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Genetic causes of Parkinson's disease and their links to autophagy regulation

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SUMMARY

Genetic studies over the past 15 years have revolutionized our understanding towards the etiology of Parkinson's disease (PD). These studies have discovered many disease-linked genetic loci (PARK 1 to 18), which are now being interrogated for cellular pathways contributing to PD. Various pathogenic pathways were proposed but validation of each pathway awaits rigorous experimental testing. Here we review recent progress in understanding the influence of disease risk genes on cellular functions, specifically, autophagy pathways. Autophagy is a cell self-eating, lysosomal degradation system that plays an important role in cell homeostasis and survival. Neurons are post-mitotic cells and particularly vulnerable to the impairment of autophagic degradation due to their inability to redistribute damaged proteins and organelles to daughter cells. Emerging evidence has implicated dysfunctional autophagy in a growing number of neurodegenerative diseases including PD. We will also discuss the prospect of intervening autophagy pathways as a potential strategy to treat PD.

1. Introduction

Autophagy, which refers to "self-eating" from the Greek terms, describes the cellular catabolic process in which cytosolic components, including mainly long-lived proteins and organelles, are transported to lysosome for degradation. Although autophagy was described as early as the 1960s, the physiological function of autophagy, especially its role in neurological diseases, has just started to be understood in the recent decade [1].

Depending on the means of cellular compartments being delivered to lysosomes, autophagy pathways can be separated into three major subtypes: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).

Macroautophagy, which is the most common form of autophagy, involves the initial elongation of the double membrane structure, known as the "phagophores" (or pre-autophagosomal structure, PAS). A portion of the cytoplasm is then sequestered during elongation to form the autophagosome, a-double membraned vesicle frequently used as a clue for identifying altered autophagy in disease models. Autophagosomes subsequently fuse with endosomes and lysosomes, where the cytoplasmic contents are degraded within the acidic environment.

Microautophagy is the least understood phenomenon in mammalian cells. It is characterized by direct invagination of the lysosomal membrane during sequestration of cytoplasmic contents.

In contrast to macro- and microautophagy, **CMA** features the most selective translocation of the cytosolic proteins to the lysosomal lumen. A complex of cytosolic chaperones is responsible for recognition of a specific protein motif and for unfolding, delivery of the protein to the receptor at lysosomal membrane.

All three types of autophagy coexist in neurons to maintain intracellular homeostasis; aid in the clearance of misfolded proteins, disease-prone aggregates and damaged organelles; as well as in support of neurodevelopment and plasticity. Alteration in macroautophagy or CMA has been implicated in neurodegenerative diseases including Parkinson's disease (PD) [2].

PD is characterized pathologically by the selective dopamine neuron loss in the nigrostriatal pathway and the presence of Lewy bodies (LB) in surviving neurons. The vast majority of PD cases are sporadic with unknown etiology. However, approximately 5% of cases are inherited. Recent human genetic studies have identified a number of genes linked to familial forms of PD. The known-to-date PD causative genes include autosomal dominant mutant *SNCA* (PARK1/4), which is the gene that encodes a major component of LB, α-synuclein; and *LRRK2* (PARK8), which encodes a multi-domain large protein, whose G2019S mutant alone accounts for most of the dominantly inherited PD cases. The autosomal recessive mutants, such as *PINK1* (PARK6) and *parkin* (PARK2) are genes closely related to mitochondrial function and maintenance. Their loss-of-function mutations cause early-onset PD. Several other genes were suggested by association studies or linkage analysis to cause Parkinsonism or Parkinson-plus syndromes [3]. These genes include *VPS35* (vacuolar protein sorting 35), *GBA* (glucocerebrosidase) and *ATP13A2* (at PARK9 locus, encodes a P-type transport ATPase), *MAPT*

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(microtubule-associated protein tau), *EIF4G1* (eukaryotic translation initiation factor 4 gamma 1).

