Abstract Parkinson’s disease is a progressive neurodegenerative disease characterized by Lewy body pathology of which the primary constituent is aggregated misfolded alpha-synuclein protein. Currently, there are no clinical therapies for treatment of the underlying alpha-synuclein dysfunction and accumulation, and the standard of care for patients with Parkinson’s disease focuses only on symptom management, creating an immense therapeutic gap that needs to be filled. Defects in autophagy have been strongly implicated in Parkinson’s disease. Here, we review evidence from human, mouse, and cell culture studies to briefly explain these defects in autophagy in Parkinson’s disease and the necessity for autophagy to be carefully and precisely tuned to maintain neuron survival. We summarize recent experimental agents for treating alpha-synuclein accumulation in α-synuclein Parkinson’s disease and related synucleinopathies. Most of the efforts for developing experimental agents have focused on immunotherapeutic strategies, but we discuss why those efforts are misplaced. Finally, we emphasize why increasing autophagy flux for alpha-synuclein clearance is the most promising therapeutic strategy. Activating autophagy has been successful in preclinical models of Parkinson’s disease and yields promising results in clinical trials.

Key Points

Evidence points to defects in protein clearance in Parkinson’s disease.

Disposal of neurotoxic proteins may provide a therapeutic option for Parkinson’s disease.

Activation of the cellular disposal machine may halt disease progression.

1 Introduction

Autophagy is a cellular mechanism involved in recycling materials, including proteins, to maintain cell survival and homeostasis. Alteration of autophagy may be a key factor that contributes to the pathogenesis of Parkinson’s disease (PD), which involves accumulation of misfolded alpha-synuclein, among other proteins, in cellular inclusions called Lewy bodies. Genetic, environmental, aging, or other factors may predispose to alpha-synuclein accumulation, but the overwhelming evidence suggests that the process of autophagy is defective in PD. Alpha-synuclein may disrupt autophagy and accumulate in cytosolic vacuoles that are unable to be efficiently degraded in the lysosome. Either an increase or an inhibition of autophagy flux can be detrimental to neuronal survival, thus regulation of autophagic activity is critical to maintain efficient alpha-synuclein clearance.

This review highlights important issues that pertain to autophagic clearance of alpha-synuclein and suggests a
Autophagy is an evolutionarily conserved quality-control mechanism shared by mitotic and post-mitotic cells and it suppresses mitosis and/or mitigates oncogenes [9–11]. In mitotic cancer cells, cell division and apoptosis are mediated by signaling mechanisms via the endosomal (early and recycling) system [12]. Under normal conditions, excess proteins are recycled via the endosomal-lysosomal system to prevent protein accumulation and/or secretion [12–14]. Degradation of proteins such as alpha-synuclein (Fig. 1) may be regulated via a ubiquitination/de-ubiquitination cycle to be cleared via proteasomes or autophagy [15–17]. In contrast to other cell types, neurons are irreplaceable and autophagy must be tuned to maintain cell survival. Therefore, failure of cellular quality-control mechanisms leads to inefficient protein degradation via the proteasome and/or autophagy [18], resulting in an intracellular accumulation of neurotoxic proteins including alpha-synuclein. Alpha-synuclein accumulation in Lewy bodies may be caused by a lack of protein clearance via chaperone-mediated autophagy and reduced lysosomal functioning [19–21].

3 Autophagic Defects in Parkinson’s Disease

Defects in autophagy are well established in neurodegenerative diseases [22–28] and are characterized by un-degraded autophagic vacuoles that accumulate in the cytosol of surviving neurons [23, 24, 29]. Accumulation of autophagosomes has been described in multiple brain disorders [30–32], including advanced stages [33] and animal models [34] of Alzheimer’s disease (AD), suggesting that reduced lysosomal clearance is taking place [35]. Dysfunction in autophagy appears to be a common denominator in neurodegenerative diseases [24, 36–38], including PD and Lewy body dementia [19–21].

