Trends in Molecular Medicine

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Special Issue: Mitochondria - From Diagnosis to Treatment

Opinion

Mitophagy and Neuroprotection

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Neurodegenerative diseases are strongly age-related and currently cannot be cured, with a surge of patient numbers in the coming decades in view of the emerging worldwide ageing population, bringing healthcare and socioeconomic challenges. Effective therapies are urgently needed, and are dependent on new aetiological mechanisms. In neurons, efficient clearance of damaged mitochondria, through the highly evolutionary conserved cellular process termed mitophagy, plays a fundamental role in mitochondrial and metabolic homeostasis, energy supply, neuronal survival, and health. Conversely, defective mitophagy leads to accumulation of damaged mitochondria and cellular dysfunction, contributing to ageing and age-predisposed neurodegeneration. Here, we discuss the contribution of defective mitophagy in these diseases, and underlying molecular mechanisms, and highlight novel therapeutics based on new discovered mitophagy-inducing strategies.

Highlights

Mitophagy, an evolutionally conserved cellular self-degradation of damaged mitochondria, is impaired in major neurodegenerative diseases, including AD, PD, ALS, FTD, and HD.

Defective mitophagy in postmortem brain samples from AD patients and in AD animal models is caused by several mechanisms, including by the inhibition of the ULK1/TBK1-dependent initiation of the mitophagic machinery via Tau/Aβ proteinopathies.

In mice and rhesus monkeys, *PINK1* deletion does not induce significant mitophagy impairment at physiological conditions, suggesting a compensatory response by other mitophagy pathways. Studies in the rhesus monkeys also suggest the existence of none–mitophagy–regulatory role(s) of PINK1.

Therapeutic strategies targeting on mitophagy induction ameliorate disease pathology and inhibit neuronal loss in both AD and PD mouse models.

Mitochondrial Maintenance through Mitophagy: More Than the Homeostasis of Cellular Powerhouses and Metabolism

Mitochondria produce ATP and are indispensable for life and health in most eukaryotic organisms [1–5]. Decades of studies on cell fates (**apoptosis, necrosis,** and **autophagy**; see Glossary) and diseases have greatly expanded our understanding of mitochondria as multifaceted organelles displaying critical roles in metabolic homeostasis, regulation of Ca^{2+} and redox signalling, mitochondrial–nuclear communications, and the arbitration of cell survival/stress resistance and death [4,6,7]. Mitochondrial dysfunction has been associated with a broad variety of human diseases. While mutations of nuclear or mitochondria-encoded mitochondrial proteins cause rare mitochondrial disorders [3], mitochondria-mediated ATP deprivation, oxidative stress, and impaired cell signalling have been linked to the pathogenesis of prominent metabolic and neurodegenerative diseases [e.g., Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS)] [8–12] (see Clinician's Corner).

Cells have developed sophisticated, interconnected regulatory pathways to maintain mitochondrial homeostasis through balanced mitochondrial biogenesis and clearance of damaged mitochondria. **Mitophagy**, a form of autophagy, mediates the removal of defective/superfluous mitochondria, and is the only known pathway through which whole mitochondria can be selectively removed (Figure 1). Mitophagy is responsible for the basal mitochondrial turnover that eliminates dysfunctional mitochondria; however, mitophagy can also be induced under certain physiological conditions whereby healthy mitochondria are degraded. For example, during the maturation of erythrocytes, when mitochondria and other organelles are removed from the cell, and in the development of fertilized oocytes, when paternal mitochondria are eliminated [13,14]. Additionally, mitophagy can be induced as a stress-response mechanism to inhibit mitochondria-dependent apoptosis. Current known mitophagy pathways are summarized here (Figure 2) as well as in recent reviews [7,9,15].

In this review, we summarise the recent progress of mitophagy in neuroprotection, covering topics from the changes of mitophagy in normal ageing and accelerated ageing conditions, as well as in the common neurodegenerative disorders, discuss mitophagy induction as a therapeutic strategy, as well as bring opinion on outstanding questions and future perspectives in this field.

Mitophagy and Healthy Longevity

Mitophagy and the Ageing Process

While ageing is the primary driver of the sporadic cases (which are dominant compared to familial ones) of neurodegenerative diseases, the underlying molecular mechanisms are elusive.