The identification of PD risk genes provides unprecedented opportunities for dissection of PD pathogenic mechanisms and development of drug targets. Emerging evidence has suggested that aberrant autophagy is one of the underlying mechanisms for inherited forms of PD. In this review, we will provide an overview of how different genetic causes influence macroautophagy and CMA.

2. Autosomal dominant genes and macroautophagy

2.1. SNCA

α-Synuclein protein levels are considered a major determinant of its neurotoxic potential and are related to the formation of LB. Indeed, multiplication of wild-type *SNCA* gene causes familial forms of autosomal dominant PD. Removing this toxic mediator of the pathology in PD has become the most important task in PD research. A recent study suggested a regulatory role of a deubiquitinase USP9X in determining the fate of α-synuclein clearance pathway. It showed that deubiquitinated α-synuclein is mostly degraded by autophagy, while monoubiquitinated α-synuclein is preferentially removed by proteasome [4]. In an *in vivo* study using α-synuclein transgenic mice, the ubiquitin–proteasome system (UPS) was demonstrated to be the main route for protein degradation under normal conditions. In case of an increased α-synuclein burden, the autophagy pathway may be recruited [5].

Current evidence also implicates a role of α-synuclein in interfering with autophagy, but the exact mechanism remains to be clarified. Results obtained from mammalian cells and in transgenic mice suggest that overexpression of α-synuclein compromises autophagy via inhibition of Rab1a and alteration of the autophagy protein Atg9 localization [6]. Using cultured cells or neurons treated with pre-formed α-synuclein fibers, a more recent study found that once formed, α-synuclein inclusions/aggregates are refractory to degradation by autophagy. In fact, the α-synuclein inclusions impair macroautophagy by reducing the autophagosome clearance [7]. Furthermore, mutant A53T α-synuclein blocks CMA, which will be discussed below (section "Effect of α-synuclein and LRRK2 on chaperone-mediated autophagy"). In contrast, another study showed that expressing either wild-type or A53T mutant α-synuclein enhanced autophagosome formation. Knocking down α-synuclein disrupts autophagy and results in accumulated α-synuclein oligomer [8].

2.2. LRRK2

Dominantly inherited mutations in LRRK2 are linked to the most common familial forms and some sporadic cases of PD. Several reports showed that LRRK2 may regulate macroautophagy. Disruption of *LRRK2* in mice causes age-dependent bi-phasic alteration of autophagy pathway in kidney [9]. Moreover, LRRK2 knockdown resulted in increased autophagic flux under starvation conditions in a human embryonic kidney cell line (HEK293) [10]. However, evidence for such regulation by LRRK2 in the brain is lacking. The pathogenic mutation G2019S of LRRK2 causes neurite shortening in differentiated neuroblastoma cells involving active autophagy [11]. LRRK2 seems to activate the CaMKK-β/AMPK pathway, which is followed by increased number of autophagosomes [12]. Using a LRRK2 pharmacologic inhibitor, a recent study showed that blocking of LRRK2 kinase activity stimulates macroautophagy [13].

2.3. VPS35

Vacuolar protein sorting 35 (VPS35) is a critical component in the retromer system for mediating intracellular retrograde transport of endosomes to the trans-Golgi network. Its mutation at D620N has been shown to cause late-onset autosomal dominantly inherited PD [3]. VPS35 has been known to regulate trafficking within the lysosome-dependent pathway; in particular, recycling cargos such as SNARES (N-ethylmaleimide-sensitive factor attachment protein receptor) between endosomes and trans-Golgi networks [14]. Although the components that participate in fusion of autophagosomes and lysosomes or endosomes are poorly characterized, the impairment in SNARE recycling was known to affect mammalian autophagic fusion. Nevertheless, the precise role of VPS35 in macroautophagy is yet to be established.

