CELL BIOLOGY

Regulation of α-synuclein homeostasis and inflammasome activation by microglial autophagy

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Autophagy clears protein aggregates, damaged cellular organelles, and pathogens through the lysosome. Although autophagy is highly conserved across all cell types, its activity in each cell is specifically adapted to carry out distinct physiological functions. The role of autophagy in neurons has been well characterized; however, in glial cells, its function remains largely unknown. Microglia are brain-resident macrophages that survey the brain to remove injured neurons, excessive synapses, protein aggregates, and infectious agents. Current studies have demonstrated that dysfunctional microglia contribute to neurodegenerative diseases. In Alzheimer's disease animal models, microglia play a critical role in regulating amyloid plaque formation and neurotoxicity. However, how microglia are involved in Parkinson's disease (PD) remains poorly understood. Propagation of aggregated α-synuclein via cell-to-cell transmission and neuroinflammation have emerged as important mechanisms underlying neuropathologies in PD. Here, we review converging evidence that microglial autophagy maintains α-synuclein homeostasis, regulates neuroinflammation, and confers neuroprotection in PD experimental models.

INTRODUCTION

Macroautophagy (hereinafter referred to as autophagy) is a self-digestion process that recycles cellular waste and provides an energy source to maintain cell survival. Autophagy is activated by a variety of stressful conditions, including nutrient starvation. During starvation, autophagy initiates a self-eating process by engulfing organelles through autophagosomes, which then fuse with the lysosome. The molecular mechanisms that mediate autophagic induction during starvation have been extensively studied (1). Autophagy is also active at a basal level to enable the clearance of aggregated/misfolded proteins, damaged organelles, and invading foreign pathogens such as bacteria (2, 3). In the brain, neuronal autophagy is compartmentalized and plays an essential role in maintaining axonal and dendritic homeostasis (4, 5). It also contributes to the clearance of disease-associated protein aggregates, such as α-synuclein, β-amyloid (Aβ), tau, and huntingtin proteins (6–10). In the absence of autophagy, neurons accumulate polyubiquitinated protein aggregates, leading to dysfunction and degeneration (11, 12). In addition, autophagy is required for maintaining neuronal excitability and synaptic transmission by regulating the function of the inwardly rectifying potassium channel, Kir2, and calcium release from the endoplasmic reticulum (13, 14).

Microglia are brain-resident macrophages that continuously scan the brain and remove neuronal cell debris, excessive synapses, and aggregated/misfolded proteins in physiological and diseased conditions (15). Microglia can be both beneficial and detrimental in diseases (16). Emerging evidence has shown that loss of microglial activity can worsen disease-associated phenotypes in Alzheimer's disease (AD) models. Depletion of microglia or loss of Trem2 (Triggering receptor expressed on myeloid cells 2) in microglia increases neuronal damage and promotes phosphorylated tau around amyloid plaques, caused by the lack of Aβ isolation by microglia (17–19). Interestingly, loss of Trem2 leads to aberrant elevation of autophagy in microglia in a similar AD mouse model that is

associated with aggressive amyloid plaque formation (20). In contrast, several reports have shown that microglia promote neurodegeneration. For example, loss of Trem2 in microglia or microglia carrying AD-associated TREM2 variant (R47H) protect neurons from degeneration by reducing inflammation and astrocytic gliosis in a tauopathy mouse model (21, 22). Also, a recent study has demonstrated that microglia promote the propagation of Aβ into unaffected brain tissue (23). Research surrounding the importance of microglia in brain health and disease is a rapidly growing field. Here, we will review the function of autophagy in microglia and how dysfunctional autophagy in microglia may contribute to the progression of Parkinson's disease (PD).

MECHANISM OF CORE AUTOPHAGY MACHINERY

Autophagy is conducted by multiple proteins encoded by autophagy-related genes (ATGs). ATGs are highly conserved from yeast to mammals (24). Unc-51–like kinase 1 (ULK1), a homolog of yeast Atg1, is a major upstream kinase that is important for the initiation of autophagy (25). The activity of ULK1 is coordinated by two nutrition sensor kinases, mammalian target of rapamycin (mTOR) and adenosine monophosphate–activated protein kinase (AMPK). In a nutrition-enriched condition, mTOR constitutively phosphorylates serine-757 (S757) of ULK1, which suppresses ULK1 activity by disrupting the interaction between ULK1 and AMPK. During starvation, AMPK phosphorylates serine-317 (S317) of ULK1, leading to ULK1 activation (26). Once activated, ULK1 forms a complex with autophagy-related protein 13 (ATG13) and FAK family kinase–interacting protein of 200 kDa (FIP200). This complex initiates the formation of a phagophore, the initial membrane structure of autophagosome, by activating a class III phosphatidylinositol 3-kinase (PI3K) lipid kinase called a vacuolar protein sorting 34 (VPS34) (27, 28). VPS34 forms a complex with Beclin-1, VPS15, ATG14, and NRBF2, which regulates VSP34 activity and increases the level of phosphatidylinositol 3-phosphate (PI3P) at the omegasome—a precursor of autophagosomes (29–31). A local increase of PI3P at the omegasome recruits PI3P-binding proteins, such as WD repeat domain phosphoinositide-interacting proteins (WIPIs) and zinc-finger

