7* in DAergic neurons leads to a moderate cell loss at an age of 9 mo. The majority of DAergic cell bodies are surprisingly resistant to the long-term stress inflicted by the loss of autophagy, despite pervasive ubiquitinated-SQSTM1/p62 inclusions present at an early age (1–4 weeks). Interestingly, the delayed loss of DAergic neurons is accompanied by impaired locomotor behavior of the mutant mice at an age of 9 mo, as shown by the open field and challenging beam tests. The late-onset of neurodegeneration and motor deficits in autophagy-disrupted mice suggest that aging significantly enhances the susceptibility of DAergic neuron to loss of autophagy and death.

Furthermore, autophagy inhibition leads to the early appearance of dystrophic axonal swellings and the subsequent degeneration of presynaptic DAergic terminals, which is associated with the reduction of striatal dopamine levels. This result, along with our previous study in Purkinje cells, suggests that autophagic clearance plays a crucial role in axonal homeostasis. In addition, midbrain DAergic dendrites show remarkable sensitivity to autophagy impairment, exhibiting numerous varicosities that contain massive ubiquitinated inclusions. The appearance of the dendritic inclusions resembles those in cell bodies, suggesting that the inclusions in the two compartments share a similar nature in their composition. The progressive degeneration of DAergic axon terminals and dendritic arbors are among the earliest pathological events in mice with *Atg7* disruption in DAergic neurons and may eventually contribute to the loss of midbrain neuron cell bodies.

Although autophagy inhibition in DAergic neurons results in ubiquitinated-SQSTM1 inclusions and loss of DAergic neurons, no abnormal accumulation of SNCA in TH^+ cell bodies was observed, even as late as 20 mo of age. However, accumulation of endogenous SNCA was detected in striatal axonal terminals of 20-mo old mutant mice, providing in vivo evidence for autophagic control of SNCA protein homeostasis in axons in an age-related manner. While autophagy may be involved in the degradation of particular forms of pathogenic SNCA within specific cellular compartments (e.g., axon terminals), it is important to note that other catabolic pathways, such as the ubiquitin-proteasome system (UPS) or chaperone-mediated autophagy (or another lysosomal pathway), rather than macroautophagy, might serve as the primary mechanisms for SNCA turnover in the soma under normal conditions. Therefore, the loss of autophagic activity may be one of several cellular systems that deteriorates with age and contributes to the pathogenesis of late-onset PD.

Mutations in the *LRRK2* gene are linked to the most common genetic cause of PD, but the precise pathological role of LRRK2 is poorly understood. Little is known about the regulation of LRRK2, although previous evidence suggests that the UPS degrades LRRK2. We show for the first time that autophagy deficiency leads to increased LRRK2 protein levels and accumulation in the brain, especially within the cerebellar Purkinje cells, and fibroblast cell lines. However, LRRK2 protein accumulation is not the consequence of impaired protein turnover, but rather appears to be caused by the increase in *LRRK2* mRNA levels. These results suggest that *LRRK2* levels are upregulated in certain cell types in response to loss of autophagy. The potential role for autophagy in regulating *LRRK2* levels is noteworthy, and future experiments should investigate the underlying mechanism. Interestingly, recent data suggest that overexpression of LRRK2 inhibits the clearance of proteasome substrates upstream of proteasome catalytic activity, favoring the accumulation of proteins and aggregate formation. One might speculate that an age-dependent decline of autophagy activity in LRRK2 PD patients might cause upregulation of mutant LRRK2 levels and accelerate its neurotoxicity and pathological process.

In summary, our recent report presents a unique model with progressive and slow degeneration of DAergic midbrain neurons and indicates that the inactivation of autophagy in animal models predisposes to PD-related pathological events. Our results are in line with recent findings that alterations in autophagic activity are involved in the pathogenic role of several PD-related genes. Therefore we propose that insufficient autophagic clearance in CNS neurons, particularly midbrain DAergic neurons, represents a significant risk to the development of parkinsonian-like disease pathology, even in the absence of PD-related gene mutations. Therefore, the manipulation of autophagic activity can be explored as a therapeutic strategy for the treatment of PD.