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Mitochondrial defects are accompanied by a plethora of age-related pathological conditions [3,7,9]. Impaired energy metabolism, which is characterized by accelerated mitochondrial DNA (mtDNA) mutation rate, reduced electron transport chain (ETC) function, elevated reactive oxygen species (ROS) levels, defective cytoplasmic calcium buffering, and enhanced release of proapoptotic factors, is a hallmark of ageing [16,17].

During the last few decades, multiple lines of experimental evidence in several model organisms, including yeast, flies, nematodes, and mice, have highlighted the vital contribution of diminished mitochondrial activity in age-associated disorders [4,7,18]. Uncontrolled accumulation of dysfunctional organelles has been demonstrated in several types of cells and tissues during ageing [2,19]. Cellular inability to discard impaired organelles, potentially due to the age-dependent decline of mitophagy or severe defects in macroautophagy, exacerbates energy homeostasis collapse through the accumulation of damaged mitochondrial population [20]. A recent study demonstrates that compromised mitochondrial function promotes a senescence response both in murine and human cells highlighting its association with the development and progression of ageing phenotypes [21]. Taken together, mitophagy might be a 'gate-keeper' of energy metabolism sustaining cellular function, tissue physiology and organismal healthspan.

Several conserved longevity pathways, including low insulin/IGF-1 signalling, caloric restriction, and moderate levels of mitochondrial dysfunction among others, converge on mitophagy stimulation to regulate lifespan. In *Caenorhabditis elegans*, mitophagy is induced in response to stress conditions eliminating damaged mitochondria and thereby promoting cellular homeostasis and viability [19]. Mitophagy deficiency mediates runaway accrual of dysfunctional organelles resulting in altered mitochondrial metabolism and increased susceptibility to stress and infection [18,19,22]. Experimental evidence supporting the vital role of mitophagy in the maintenance of energy metabolism comes from recent studies showing that mitochondrial removal is declined with age in mice and human cells [23,24]. Hippocampal neurons present approximately 70% reduction of mitophagic events in old mice. These results signify the crucial role of mitophagy in this particular brain region that is especially vulnerable to deterioration at early stages of AD. However, we should be cautious in data extrapolation based on these experiments since these mt-Keima mice have early neurodegeneration and impaired circadian biology, compared with commonly used C57BL6/J mice [25]. Recent proof-of-concept studies demonstrate that mitophagy is blocked both in Tau and amyloid-β (Aβ)-based *C. elegans* and mouse models of AD, as well as in postmortem hippocampal brain tissues from AD patients, highlighting mitophagy defects as a common feature of AD pathogenesis [10,26].

Taken together, mitochondrial metabolism and mitophagy hold a pivotal role in the maintenance of organismal homeostasis and ageing. Alterations in mitophagy balance result in the accumulation of defective mitochondria that contribute to the development and progression of age-associated pathologies [7]. Thus, the delineation of the molecular underpinnings and the identification of genetic and/or pharmacological interventions that influence mitophagy and mitochondrial function could be beneficial for cellular homeostasis and organismal healthspan.

Impaired Mitochondrial Clearance in Premature Ageing Diseases with Neurodegeneration

Premature ageing diseases are rare autosomal-dominant genetic disorders in which clinical syndromes are manifested at an early age, resembling aspects of ageing [5,18,27]. Mutations of genes involved in DNA repair are the primary cause of premature ageing diseases, such as the mutations of genes encoded for xeroderma pigmentosum (XP) proteins, ataxia telangiectasia (AT)-mutated (ATM), and Cockayne syndrome group A (CSA) or B (CSB) [5,18,27]. Recent studies have indicated that mitochondrial dysfunction is also a major contributor to premature ageing in these pathological conditions. In XP group A (XPA), AT, and CSB, accumulation of nuclear DNA damage drives depletion of intracellular NAD^+ levels, leading to impairment of mitophagy and subsequent accrual of defective mitochondria via reduction of the NAD^+/sirtuins pathway [4,5,18]. Defective mitophagy has also been reported in other DNA-repair-deficiency-related diseases, such as Fanconi anaemia [28]. These data