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FYVE domain–containing protein 1 (DFCP1) (*32*). These further recruit ubiquitin-like LC3-conjugation machinery, composed of ATG5, ATG12, and ATG16L1, and add phosphatidylethanolamine (PE) to LC3I during a process called LC3 lipidation (*33*). The lipidated form of LC3I, known as LC3II, is incorporated into the elongating phagophore, forming an autophagosome (*34*). Selective autophagy receptors, such as p62/SQSTM1 and optineurin, recognize ubiquitinated targets through the ubiquitin-binding domain (UBD) and interact with LC3 via LC3-interacting motif (LIR), conferring selectivity during autophagic degradation.

α-SYNUCLEIN AND PD

PD is a common, age-related, neurodegenerative disease that affects approximately 1 million people in the United States and 10 million people worldwide (*35*). A primary pathological feature of PD is the loss of dopamine-producing (dopaminergic) neurons in substantia nigra pars compacta (SNpc). Depletion of SNpc dopaminergic neurons results in the classic motor-related symptoms of PD: tremor, rigidity, and slowness of movement (bradykinesia) (*36*). During the late stages of PD, patients frequently experience cognitive problems, including memory loss and dementia, called PD with dementia (PDD). PD is neuropathologically characterized by the presence of intracellular inclusions known as Lewy bodies and Lewy neurites. These protein aggregates are composed, in part, of the misfolded α-synuclein. α-Synuclein, encoded by the *SNCA* gene, is localized to presynapses of neurons and may account for 1% of the total cytosolic protein in the brain (*37*). Importantly, enhanced *SNCA* expression is associated with PD onset (*38–40*). Missense mutations in the *SNCA* have also been described in familial PD. A number of these *SNCA* mutations, including A53T, A30P, and E64K, have been shown to promote misfolding and aggregation of α-synuclein (*41, 42*).

In 2003, Braak and colleagues proposed a staging system for PD neuropathology based on a rostral to caudal progression of Lewy bodies in PD brains (*43*). They hypothesized that aggregated species of α-synuclein may spread through the anatomically connected nervous system, leading to a progressive worsening of PD-associated symptoms (*43*). Subsequent analysis of postmortem PD brains that received fetal midbrain grafts also described Lewy bodies within grafted neurons with a similar distribution and morphology to those in the surrounding host brain (*44, 45*). An observation that supports the hypothesis of α-synuclein intercellular transmission was subsequently validated in rodent models (*46, 47*). Pathological spreading of α-synuclein has been successfully recapitulated in rodents through the injection of preformed fibrils (PFFs) of α-synuclein (*48*). Striatal injection of PFF causes the formation and propagation of Lewy body–like pathology throughout anatomically interconnected regions, including SNpc and cortex. Progressive accumulation of α-synuclein pathology leads to degeneration of dopaminergic neurons and PD-like symptoms in mice (*48*). In addition, injection of PFF in the gastrointestinal tract led to the progressive spreading of α-synuclein pathology from the gut to the brain (*49, 50*). These experimental data support the gut-brain axis of transmission of α-synuclein pathology during PD progression as originally proposed by Braak and colleagues.

Despite an emerging view that α-synuclein pathology can spread via cell-to-cell transmission, the exact molecular and cellular mechanism underlying the propagation of α-synuclein remains unclear.

Multiple studies have shown that α-synuclein can be secreted from neurons (*51, 52*). Indeed, α-synuclein is found in plasma, cerebrospinal fluid, and brain interstitial fluid (*53–55*). The extracellular α-synuclein can be taken up by neurons and glia, which may regulate the homeostasis of α-synuclein in the brain (*56*). A previous study showed that depletion of microglia enhances the propagation of α-synuclein (*57*), demonstrating the importance of microglia in preventing the spreading of α-synuclein pathology. Therefore, understanding the degradation pathways of α-synuclein in microglia will provide insight into the transmission of α-synuclein pathology and the pathogenesis of PD.