We previously investigated the role of autophagic clearance in postmortem nigrostriatal tissues from 22 patients with non-familial sporadic PD and 15 control subjects [39]. We previously found that alpha-synuclein accumulates in pre-lysosomal vacuoles that possibly include phagophores and autophagosomes but not lysosomes, indicating defects in autophagic flux in post-mortem brains [39]. Further examination of the same post-mortem brain tissues with transmission electron microscopy (Fig. 2) shows no noticeable accumulation of autophagic vacuoles in the caudate (Fig. 2a) and midbrain (Fig. 2b, c) of healthy human subjects, but PD brains show an appreciable accumulation of autophagic vacuoles in the cytosol in the caudate (Fig. 2d, e) and midbrain (Fig. 2f). It is noticeable in the PD brains that cellular materials and perhaps mitochondria and other cell organelles remain undegraded within these large accumulating cytosolic vacuoles. Subcellular fractionation of these vacuoles in human [39] and mouse models showed that alpha-synuclein accumulates in the striatum or substantia nigra in autophagosomes but not lysosomes, while stimulation of autophagy flux via parkin over-expression or with tyrosine kinase inhibitors (TKIs), including nilotinib or bosutinib, leads to alpha-synuclein clearance and disappearance of the vacuoles [2, 39, 40]. Alpha-synuclein-containing vacuoles may be immature autophagosomes or lysosomes that fail to deposit their contents into the lysosome for degradation [3]. Therefore, a therapeutic strategy that can facilitate autophagic flux and promote autophagosome maturation to dump misfolded alpha-synuclein and other neurotoxic proteins in the lysosome has a disease-modifying potential and may arrest PD progression.

Impairment of the autophagy-lysosome pathway could come from dysfunction in any of several steps of autophagy (Fig. 1). Induction of autophagy can become
detrimental when un-degraded autophagosomes accumulate and overwhelm the lysosome in a process that may potentially lead to apoptotic cell death. Inhibition of the mammalian target of rapamycin (mTOR) stimulates autophagy [41–43] and increases protein degradation via the lysosome [44]. Conversely, expression of intraneuronal neurotoxic proteins exacerbates accumulation of autophagic vacuoles [2, 40, 45, 46], indicating that autophagy may be impaired by protein accumulation. Additionally, lack of recognition of aggregate proteins was described in models of neurodegeneration [47] whereby molecular steps of autophagy are activated but autophagosome clearance due to inefficient fusion with the lysosome is defective [48]. In neurodegeneration, the levels of a key autophagy protein complex known as Beclin-1 are reduced [49], suggesting inefficient execution of Beclin-1-dependent autophagy. Lentiviral delivery to express Beclin-1 activates autophagy and improves neurodegenerative pathology in alpha-synuclein mouse models of PD [50]. We previously demonstrated that parkin interaction with Beclin-1 is a trigger to facilitate autophagic flux and neurotoxic protein clearance in several animal models of neurodegeneration, including PD [2].

The final step of autophagy following induction and autophagosome maturation may include defects in degradative enzymes of the lysosome. However, there is no known evidence of lysosomal defects in sporadic PD to date, and it appears that impairment of autophagic flux may be owing to misfolded protein accumulation that overwhelms efficient lysosomal function. Mutations in glucocerebrosidase that result in a lysosomal storage disease known as Gaucher disease is associated with autosomal-recessive PD [51, 52], further suggesting the involvement of autophagy in PD pathogenesis. Sanofi-Genzyme has a current clinical study (NCT02906020) to determine the efficacy of a drug called GZ/SAR402671 in patients with early-stage PD who carry a glucocerebrosidase mutation. Further, there is emerging evidence that the greatest known genetic contributor to PD, leucine-rich repeat kinase 2 protein, is involved in molecular trafficking that mediates chaperone-mediated autophagy and interaction between autophagosomes, endosomes, and lysosomes [53]. More evidence from genome-wide association studies also suggests that several key genes involved in the regulation of autophagy are altered in post-mortem PD brains [54].

Fig. 1 Under normal conditions, alpha-synuclein clearance may be regulated via an ubiquitination/de-ubiquitination balance and recognized by the proteasome and/or autophagy for degradation and recycling into individual amino acids. One method of activating normal autophagy is via the interaction of Beclin-1 and parkin (which appears to be inactive in sporadic Parkinson’s disease) to sequester excess or misfolded alpha-synuclein into phagophores, which then fuse with endosomes or lysosomes to be degraded in autolysosomes. In Parkinson’s disease, misfolding or aggregation of alpha-synuclein may be exacerbated by imbalances in the ubiquitination/deubiquitination cycle, inhibition of the proteasome, or reduced flux of autophagy, leading to failure of alpha-synuclein clearance.
4 Molecular Pathways for Maturation of Autophagic Vacuoles