Glossary

AMPK: an evolutionary conserved and fundamental metabolic regulator that senses changes in the intracellular energy. In response to energy deprivation, AMP, ADP, and the newly discovered fructose-1,6-bisphosphate, and aldolase activate AMPK for glucose and fatty acid uptake and oxidation.
Apoptosis: process of programmed cell death occurring in multicellular organisms, with distinct morphological characteristics and energy-dependent biochemical mechanisms. Classical features of apoptotic cells include cell shrinkage, blebbing, and nuclear fragmentation.
Autophagy: cellular self-clearance system which delivers unnecessary/damaged cytoplasmic substrates to lysosomes for degradation. Autophagy can be further classified into macroautophagy, microautophagy, and chaperon-mediated autophagy. Autophagy plays a fundamental role in lifespan and health, including neuronal development and survival.
Mammalian target of rapamycin (mTOR): kinase in the PI3K-related kinase family, regulating a broad spectrum of cellular processes. Through the binding of two distinct protein complexes, mTOR can be further classified as mTOR complex 1 and mTOR complex 2. The major functions of mTOR are the regulations of growth, proliferation and motility of cells and body. mTOR inhibition activates autophagy.
Mitochondria-derived vesicles (MDVs): While cells initiate mitophagy to eliminate severely damaged whole mitochondria, cells can also remove damaged cargos from a mildly defective mitochondrion, through the generation of MDVs.
Mitophagy: subtype of macroautophagy by which the double-membraned cup-shaped phagophore recognises damaged mitochondria for lysosomal degradation. The first and most well-defined mitophagy pathway is the PINK1/Parkin-dependent pathway whereas mutations of the genes *PINK1* and *Parkin* link to PD.

strongly suggest a role of nuclear DNA damage signalling to mitochondria (N-M signalling) in mitophagy [4], and highlight an additive/synergistic role with genetic-oriented nuclear DNA damage to promote premature ageing, as well as related neurodegeneration phenotypes.

Defective Mitophagy in Neurodegenerative Diseases

Mitochondria are the major producers of ATP that is essential for the excitability and survival of neurons. Mitochondrial signalling pathways regulate synaptic signalling and related long-term modifications in neuronal structure and function [6,9]. Thus, mitochondrial dysfunction, as well as inefficient clearance of impaired mitochondria, is detrimental to neurons and normal brain function [6,9].

Defective Mitophagy in AD

AD is the most common neurodegenerative disorder, resulting in severe memory loss and cognitive dysfunction. The principal pathological hallmarks of AD are intracellular neurofibrillary tangles (NFTs), mostly constituted by hyper-phosphorylated Tau protein (pTau), and extracellular Aβ plaques that derive from proteolytic processing of amyloid precursor protein (APP) [9,10,29]. Genetic and animal studies suggest that impairment of the autophagic/lysosomal/endosomal system contributes to accumulation of Tau- and Aβ-based **proteinopathies** [30,31]. In addition to pTau and Aβ, mitochondrial dysfunction with accumulation of defective organelles are common features in brain tissues from AD patients as well as in experimental AD mouse models [9,10,32,33]. Mounting evidence argues that alterations in mitochondrial dynamics, morphology, motility, and activity are linked to AD, and together with oxidative stress seem to be important early events in disease development [9,10,32,33]. Since mitochondrial dysfunction is a hallmark of AD, and neuronal autophagy is also impaired in affected neurons, we have suggested a theory of defective mitophagy in AD [9]. Indeed, the basal level of mitophagy is dramatically reduced in postmortem hippocampal tissues from AD patients, AD-induced pluripotent stem cell (iPSC)-derived cortical neurons, as well as in the APP/PS1 and the 3xTg AD mouse models [10]. At the molecular level, the mitophagy machinery is hampered, as demonstrated by impaired activation of two autophagic/mitophagic initiator machinery proteins; ULK1 (low phosphorylated serine 555 site) and TBK1 (low phosphorylated serine 172 site) [10]. Furthermore, lower basal as well as an impaired mitochondrial-stress-induced mitophagy is evident in pan-neuronal pTau- or Aβ-expressing nematodes [10], indicating that Tau- and Aβ-based proteinopathies can affect the efficiency of mitophagic machinery. Possible additional mechanisms of defective mitophagy (accumulation of damaged mitochondria) in AD include: impaired Parkin–presenilin 1 (PS1)/γ-secretase–amyloid precursor protein intracellular domain (AICD)–PINK1 transcription axis [34]; β-secretase-derived APP fragment/CTFβ (C99)-induced endosomal–autophagic–lysosomal dysfunction [35]; dysfunctional over-sized lysosomes [36]; defective retrograde transport of damaged mitochondria to the soma for mitophagic degradation [37]; and impaired mitochondrial stress response [38].