α-SYNUCLEIN AND AUTOPHAGY

Genetic and genomic studies have implicated dysfunctional autophagy in the pathogenesis of PD. Genome-wide association studies (GWAS) have nominated a number of risk genes such as *BECN* (encoding Beclin-1), *ATP13A2*, *CSTB* (encoding cystatin B), and *GBA* (encoding glucocerebrosidase), which play a key role in autophagy and lysosome homeostasis (*58*). A recent study has also reported DNA hypermethylation of autophagy/lysosomal genes in the appendices and brains of PD patients, which are associated with altered expression of genes and proteins in the autophagy/lysosomal pathway (*59*). Furthermore, DNA hypermethylation proceeded the appearance of Lewy bodies, suggesting the importance of autophagy/lysosome functions in α-synuclein homeostasis (*59*).

Several studies have suggested that α-synuclein is degraded by autophagy, chaperone-mediated autophagy (CMA), and the proteasome in neurons (*60–64*). Pharmacological experiments using proteasome inhibitors, such as epoxomicin, clasto-lactacystin β-lactone, and MG132, were shown to block the degradation of α-synuclein in animals and differentiated neuronal cell lines (*63, 64*). CMA is a chaperone-mediated lysosomal degradation pathway for the clearance of short-lived soluble cytosolic proteins (*65*). CMA substrates contain a pentapeptide motif that is recognized by the molecular chaperone, heat shock cognate 70 (HSC70). Substrates that are bound to HSC70 are selectively trafficked across the lysosomal membrane through interactions with the lysosomal receptor LAMP2A. α-Synuclein contains a CMA-targeting motif and can be directly transported to the lysosome (*60*). Disruption of the CMA-targeting pentapeptide motif in α-synuclein or genetic down-regulation of LAMP2A resulted in the accumulation of α-synuclein in neuronal cell lines and primary neurons (*62*). Furthermore, autophagy was also shown to degrade α-synuclein as evidenced by the accumulation of α-synuclein in cells following treatment with pharmacological inhibitors of autophagy (*62*). However, evidence for autophagic degradation of α-synuclein is largely based on cultured neurons or neuron-like cell lines and chemical inhibitors with poor specificity (e.g., 3-methyladenine) (*66, 67*). To address this, several research groups have examined the effects of neuron-specific deletion of *Atg7* in α-synuclein in vivo (*6, 68, 69*). Autophagy deficiency in dopaminergic neurons causes the accumulation of ubiquitinated inclusions and age-dependent neurodegeneration. Aggregated α-synuclein was observed at presynaptic terminals of dopaminergic neurons in aged mice but not in young mice (*6*). Curiously, total levels of α-synuclein in the brain were not altered by autophagy deficiency in neurons (*68*), suggesting that the majority of soluble α-synuclein is not degraded through neuronal autophagy. Furthermore, autophagy machinery in neurons, such as p62, failed to recognize α-synuclein in neurons (*68*), although

this might be dependent on the specific structure of α-synuclein (70). Consistent with these findings, a previous study suggested that the proteasome is the main degradation pathway for endogenous α-synuclein in neurons, but autophagy-lysosomal pathway may participate in α-synuclein degradation through digesting modified α-synuclein (e.g., aggregates or oligomers) at late stages of disease (71). Other studies have also shown that stimulation of autophagy by overexpressing Atg7 or Beclin-1 lowers levels of α-synuclein and neurodegeneration in α-synuclein transgenic mice (72, 73). Together, the evidence has indicated that neuronal autophagy plays a negligible role in endogenous α-synuclein degradation, particularly at young ages, but contributes to the degradation when α-synuclein protein is modified or overloaded at an advanced age. However, the experimental outcomes are complex depending on the levels and structural status of α-synuclein or the severity of neurodegeneration at the time of observation in animals.