Several molecules can regulate the maturation of autophagosomes, including the AAA ATPase SKD1, the small GTP-binding protein Rab7, and the AD-linked presenilin 1 [55]. Autophagosome accumulation could be the result of a lack of maturation, leading to their inefficient fusion with lysosomes. Ubiquitination may facilitate recognition between components of the autophagic machinery [56, 57], and may promote autophagosome maturation [58, 59]. Autophagy-related proteins (Atgs) are critical to determining autophagic vacuole formation [60]. Specifically, Atg5, Atg7, and Atg12 determine the sequestering phagophore formation [61–66]. Light chain (LC)-3 protein is initially synthesized in an unprocessed form, proLC3, but a sequential ubiquitination-like reaction and conjugation of Atgs lead to its modification (via lipidation) into LC3-II, which is a marker of mature autophagosomes [67, 68]. Reduced turnover of autophagosomes can be due to inhibition of their maturation, leading to an inability to fuse with the lysosome [69], which is evident in PD (Fig. 1).

Parkin is an E3 ubiquitin ligase that facilitates proteasomal degradation of misfolded proteins [70]. Loss-of-function mutations in the gene coding for the E3-ubiquitin ligase parkin [i.e., threonine to arginine (T240R)] are associated with juvenile-onset autosomal recessive PD [71, 72]. Parkin is inactivated in the nigrostriatal regions of sporadic PD [73, 74] and decreased parkin levels are detected in AD brains [75]. The role of parkin as a key component of the molecular cascade to activate autophagy is now widely recognized. First, parkin over-expression attenuates β-amyloid (Aβ) and alpha-synuclein toxicity in human M17 neuroblastoma cells over-expressing 4R wild-type tau [76]. Parkin overexpression also activates Beclin-1-mediated autophagy in triple transgenic AD mice [77]. Activation of autophagy improves dopaminergic cell survival in parkin-deficient and tau over-expressing mice [78]. Second, notwithstanding the role of parkin and PTEN-induced putative kinase 1 regulation of mitophagy [79–83], parkin has a broader role in neurodegeneration (reviewed in [84]). In mitophagy, parkin ubiquitinates proteins of...
critical role in autophagy in PD. Therefore, parkin effects on autophagosome maturation via activation of Atgs and conversion of LC3-I to LC3-II, leading to restoration of normal autophagy in animal models of neurodegeneration. These findings indicate that parkin activation leads to autophagosome maturation and clearance via the lysosomes. Tyrosine kinase inhibitors are effective and well-tolerated treatments for chronic myelogenous leukemia. Tyrosine kinase inhibitors nilotinib (BCR-ABL) and bosutinib (SRC-ABL) inhibit Abl, which is activated in sporadic PD brains, and activate parkin, leading to interaction with Beclin-1 and autophagic protein clearance. These mechanisms can lead to the formation of autophagosomes, including mTOR-dependent and mTOR-independent autophagy, as well as Beclin-1-independent autophagy. Autophagosomes may recruit lysosomes via retrograde transport on microtubules, requiring an intact microtubule cytoskeleton and cytoplasmic histone deacetylase (HDAC6) to mediate the fusion of autophagosome with the lysosome. Increased tau expression is also detected in the PD brain, mainly the striatum, suggesting that common mechanisms of increased expression underlie the association of the microtubule-associated protein tau gene with PD. Parkinson’s disease with parkin mutations as well as some familial frontotemporal dementia with parkinsonism (FTDP-17) often exhibit tau pathology, mainly in the striatum and basal ganglia. Genome-wide association studies identified microtubule-associated protein tau as a genetic risk factor for PD. However, inconsistent tau pathology in idiopathic PD makes the genetic link puzzling, especially as microtubule-associated protein tau has not been identified as a risk factor in other genome-wide association studies of PD. Importantly, the effects of tau on pathological mechanisms in PD may be a common denominator owing to its role in neuronal transport and the execution of autophagy. The effects of tau may be downstream in neurodegenerative pathologies as transgenic animals over-expressing malfunctioning hyper-phosphorylated tau or Tau−/− mice develop autophagic defects in response to alpha-synuclein accumulation. Tau−/− mice provide an important insight into the role of tau as a neuronal microtubule-associated protein involved in the stabilization of axonal microtubules. We previously demonstrated that tau modification and cell death in lentiviral gene transfer animal models occur as a result of the expression of intracellular Aβ or alpha-synuclein, suggesting that tau modification can destabilize microtubules that provide the railway for autophagosomal transport to fuse with the lysosomes.