It remains to be determined whether the presence of impaired mitophagy is a consequence of Tau/Aβ proteinopathies, or whether inefficient mitophagy is an early event preceding and causing Tau/Aβ proteinopathies. Data from interventional studies, where mitophagy was reinstalled pharmacologically, favour the latter [10]. There are limitations to the use of transgenic Tau/Aβ animal models to address this puzzle. In AD mouse models, Tau/Aβ is already artificially overexpressed from the day of birth, a time-point several months ahead of disease pathologies. Studies using sporadic AD iPSC-derived neurons or other similar models are necessary to further dissect a confounding question: whether defective mitophagy plays a causative role or serving as a compensatory response in Tau/Aβ proteinopathies.

Defective Mitophagy and Imbalanced Mitochondria-Derived Vesicle Formation in PD

The symptoms of PD are caused by a loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) resulting in a decrease of dopamine (DA) levels in the striatum. Mitochondrial dysfunction and its related oxidative stress and inflammation are common features in PD patient brain samples, PD iPSC-derived neurons, and/or PD animal models [11,12,39,40]. Pathological studies in the substantia

NAD^+: it is an essential molecule (cofactor) for numerous proteins involved in cellular energy metabolism, healthspan, and longevity. NAD^+ depletion is a fundamental feature of ageing that may lead to broad chronic diseases, including neurodegenerative disorders.
Necrosis: acute cell injury resulting in both regulated and unregulated cell death. Classical features of necrosis include DNA damage and degradation, nucleus shrinks, plasma membrane rupture, and inflammatory responses.
NLRP3: nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 or Nod-like receptor protein 3. It is an inflammasome involved in a wide range of autoimmune diseases, autoinflammatory diseases, and neurodegenerative diseases, as it can sense inflammatory proteins and aggregated proteins, including Aβ.
Proteinopathies: type of pathological feature with intracytoplasmic protein misfolding and aggregation. They are common characteristics of many late-onset neurodegenerative diseases, including AD, PD, and HD.
Stimulator of interferon genes (STING): also known as TMEM173. Activated by intracellular/extracellular pathogens, STING participates in innate immunity through the induction of type I interferon. Studies using PD animal models indicate STING activation is a driver of PD.