MICROGLIAL AUTOPHAGY IN α-SYNUCLEIN CLEARANCE AND PD

As brain-resident macrophages, microglia constitutively monitor their local environment and scavenge neuronal debris, such as myelin and synapses (74, 75). Accumulating evidence has shown that autophagy plays an important role in phagocytosis and subsequent degradation of engulfed materials in microglia. Atg7-deficient microglia exhibit impairments in synaptosome degradation, which causes alterations in spine density and functional connectivity in the brain. These defects subsequently manifest as autism-like social behavioral defects in mice (76). Also, increased myelination and longer nodes of Ranvier are observed in mouse brains harboring Atg7-deficient microglia, which may lead to an increased seizure susceptibility (77). Furthermore, microglial autophagy is shown to regulate the clearance of disease-related proteins. Depletion of Beclin-1, a component of the VPS34 kinase complex required for autophagy initiation (78), impairs phagocytosis in microglia by reducing receptors, such as CD36 and Trem2, resulting in defective extracellular Aβ clearance and reduced phagocytosis capacity (79). Knockdown of Atg7 and Map1lc3b, a gene encoding LC3, suppresses extracellular Aβ clearance in microglia and increases the inflammation in Aβ-injected brains (80). In addition, Atg7 deletion from microglia enhances abnormal tau phosphorylation, accumulation, and spreading in the PS19 tau transgenic mice as well as mice injected with exogenous tau (81). Together these observations demonstrate that autophagy is essential for maintaining homeostatic functions in microglia.

Several studies have characterized the effects of α-synuclein on microglial inflammation; however, it is largely unknown how α-synuclein is degraded in microglia. Our study has shown that microglia take up α-synuclein released from neurons and degrade the ingested α-synuclein through p62-mediated selective autophagy—a process called synucleinphagy (82, 83). Our report used two different PD animal models expressing human α-synuclein in a neuron-specific manner. For an acute model of PD, degeneration of dopaminergic neurons was induced through an adeno-associated virus (AAV) that robustly expresses human wild-type (WT) α-synuclein. For a chronic PD animal model, a transgenic mouse line that expresses human WT α-synuclein at lower levels under the Thy1 promoter (hα-Syn-Tg) was used. Importantly, this transgenic line does not typically exhibit a neurodegenerative phenotype. In these two PD models, microglia were shown to engulf α-synuclein released

from neurons (82). When crossed with these PD animal models, microglial Atg7-deficient mice showed increased levels of insoluble α-synuclein and phosphorylated α-synuclein (p-S129), indicative of misfolded/aggregated α-synuclein. Furthermore, depleting autophagy in the microglia from the hα-Syn-Tg PD model led to the loss of dopaminergic neurons, indicating that loss of microglial autophagy predisposes the brain to PD-like pathology. This result demonstrates that autophagy in microglia fulfills an important neuroprotective function to safeguard against the onset of PD.

A mechanistic study demonstrated that p62, a selective autophagy receptor, is required for degrading ingested α-synuclein. In response to α-synuclein treatment, microglia increased levels of p62/Sqstm1 mRNA and protein through nuclear factor κB (NF-κB)–mediated transcriptional activation (82). Furthermore, internalized α-synuclein was shown to interact with p62 and associate with p62-positive and ubiquitin-positive aggregates in microglia. As a selective autophagy receptor, p62 is known to interact with its targets to form ubiquitinated aggregates by an oligomerization process, which is necessary for autophagic degradation (84). p62-knockout (KO) microglia failed to form α-synuclein–positive and ubiquitin-positive small aggregates, suggesting that p62 functions as a selective autophagy receptor for α-synuclein. In line with these observations, a previous report showed that p62 deficiency increases the number of α-synuclein inclusion and the overall levels of α-synuclein in the brains of α-synuclein–overexpressing transgenic mice (85). However, cell type–specific roles of p62 should be examined to further determine the effect of microglial p62 on α-synuclein homeostasis over other cell types. Together, these findings highlight the importance of p62 in maintaining α-synuclein homeostasis.

TLR2 AND TLR4, POTENTIAL RECEPTORS FOR α-SYNUCLEIN

Toll-like receptors (TLRs) play an important role in sensing pathogen-related molecular patterns and intrinsic danger signals to elicit immune responses in the innate immune system (86). Multiple reports have demonstrated that TLR4 is linked with α-synuclein–mediated activation of microglia. Both monomeric and aggregated forms of α-synuclein were shown to induce the secretion of inflammatory cytokines and chemokines, including interleukin-1β (IL-1β), tumor necrosis factor–α (TNF-α), and CXCL1, and reactive oxygen species (ROS) through TLR4 (87). Oligomeric α-synuclein also increased the levels of TNF-α and ROS through TLR4 (88). In the absence of TLR4, microglia failed to increase the levels of p62 mRNA and proteins in response to α-synuclein treatment (82). These findings may be linked with increased expression of TLR4 in the SNpc, relative to other regions of the human brain (88).