6 Mitophagy in Parkinson’s Disease

Parkin activity is regulated by associated proteins, post-translational modification, and self-regulation through intramolecular interactions. Parkin modifies mitochondrial outer membrane proteins and promotes the removal of dysfunctional mitochondria. In response to mitochondrial depolarization, PTEN-induced putative kinase 1 phosphorylates serine 65 in the ubiquitin-like domain of parkin, leading to activation. A recent structural analysis of parkin in an auto-inhibited state provided further insights into how phosphorylation and ubiquitination may activate parkin. PTEN-induced putative kinase 1 modifies ubiquitin at serine 65, which is homologous to the site phosphorylated in the tau ubiquitin-like domain. Phosphorylation of both ubiquitin and parkin are necessary to overcome parkin auto-inhibition, allowing parkin self-ubiquitination and recruitment of substrates. Auto-ubiquitination activates parkin to subsequently recruit TDP-43 for ubiquitination. The link between parkin ubiquitination and its E3 ubiquitin ligase activity was demonstrated by several studies showing that the regulation of parkin ubiquitination affects its activity and stability. Taken together these findings provide evidence that parkin is a quality-control protein that monitors physiological perturbations and regulates autophagy in neurodegeneration and beyond.
7 Current Therapies in Parkinson’s Disease

Alpha-synuclein has long been implicated in the underlying neuropathology of PD [122, 123]. Mutations and duplications in the SNCA gene have been coined as hallmark genetic underpinnings in cases of familial PD [124–126]. The abundance of this genetic and neuropathological evidence implicating alpha-synuclein in PD have made it the most promising therapeutic target. Aims to prevent alpha-synuclein dysfunction, and subsequent protein aggregation, can include reducing SNCA expression, inhibiting toxic prion-like activity, reducing aggregates, or activating autophagy [127–129]. Most of the current research has been swayed towards the direction of developing antibodies against alpha-synuclein. In neurodegeneration, un-degraded neurotoxic proteins may be secreted from dying neurons and may take advantage of cellular contiguity and trans-synaptic propagation along neuroanatomical pathways [130], perhaps leading to the progressive spread of disease and cell death. The discovery of the secretion of neurotoxic proteins such as Aβ in AD triggered enormous research efforts via the development of antibodies to clear extracellular proteins. However, the propagation hypothesis of prion-like proteins in AD may not be simply extrapolated to PD in which no extracellular alpha-synuclein in the interstitial space is appreciably detected. It is possible that cell-to-cell propagation of alpha-synuclein may be a trans-synaptic process, but there is a lack of evidence suggesting that alpha-synuclein is secreted into the extracellular space. Therefore, an antibody may have to capture misfolded alpha-synuclein en route across the synapse or enter the neuron.

These issues are fraught with conceptual and mechanistic problems for anti-alpha-synuclein antibodies. First, anti-alpha-synuclein antibodies must gain entry to the affected neurons, and it is very challenging to develop an antibody that can cross the lipid bilayer of cellular membranes. Second, if an antibody is able to gain neuronal entry, there is a likelihood of it being destroyed by the lysosome, which may still be functional. Taken together, (1) the absence of misfolded alpha-synuclein accumulation in extracellular aggregates, (2) the difficulty of antibody passage into the cell, (3) the risk of antibody destruction in the lysosome, and (4) the possible targeting of monomeric and unfolded alpha-synuclein all constitute significant hurdles to potential immunotherapies.

A number of studies have explored these options of targeting alpha-synuclein at its various stages of production and dysfunction, though none have so far led to disease-modifying therapies for use in humans. Immunotherapy strategies have been adopted by three clinical trials for alpha-synuclein clearance, two of which are currently underway and one slated to start soon (Table 1). Prothena Biosciences demonstrated that an antibody directed against the carboxyl terminal of alpha-synuclein alleviated motor dysfunction and Lewy body pathology in mice [131, 132]. The anti-alpha-synuclein antibody (9E4) was tested in two transgenic models of PD that overexpress alpha-synuclein in the cortical and subcortical regions with accompanying striatonigral degeneration and was successful in reducing the accumulation of calpain-cleaved and oligomerized species of alpha-synuclein [131, 133, 134].