3. Autosomal recessive mutations and mitophagy

3.1. Parkin and PINK1

Mitophagy is the selective engulfment of mitochondria by autophagosomes and their subsequent catabolism by lysosomes. Several autosomal recessive PD-related genes were known to be involved in mitophagy. Parkin, a cytosolic E3-like ubiquitin ligase, was mutated in nearly 50% of autosomal recessive, and 10–15% of sporadic early-onset PD [15]. At resting state, Parkin is cytosolic, and has a previously known function on ER and plasma membrane in addition to mitochondria. The Youle group and others have demonstrated redistribution of Parkin to mitochondria following the treatment of a mitochondrial uncoupler (CCCP) [16]. Further analysis showed that Parkin was selectively recruited to mitochondria fragments and mitochondria with impaired membrane potential. These Parkin-labeled mitochondria then recruit autophagy components, bringing themselves to the ultimate fate in the autophagy–lysosomal pathway.

Parkin interacts with PINK1 (PTEN-induced kinase 1), a mitochondrial membrane-anchored serine/threonine kinase whose mutation is also linked to separate autosomal recessive cases of PD [17]. When mitochondrial membrane depolarizes, PINK1 accumulates on the outer membrane of damaged mitochondria to achieve selectivity in recruiting Parkin. Earlier studies on loss-of-function mutations in *Drosophila melanogaster* found that the defects in mitochondria are very similar between Parkin and PINK1 knockout animals [18]; however, only overexpression of Parkin can rescue the PINK1 null phenotype. The results also support the notion that the two proteins act in the same pathway and PINK1 is an upstream regulator of Parkin function.

After Parkin translocation to mitochondria, numerous outer mitochondrial membrane proteins were ubiquitinated by Parkin and in turn recruited other proteins to initiate mitophagy [19]. Despite extensive investigation, detailed steps in the mitophagy pathway still remain elusive. In addition, whether or not dysfunctional mitophagy is the cause of the majority of Parkinson's cases remains to be determined.

4. Effect of α-synuclein and LRRK2 on chaperone-mediated autophagy

4.1. SNCA

α-Synuclein, encoded by *SNCA*, can be degraded in its native form by CMA [20]. The protein contains a pentapeptide motif (KFERQ), which can be recognized by the chaperone protein HSC70. α-synuclein can then bind to lysosomal membrane receptor LAMP-2A for transporting into the lysosome for digestion. Mutant forms of α-synuclein (A30P, A53T) bind much more strongly to LAMP-2A, however failed to be transported across the membrane [21]. Such interference with normal function of CMA translocation complex reduces degradation of other CMA substrates, including damaged and misfolded proteins, resulting in cytotoxicity. α-Synuclein can be modified by oxidized dopamine, creating DA-α-synuclein, which

displays a phenotype closely resembling that of the mutant α-synuclein. In various experimental conditions, CMA defect was observed when expressing DA-α-synuclein [21]. Whether the aforementioned α-synuclein-mediated toxicity underlies the pathogenic mechanism of PD remains to be tested particularly in vivo.

4.2. LRRK2

One recent study from the Cuervo group indicated that LRRK2 and its PD-related variants are degraded by CMA [22]. LRRK2 has eight putative CMA-targeting motifs, one of which is required for binding to the chaperone protein HSC70. While wild-type LRRK2 can be degraded through either UPS or CMA, pathogenic mutant LRRK2 inhibits CMA in cellular models of PD. The mechanism of CMA inhibition mediated by LRRK2 is different from that of α-synuclein. For example, the binding of mutant LRRK2 to lysosomal membranes is enhanced rather than decreased (as in the case of α-synuclein) in the presence of other substrates. Although PD mutants of both LRRK2 and α-synuclein impair the CMA pathway, their functional interaction in the PD process, especially in an *in vivo* model, remains to be further addressed [23].