Several lines of evidence have also suggested that TLR2 is a major receptor for α-synuclein. In α-synuclein–overexpressing SH-SY5Y cells, a dopaminergic neuronal cell line, secreted α-synuclein activates microglia through TLR2 (51). Furthermore, TLR2 was shown to be necessary for microglia to uptake SH-SY5Y–secreted α-synuclein (51). A recent study has further reported that α-synuclein can be detected in plasma exosomes derived from PD patients. Exosomal α-synuclein was taken up by microglia, and the ingested exosomal α-synuclein was transferred to neurons following microglial activation through TLR2 (89). Another study showed that both monomeric and oligomeric species of α-synuclein activate the NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome via TLR2 and TLR5 in microglia (90). Notably, TLR2 expression was

up-regulated in dopaminergic neurons, but not in microglia, in PD patients as the disease progresses (*91, 92*). Furthermore, TLR2 activation in neurons is known to cause increased levels of inflammatory cytokines and α-synuclein expression, leading to α-synuclein aggregation (*91, 93*). These observations suggest a direct role for neuronal TLR2 in α-synuclein homeostasis. In support of the hypothesis that TLR2 activation is central to PD pathogenesis, deletion or pharmacological blockade of TLR2 in α-synuclein–overexpressing mice has been shown to lessen several PD-associated phenotypes (*93, 94*). A caveat of this approach, however, is that the relative contributions of TLR2 signaling in different cell types cannot be accurately determined.

By contrast, our study directly examined a differential preference of α-synuclein between TLR4 and TLR2 (*82*). Although α-synuclein failed to induce p62 up-regulation, it caused a small increase in the cytokines *Il-1b* and *Tnf* mRNA in *Tlr4*-KO microglia following α-synuclein treatment. This increase of *Il-1b* and *Tnf* mRNA was abolished following TLR2-blocking antibody treatment, indicating a partial involvement of TLR2 in α-synuclein–induced microglial activation in the absence of TLR4. Furthermore, TLR2-blocking antibody exhibited a stronger inhibitory effect on microglial activation in *Tlr4*-KO microglia than in WT microglia. In the human embryonic kidney (HEK) 293T–NF-κB–luciferase system, α-synuclein was able to activate TLR4 and TLR2 separately, but preferred TLR4 when both receptors are coexpressed. The above observations suggest a preference of α-synuclein for TLR4 over TLR2. However, future studies should investigate the effects of different species of α-synuclein in this regard. Interestingly, a previous study showed that *Tlr4* deficiency causes loss of dopaminergic neurons and impairment of motor behaviors in α-synuclein–overexpressing mice. Importantly, the authors also demonstrated that loss of *Tlr4* reduces the clearance of α-synuclein by microglia (*95*). Therefore, the α-synuclein–TLR4–p62 axis is essential for maintaining α-synuclein homeostasis in microglia.

MICROGLIAL AUTOPHAGY IN INFLAMMASOME CLEARANCE AND PD

The inflammasome is a multiprotein complex that regulates caspase activation and IL-1β and IL-18 maturation in immune cells in response to pathogens and endogenous stress signals (*96*). Inflammasome activation requires a "primed" stage with an increased expression of NOD-like receptor (NLR) family, such as NLRP3, through TLR activation. The inflammasome can be triggered by a variety of stimuli including, but not limited to, ROS from mitochondria, cathepsin released from lysosomal rupture, and the efflux of potassium through ion channels. Once activated, inflammasome complexes are formed through recruitment of the inflammasome adaptor protein ASC (apoptosis-associated speck-like protein containing CARD) and pro–caspase-1 to the NLR family, generating an active form of caspase-1. In turn, active caspase-1, a cysteine-dependent protease, produces mature IL-1β and IL-18 by cleaving pro–IL-1β and pro–IL-18, respectively. Inflammasome activation also induces pyroptosis, a lytic programmed cell death that is part of the antimicrobial response. However, uncontrolled and excessive inflammasome activation has been associated with neurodegenerative diseases including PD (*97*). Soluble and insoluble α-synuclein were known to cause NLRP3 inflammasome activation in primary mouse microglia (*90*), human microglia (*98*), and peripheral blood

mononuclear cells (PBMCs) (*99*). The levels of NLRP3, ASC, and CASPASE-1 mRNA and protein were increased in PBMCs derived from PD patients compared to healthy controls (*100*). Furthermore, levels of plasma α-synuclein were higher in PD patients than in controls and positively correlated with levels of IL-1β and severity of motor symptoms (*100*). A previous study reported an increase of cleaved caspase-1 and ASC in PD animal models and SNpc in PD patients (*101*). Importantly, administration of a small-molecule NLRP3 inhibitor, MCC950, blocked inflammasome activation and release of ASC in fibrillar α-synuclein–treated microglia (*101*). Suppression of inflammasome activation was also shown to ameliorate accumulation of α-synuclein and prevented dopaminergic neuron loss and motor deficits in PD animal models (*101*).