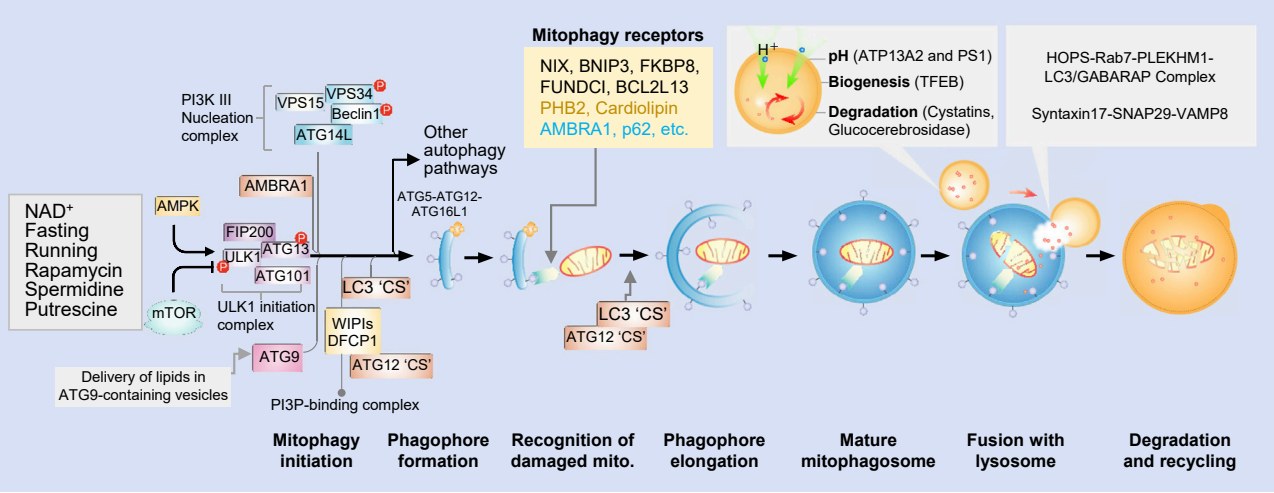
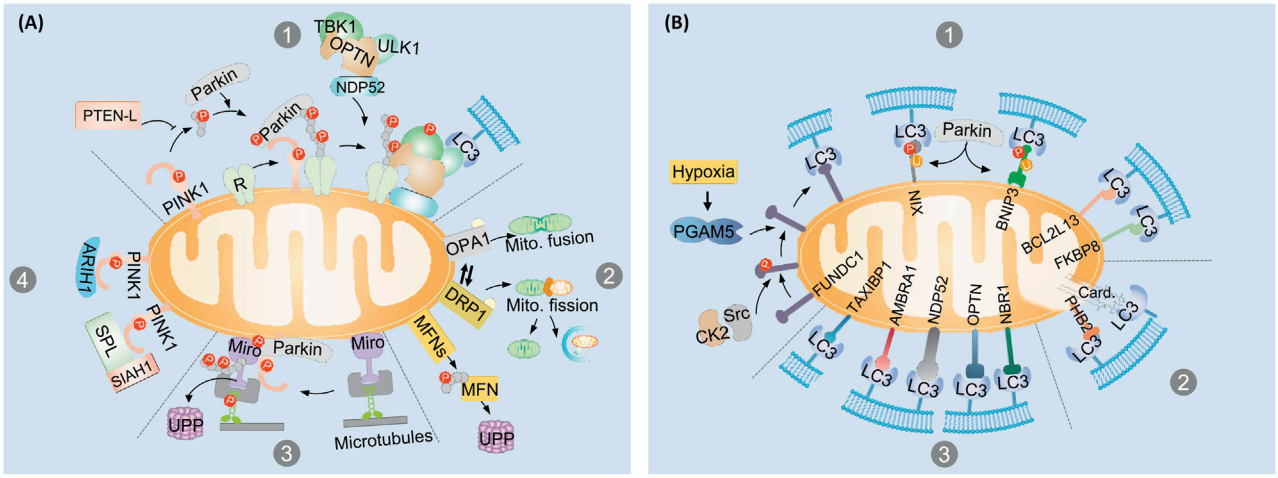


Figure 1. Overview of Mitophagy Pathway.

Mitophagy comprises initiation, membrane nucleation and phagophore formation, phagophore expansion, fusion with the lysosome, and finally, degradation. Under different mitophagy-induction conditions which normally activate AMPK and/or inhibit mTOR, resulting in the activation of ULK1 and following formation of the ULK1 initiation complex. Activated ULK1 phosphorylates Beclin1 (the mammalian orthologue of yeast Atg6) and activates VPS34 lipid kinase (a class III phosphatidylinositol 3-kinase/PI3K), leading to the formation of the PI3KIII nucleation complex. AMBRA1 may also participate in these processes, including stabilization of ULK1 and binding to the PI3KIII complex. The PI3KIII nucleation complex induces the synthesis of phosphatidylinositol-3-phosphate (PI3P) which creates platforms that serve to recruit specific effectors for membrane trafficking events. Typical PI3P-binding complex comprises zinc-finger FYVE domain-containing protein 1 (DFCP1) and WD repeat domain phosphoinositide-interacting proteins (WIPIs). The ATG12 conjugation system (CS) then binds on DFCP1 and WIPIs. Together with the ATG12 CS and the LC3/GABARAPs conjugation system (only LC3 is shown for simplicity, LC3 CS), these processes enable the formation and elongation of the double-membrane phagophore. The ATG5–ATG12–ATG16L1 complex promotes conversion of LC3 to LC3-I, with is then conjugated with phosphatidylethanolamine (PE) to form LC3-II (similar to other Atg8 members). LC3-II is incorporated into the phagophore/autophagosome that enables autophagic recognition and engulfment of damaged mitochondria through binding on LC3-interacting motifs (LIRs) in the mitophagy receptors. Current known mitophagy receptors are listed. The mature mitophagosome can then fuse with the acidic lysosome through at least two known mechanisms as noted. Conditions which affect lysosome function are summarised.

nigra regions from six patients suggest that Complex I deficiency did not correlate with parkinsonism [41]. At the molecular level, mutations of the *PARK6* (encoding PINK1) and *PARK2* (encoding Parkin) genes cause <5% of PD cases (familial PD) [42,43]. In addition to the well-known function in mitophagy, PINK1 and Parkin repress mitochondrial antigen presentation (MitAP). Experimental studies point to a significant contribution of a hyperactivated immune system, provoked by inflammation, in PD progression [40]. A recent study shows that PINK1 and Parkin repress MitAP through inhibition of Sorting nexin 9 (Snx9)-dependent formation of **mitochondria-derived vesicles (MDVs)** [44].