5. Parkinsonism-related genes in lysosome impairment

Degradation of the autophagic cargo occurs in the acidic environment of lysosomes. Several steps of the lysosomal pathway can go wrong, which may lead to insufficient clearance and toxic substance accumulation. Fusion of the autophagosome to the lytic compartments (lysosomes or endosomes) requires a number of fusion proteins, such as ESCRT, SNAREs and Rab7 GTPase and class C Vps, as well as molecules that recruit the fusion machinery. In addition, changes in the lysosomal lumen, such as reduced acidification, decreased content or activity of hydrolases, have been described behind autophagic failure. These conditions may fall into the category of lysosome storage disorders; however, the pathophysiology is at least in part associated with several neurodegenerative diseases including PD. To date, two genes that encode lysosomal proteins, the enzyme glucocerebrosidase (GBA) and lysosomal type 5 P-type ATPase (ATP13A2) have been linked to parkinsonism.

5.1. GBA

GBA catalyzes the conversion of glucosylceramide into glucose and ceramide inside lysosomes. Mutant GBA (N370S, L444P) allele is a significant risk factor for PD and for dementia with LBs. Patients with Gaucher disease (caused by GBA homozygous deletion or mutation) also frequently exhibit parkinsonism and loss of dopaminergic cells [3]. Mechanistic studies indicate that loss of GBA activity results in glucosylceramide accumulation and decreased lysosomal degradation in neurons. This promotes α-synuclein oligomer formation which in turn impairs GBA trafficking from ER and Golgi to lysosomes [24]. This positive feedback loop was proposed to lead to neurodegeneration in the disease.

5.2. ATP13A2

The ATP13A2 gene encodes a lysosomal ATPase involved in selectively active transport of cations across the membrane. Mutations in this gene have been linked to autosomal recessive Parkinsonism with nigrostriatal-pallidal pyramidal neurodegeneration (Kufor–Rakeb syndrome [KRS]) [25]. Studies in ATP13A2-mutant or -deficient cells revealed a general lysosomal impairment characterized by instability of the lysosomal membrane, impaired lysosomal acidification, diminished clearance of autophagosomes and marked accumulation of α-synuclein [26].

6. Therapeutic implications of enhanced autophagy

Basal autophagy is required to maintain neuronal homeostasis, and thus is a neuroprotective mechanism. Enhancement of autophagic degradation is being explored as a therapeutic strategy when neurons are overloaded with disease-causing, aggregated proteins. Indeed, in some cases autophagy activation in response to increased protein burden and aggregates is mainly considered compensatory and neuroprotective at early stages of the disease, however, becomes the culprit for disease progression when lysosomal clearance is compromised. Therefore, autophagy as an attractive target for PD should aim at fixing the stages that are specifically disrupted in each case.

Two kinase complexes play critical roles in autophagy regulation: ULK1–Atg13–FIP200 complex and Beclin 1–Vps34 complex (PI3K-III complex). Overexpression of Beclin 1 through gene transfer into the brain of α-synuclein transgenic mice ameliorated the synaptic and dendritic pathology in the mice and reduced the accumulation of α-synuclein in the limbic system without any significant deleterious effects [27]. Several pharmacological autophagy-inducing agents have been analyzed more recently and they were found to cause a reduction of α-synuclein levels in animal models [28,29].

Enhancing lysosomal capacity is another promising strategy targeting α-synuclein protein levels. Transcription factor EB (TFEB), when translocated to the nucleus, coordinately upregulates expression of most genes involved in lysosome biogenesis and additional genes required for autophagosome formation. TORC1 negatively regulates this nuclear translocation through the amino acid–Rag GTPase pathway. A recent study using overexpression of TFEB in an *in vivo* model of α-synuclein toxicity demonstrated robust neuroprotection via the clearance of α-synuclein oligomers in midbrain dopamine neurons [30].

Finally, enhancement of selective autophagy by targeting the CMA pathway, such as the CMA receptor and its chaperone complex, is under extensive investigation. Promising as these beginnings are, researchers are facing daunting clinical challenges such as developing biomarkers for evaluating *in vivo* efficacy of autophagy drugs as well as modifying chemicals to break the blood–brain barrier. Nevertheless, great promise stands that autophagy regulation may eventually become a useful and effective way to prevent and treat PD.

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Conflict of interests

The authors have no conflicts of interest to declare.

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