Autophagy acts as an important regulator of inflammasome. Loss of ATGs, such as *Atg16L1* and *Atg7*, resulted in an increased production of inflammatory cytokines, IL-1β and IL-18, in response to lipopolysaccharides (LPS), a well-known TLR4 agonist, in macrophages (*102*). Deficiency of *Atg16L1* in hematopoietic cells increased the susceptibility of mice to dextran sulfate sodium–induced colitis, suggesting that mice harboring autophagy-deficient immune cells may be more sensitive to inflammatory stimuli (*102*). Similarly, a reduction of either p62 or Beclin-1 increased the levels of mature IL-1β and active caspase-1 in the THP-1 monocyte cell line (*103*).

Several studies have indicated cross-talk between inflammasome and autophagy. LPS is known to prime the inflammasome by inducing transcription of NLRP3 and IL-1β through activation of the NF-κB pathway (*104*). In addition, TLR4 activation enhanced autophagy flux as evidenced by increased levels of lipidated LC3 and LC3-positive puncta in human alveolar macrophages and the macrophage cell line, RAW264.7 (*105*). LPS can also induce p62 in primary macrophages (*106*). Interestingly, p62 plays a role in preventing excessive activation of the inflammasome by clearing damaged mitochondria through autophagy, a process known as mitophagy (*106*). As a result, myeloid cell–specific *p62*-deficient mice demonstrated enhanced vulnerability to inflammasome-mediated models of inflammation such as alum-induced peritonitis and fulminant hepatitis (*106*). Components of the inflammasome complex, including NLRP3 and AIM2, were also shown to colocalize with LC3-positive autophagosome and p62 upon inflammasome activation (*103*). Together, these studies support the hypothesis that autophagy is an important regulator of inflammasome activation in immune cells.

Autophagy in microglia is suggested to play a key role in curtailing excessive inflammation in the brain and preventing disease onset. Deletion of *Atg5* in microglia was shown to cause NLRP3 inflammasome activation through the PDE10A (phosphodiesterase 10A)–cyclic adenosine monophosphate (cAMP) pathway, resulting in increased IL-1β production (*107*). Notably, microglia-specific *Atg5*-deficient mice developed motor impairments and dopaminergic neuron loss in SNpc. Pharmacological inhibition of NLRP3 lowered the levels of inflammatory cytokines, including IL-1β and TNF-α, and reduced neuronal loss and microgliosis (*107*). Another study has recently reported that blocking autophagy increases the levels of NLRP3, caspase-1, and IL-1β following inflammasome activation in the BV2 microglial cell line. *Atg5* deficiency in microglia worsened motor-related behaviors and promoted a loss of dopaminergic neurons in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–induced PD model (*108*). These studies highlight the importance of

microglial autophagy in controlling inflammasome activation in the context of PD.

DISCUSSION

The converging evidence has demonstrated that autophagy in microglia fulfills an important neuroprotective role through the regulation of α-synuclein homeostasis and inflammasome activation (Fig. 1) (*82*, *107*, *108*). Interestingly, a recent study showed that α-synuclein treatment suppresses autophagy flux as evidenced by an increase of p62 and reduction of LC3II by activating the Akt-mTOR pathway in microglia (*109*). The discrepancy between these observations and findings from other groups is not currently understood. Differences in certain experimental conditions, such as the source of α-synuclein and cultured microglia, may underlie several of these differences. Furthermore, a previous study showed that oligomeric α-synuclein is a major structural form of α-synuclein released from cultured neurons that overexpress human α-synuclein (*51*). As a field, we currently lack complete understanding regarding which