Although extensive mechanistic studies of the PINK1/Parkin pathway have been performed, the role of PINK1/Parkin-dependent mitophagy *in vivo* remains elusive. Both *Prkn*$^{-/-}$ and *Pink1*$^{-/-}$ mice show no substantial PD-relevant phenotypes [45,46]. In addition, basal mitophagy is comparable between PINK1 wild-type and *Pink1*$^{-/-}$ mice [47]. This might be due to sufficient compensation of the loss of PINK1-dependent mitophagy by other PINK1-independent pathways under physiological conditions. *PINK1* deletion in rhesus monkeys results in neurodegeneration; however, this phenotype is not associated with altered mitochondrial morphology in the neurons, suggesting the existence of none–mitophagy–regulatory role(s) of PINK1 [48]. PD patients show strong inflammatory phenotypes, with elevated levels of IL-1β, IL-6, TNF-α, and IFN-γ [49]. A recent study showed that under acute (exhaustive exercise) or chronic (mutation of DNA Polymerase *PolG*, named *mutator*) mitochondrial stress conditions, knock down of *Prkn* or *Pink1* in mice leads to an exacerbation of inflammation status via a **stimulator of IFN genes (STING)**-dependent manner [49]. Additional studies have linked mitochondrial dysfunction and inflammation. Accumulation of damaged mitochondria can trigger inflammation via the **NLRP3** inflammasome and/or the cGAS (a DNA sensor, also known as MB21D1)-



Trends in Molecular Medicine

Figure 2. Summary of Different Mitophagy Pathways.

(A) PINK1-dependent and Parkin-(in)dependent mitophagy pathways. From upper clockwise: (1) For the PINK1- and Parkin-dependent mitophagy pathway, reduced mitochondrial membrane potential enables the stabilization of full-length PINK1 at OMM. Full-length autophosphorylated PINK1 phosphorylates ubiquitin, enabling the binding of Parkin to phosphoubiquitin and the recruitment and binding to OMM receptors (Rs). Parkin, phosphorylated by PINK1, ubiquitinates several known Rs, including VDAC, mitofusins (MFN1 and MFN2), and the mammalian mitochondrial Rho (Miro). (2) Mitochondrial fission facilitates mitophagy since smaller mitochondria are easily engulfed and degraded. (3) PINK1 and Parkin arrest mitochondrial motility through degradation of Miro. (4) PINK1-dependent, Parkin-independent mitophagy pathway. In addition to Parkin, alternative E3 ubiquitin ligases like SIAH1 and ARIH1 participate in PINK1-dependent, Parkin-independent mitophagy. (B) Receptor-mediated mitophagy pathways. Here, receptor-mediated mitophagy pathways are divided into three groups. (1): Group 1 comprises OMM proteins, including FUNDC1, NIX (BNIP3L), BNIP3, FKBP8, and BCL2L13 (the mammalian orthologue of the yeast Atg32). (2): Group 2 contains mitophagy receptors that are IMM components, including PHB2 and cardiolipin (Card.). (3) Group 3 comprises autophagy receptors that are also involved in mitophagy, including AMBRA1, NDP52, TAXIBP1, NBR1, OPTN, and p62.

cGAMP–STING–TBK1–IRF3 pathway, linked to various human diseases, including pathological ageing and neurodegeneration [10,49–51]. In cells with mitochondrial inner membrane permeabilization, mtDNA release induces activation of the cGAS–STING signalling [51]. Strikingly, loss of STING has been shown to dramatically rescue inflammation in both *Pink1*$^{-/-}$, *Prkn*$^{-/-}$ and *Prkn*$^{-/-}$*/mutator* mouse models, and it even inhibited loss of dopaminergic neurons and motor defects observed in aged *Prkn*$^{-/-}$/mutator mice [49]. The *in vivo* mitophagy levels were performed in the heart, but not in the dopaminergic neurons in the *Pink1*$^{-/-}$ mt-Keima mice [49]. In summary, these *in vivo* data suggest that the PINK1/Parkin-dependent mitophagy may be dispensable at physiological conditions but is indeed necessary at stress/pathological conditions for the function and survival of PD-related dopaminergic neurons. More information between defective mitophagy and PD has been elegantly summarised in a recent review [52].