In 2014, Prothena Biosciences completed the phase I study (NCT02095171) of the humanized version of 9E4, compound PRX002, for safety and tolerability in healthy participants [135]. It was reported that PRX002 reduced serum alpha-synuclein levels within the first hour after injections and for 2–4 weeks afterwards [132]. In 2016, Prothena Biosciences completed a second phase I study (NCT02157714) of PRX002 for multiple ascending doses in approximately 60 participants with PD [136]. Currently, they are recruiting for a phase II study (NCT03100149) of PRX002, renamed RO7046015, in patients with early-stage PD [137].

Biogen is currently recruiting for a phase I study (NCT0249886) to assess the safety and tolerability of BIIB054, a human-derived monoclonal anti-alpha-synuclein antibody generated from B lymphocytes of healthy elderly subjects [138] in patients with early-stage PD [139]. BIIB054 was reported to have selective binding and the ability to inhibit alpha-synuclein transmission between neurons in a primary neuronal culture. BIIB054 was further assessed in mice that received intracerebral injections of preformed alpha-synuclein fibrils that seed the aggregation of endogenous soluble alpha-synuclein monomers. In this mouse model of PD, BIIB054 alleviated pathology and rescued motor deficits [138]. The most recent phase I study (NCT03272165) to use an anti-alpha-synuclein antibody is the result of a partnership between Takeda and AstraZeneca to test Medi1341 for safety and tolerability in healthy participants, but has not yet started recruiting [140].

In parallel with current immunotherapy studies, activation of autophagy to clear misfolded alpha-synuclein in PD has also been used as a potential strategy. The small molecule, nilotinib, originally intended for the treatment of chronic myelogenous leukemia, has been used to promote autophagic protein clearance in PD models [2]. We have shown that nilotinib, a second-generation BCR-ABL TKI, penetrates the brain and activates parkin, leading to Beclin-1-mediated autophagic clearance of aggregated alpha-synuclein [2, 40]. In 2014, we conducted an open-label phase I study (NCT02281474) to test low doses of nilotinib for safety and tolerability in patients with mid-advanced
We found a reduction in phosphorylated ABL, suggesting nilotinib may be inhibiting the phosphorylation of ABL [4]. Motor symptoms and cognitive performance were both monitored over the 6-month period of nilotinib administration. The motor symptoms were assessed by the Unified Parkinson Disease Rating Scale and showed an average beneficial decrease of approximately 3 points at 6 months when compared with baseline. The cognitive performance was assessed by the Mini-Mental State Examination and also showed a beneficial average increase of approximately 4 points in scores at 6 months compared with baseline [4]. Both of these measures reverted back to baseline scores after 3 months of withdrawal from nilotinib treatment [4]. We are now recruiting for a phase II study (NCT02954978) to evaluate the effects of nilotinib in a randomized double-blind placebo-controlled trial in patients with PD [142]. From a clinical perspective, it is expected that early intervention with agents that promote autophagy may be particularly promising as they may clear alpha-synuclein and halt the progression of parkinsonism via arrest of neuronal death.

8 Conclusion

There are currently no disease-modifying therapies for PD, leaving a huge therapeutic gap to be filled. Alpha-synuclein is a good therapeutic target, but the question of how to effectively target it is still being investigated. Defects in autophagy have been established in neurodegenerative diseases, but with a discrete population of neurons this process must be kept under control to maintain cell survival. The overwhelming evidence suggests that autophagy may be manipulated to facilitate the clearance of alpha-synuclein with the aid of therapeutic agents. Therefore, clearance of misfolded alpha-synuclein via manipulation of autophagy is a feasible strategy as a potential treatment for PD.
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Compliance with Ethical Standards

Conflict of interest  Charbel E.-H. Moussa is listed as an inventor on a Georgetown University intellectual property patent to use tyrosine kinase inhibitors for the treatment of neurodegenerative diseases. Alan J. Fowler has no conflict of interest directly relevant to the content of this article.

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Autophagy in Parkinsonism

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