forms of α-synuclein are present in vivo and responsible for the propagation of pathological α-synuclein. Interestingly, in vivo microdialysis experiments have shown that multimeric (~60 kDa) α-synuclein, not monomeric (~14 kDa) or dimeric (~28 kDa), is present in the brain interstitial fluid of mice (*55*). It remains to be determined whether α-synuclein forms complexes with other proteins or α-synuclein forms tetramers as suggested by the authors of this study (*110*). Increased levels of multimeric α-synuclein have also been reported in the cerebrospinal fluid of PD patients (*111*). As neurodegeneration occurs in PD, various forms of pathogenic α-synuclein in neurons may be secreted to the extracellular space and interact with neighboring cells including microglia. This hypothesis is supported by a study showing that inoculating brain lysates from symptomatic mice with neurodegeneration causes the accumulation and propagation of α-synuclein aggregates (*112*). Injection of PFFs produced from purified recombinant α-synuclein protein has been repeatedly shown to cause the spreading of pathological accumulation of α-synuclein (*48*). Interestingly, a recent study has demonstrated that microglia distribute ingested PFF to neighboring
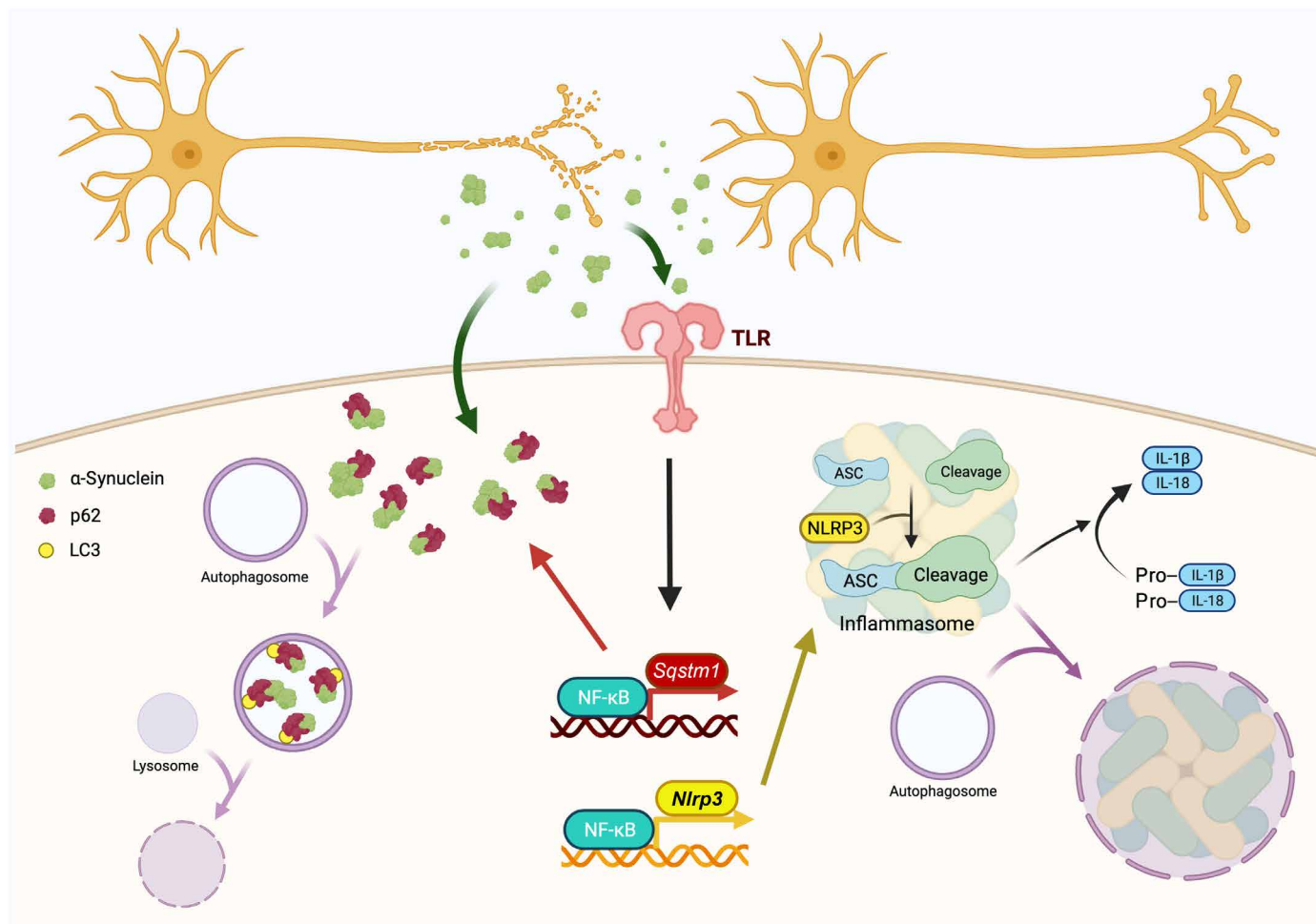