Emerging evidence from multiple studies suggests that the story is far more complex than previously appreciated with respect to PINK1 mutation, mitophagy status and PD [47,48,51,53] as PINK1 and Parkin may have potential mitophagy-independent functions [48,54]. Further understanding of PD aetiology through the investigation of different mitophagy pathways, other noncanonical mitochondrial clearance pathways, and mitochondria-unrelated pathways is necessary.

Defective Mitophagy in ALS and Other Neurodegenerative Diseases

From the studies presented above, proteinopathies with mitochondrial dysfunction seem to be common features of neurodegenerative diseases. In addition to AD and PD, other neurodegenerative disorders, such as ALS, frontotemporal dementia (FTD), and Huntington's disease (HD) share similar features.

ALS is a fatal disease characterised by the selective degeneration of motor neurons. A hallmark of the disease, as in other neurodegenerative pathologies, is the accumulation of aggregated proteins

within the affected neurons. A study using HeLa cells with protein overexpression suggests that inefficient turnover and aggregates of damaged mitochondria, may contribute to disease progression in ALS [55]. FTD affects cortical neurons and the basal ganglia, resulting in cognitive impairment, language deficiency and changes in social behaviour and conduct. Despite the differences in the types of neurons affected, both diseases show clinical and genetic overlap [56]. Many of the genes linked to ALS and FTD encode proteins involved in mitophagy/selective autophagy, including: OPTN, TBK1, p62, and receptor-interacting protein kinase 1 (PIPK1), among others [55–58], but how mutation of these genes contributes to the pathology is not fully understood.

HD is an autosomal-dominant neurodegenerative disorder caused by an expansion of the numbers of cytosine–adenine–guanine (CAG) trinucleotide repeats encoding a polyglutamine (polyQ) tract in the amino-terminal region of Huntingtin (Htt) protein. Htt is a large protein that can interact with and probably impair numerous other proteins. Interestingly, mutant Htt has also been proposed to affect the endosomal/autophagic system proteins [59]. Mitochondrial dysfunction and proteostasis alterations, particularly in the autophagic/endosomal system, including the mutation of *ATG7* [30], impairment of GAPDH-mediated mitophagy by mutant Htt [60] are linked to the pathogenesis of HD. In summary, while evidence of impaired autophagy and pathological mitophagy in HD, determination of mitophagy status (upregulated/defective) in HD and more detailed molecular mechanisms are necessary.

In summary, it is likely that impaired mitophagy is a common phenomenon in many neurodegenerative pathologies (Table 1). This opinion is based on the data predominantly from cell culture and laboratory animal models, and clear-cut postmortem evidence from patients that demonstrates dysregulated mitophagy triggers multiple forms of neurodegeneration is scarce. In view of the high instability and diversity of mitophagy status in the human brain tissues, it is essential to combine large cohort of human brain samples obtained under tightly controlled conditions and state-of-the-art tissue processing methods, as recently performed [61], to further address this question.

Targeting Clearance of Damaged Mitochondria as Therapeutic Strategy

Maintenance of a healthy mitochondrial pool may serve as a robust approach to maintain healthy ageing and high quality of life in vulnerable old age. Since mitochondrial damage is a hallmark of several neurological pathologies, chemical substances that enhance the clearance of defective mitochondria are likely to have significant therapeutic benefit [6,9,17]. For diseases with genetic mutations, the best candidates for therapy remain small-molecule drugs. Several robust mitophagy inducers have been discovered with promising benefits to the extension of healthspan and the protection of neurons in animal models and/or human cells. Among them are NAD^+ precursors, urolithin A (UA), actinonin, spermidine [62,63], and the FDA-approved drugs, rapamycin and metformin [64] (Table 2).