Fig. 1. The neuroprotective function of microglial autophagy in the clearance of α-synuclein and inflammasome regulation. (Left) The neuroprotective function of microglial autophagy in the clearance of α-synuclein and inflammasome regulation. α-Synuclein activates the NF-κB pathway through TLR2 and TLR4 and increases the level of p62 mRNA. As a selective autophagy receptor, p62 binds to internalized α-synuclein and recruits autophagy machinery. The p62–α-synuclein complex is sequestered by autophagosome and degraded by lysosome. **(Right)** Activated NF-κB pathway by TLR–α-synuclein interaction increases the level of NLRP3 mRNA, priming the activation of inflammasome. Components of the inflammasome complex are recognized by autophagy machinery and degraded by autophagosome-lysosome, preventing the excessive formation of the inflammasome in the brain.

microglia through tunneling nanotubes and jointly degrade PFF (*113*). While PFF can replicate the neuropathology of PD in animal models and has been used in microglial research, it remains unclear whether α-synuclein reminiscent of PFF exists in vivo. It also remains to be tested whether autophagy participates in the degradation of PFF α-synuclein species. The roles of different species of α-synuclein in PD have been reviewed elsewhere (*114*).

Several studies have indicated that many autophagy proteins discussed in this review have other independent cellular functions. For example, ATG7 has been implicated in LC3-associated phagocytosis called LAP, which regulates the phagocytosis process (*115*). In certain disease conditions, microglia become highly phagocytic, and LAP may contribute to the degradation process of phagocytosed materials, although in vivo evidence of LAP in microglia remains elusive. Therefore, novel cell or animal models may be required to distinguish LAP from canonical autophagy (*116*). It is worth noting that degradation of α-synuclein is blocked also in *Atg14*-deficient microglia to a similar extent as *Atg7*-deficient microglia (*82*), demonstrating the role of autophagy, rather than LAP, in α-synuclein degradation in microglia.

Multiple studies have shown that PD-linked genes directly control the autophagy-lysosomal pathway (*117*). Despite many PD-related genes being highly expressed in glial cells, including microglia, most studies have remained focused on neurons. A recent study revealed that one of the PD risk variants (rs76904798) located on a noncoding region of the *LRRK2* locus causes an increased *LRRK2* expression specifically in microglia, nominating gene expression in microglia as an important driver of PD risk (*118*). Furthermore, we recently demonstrated that *LRRK2* is highly expressed in microglia and oligodendrocyte precursor cells compared to other cell types in SN tissues from the human brain (*119*). Importantly, LRRK2 has been strongly associated with microglial functions. Loss of LRRK2 or pharmacological inhibition of LRRK2 suppressed inflammatory responses of microglia in response to various stimuli, including LPS, α-synuclein, and virus exposure (*120–122*). LRRK2 deficiency caused increased motility and migration in microglia (*123, 124*), whereas G2019S mutant of LRRK2, which increases kinase activity, suppressed the functions in microglia and monocytes differentiated from LRRK2 (G2019S) patient–derived Induced Pluripotent Stem Cells (iPSC) (*123, 125*). LRRK2 is also linked to microglia phagocytic activity (*126*). Therefore, understanding the role of PD-related genes, such as *LRRK2*, in the autophagy-lysosomal pathway in microglia will help to unravel the complexity of PD genetic influence on the development of disease.

Stimulation of the autophagy-lysosome pathway is a promising therapeutic strategy for the clearance of protein aggregates. Rapamycin, an mTOR inhibitor that induces autophagy by mimicking starvation conditions, or trehalose, an inhibitor for glucose transporter that promotes autophagy through AMPK activation (*127*), is currently in clinical trials for many age-related diseases, including AD. However, nonspecific and constitutive stimulation of autophagy may not be beneficial to all cell types and could even increase the risk of certain diseases. For example, autophagy is known to promote cancer survival and progression depending on the stage of cancer development (*128*). Also, autophagy is linked to maintaining the pluripotency and self-renewal ability of cancer stem cells and conferring the drug resistance of cancer cells (*129, 130*). Furthermore, because of the broad effects of mTOR signaling, rapamycin has many documented side effects (*131, 132*). As an immunosuppressant,

rapamycin may prevent the proper activation of microglia, which is necessary for their neuroprotective roles. Dietary trehalose is also linked with an increase in two epidemic ribotypes (RT027 and RT078) of the bacterium *Clostridium difficile*, causing inflammation, diarrhea, and death (*133*). Many studies have demonstrated the importance of the TLR4/p62-mediated selective autophagy pathway in microglia and its role in the clearance of α-synuclein and inflammasome activation as described above. Therefore, selective boosting of the TLR4-mediated p62 pathway in microglia may offer a promising avenue in the treatment of PD and other neurodegenerative diseases.

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