NAD^+ Precursors as Mitophagy Inducers

Major NAD^+ precursors include nicotinamide, nicotinamide mononucleotide (NMN), and nicotinamide riboside (NR). Because of low NAD^+ levels in tissues from laboratory AD animal models, NAD^+ replenishment through supplementation with NAD^+ precursors can inhibit Aβ and Tau pathology and reverse cognitive deficits in 3xTgAD and APP/PS1 models of AD via the NAD^+-dependent SIRT1 and SIRT3, increased activities of PI3K–AKT and MAPK/ERK1/2, and increased expression of CREB [10,65–68]. In addition to an intriguing benefit to AD, NAD^+ replenishment also rescued mitochondrial defects and dopaminergic neuronal loss in iPSC and fly models of PD [39]. A more compressive summary of multifaceted pathways between NAD^+ and mitophagy is in a recent review [69]. Several 1–3-month clinical trials have indicated that the NAD^+ precursor NR is orally bioavailable and safe (1–2 g/day/adult) [70,71] (NCT02191462[i] and NCT02303483[ii]), and it is important to see the benefits of NAD^+ replenishment in clinical trials on brain function and cognition in the elderly (NCT03562468[iii], NCT02942888[iv], and NCT03482167[v]), as well as in Chemo-induced Peripheral Neuropathy (NCT03642990[vi]).

Table 1. Proteins Involved in Mitophagy and Their Relations to Neurodegenerative Diseases

Proteins	Functions in mitophagy	Relation to diseases	Refs
Specific receptors for mitophagy			
PINK1	Ser/Thr kinase; initiates mitophagy by phosphorylating components of mitophagy machinery including Ubiquitin and Parkin among others.	PD, AD, ALS	[42,43,86]
Parkin	E3 ubiquitin ligase: initiates mitophagy by ubiquitinating proteins on the OMM. PTEN-L inhibits PINK1/Parkin dependent mitophagy via dephosphorylation of PINK1-mediated pSer65Ub.	PD, AD, ALS	[42,43,87–90]
RIPK1	Binds on PGAM5 and PINK1 to induce mitophagy	ALS, Cancer	[57,58]
PGAM5	Involved in PINK1-dependent mitophagy.	PD, ALS	[57,58]
ARIH1/ HHARI	E3 ligase, RING-between-RING E3 ligase family: participates in PINK1-dependent Parkin-independent mitophagy in Parkin-deficient cancer cells.	Cancer, especially breast and lung cancer	[91]
NIX/BNIP3L	Mitochondrial receptor protein: essential in maturation of reticulocytes and hypoxia-induced mitophagy.	AD, PD	[13,92]
BNIP3	Involved in hypoxia-induced mitophagy; regulates OPA1 disassembling and DRP1 recruitment to mitochondria to facilitate mitophagy; stabilizes PINK1.	PD?	[95–99]
FUNDC1	OMM protein: initiates hypoxia-induced mitophagy, where phosphorylation of FUNDC1 by CK2 and Src is inhibited and dephosphorylation by PAGM5 is stimulated, hereby allowing FUNDC1 to bind to LC3 and recruit DRP1/DNM1L.	—	[93–96]
BCL2L13	OMM protein: involved in mitochondrial dynamics, Parkin-independent mitophagy and cargo binding.	—	[97–101]
FKBP8/(38)	OMM protein: involved in Parkin-independent mitophagy by binding to LC3A.	—	[102,103]
PHB2	IMM protein: interacts with LC3 following mitochondrial membrane rupture; participates in Parkin-mediated mitophagy.	—	[104]
Cardiolipin	IMM phospholipid: involved in cargo labelling, via interaction with LC3 following mitochondrial membrane rupture.	—	[105,106]
Receptors for both macroautophagy and mitophagy			
AMBRA1	Working with PI3KIII nucleation complex to initiate phagophore formation; participates in PINK1/Parkin (in)dependent mitophagy.	Neural tube defects	[107–109]
ULK1	Ser/Thr kinase: activation of AMPK or inhibition of mTOR induces composition of ULK1 initiation complex.	AD, development defects of axon	[9,110–115]
p62 (SQSTM1)	Autophagy/mitophagy receptor: involved in cargo sequestration; degrades sperm-derived mitochondria in fruit flies and mammals.	Motor neuron disease	[116–118]
NDP52	Autophagy receptor: involved in cargo sequestration.	—	[86]
OPTN	Involved in cargo sequestration, where it is being regulated by TBK1.	ALS	[119]
Beclin 1	Promotes formation of PI3KC3-C1 and regulates the lipid kinase.	PD	[120]
TBK1	Kinase: binds ubiquitin chains on mitochondrial proteins, then phosphorylates autophagy receptors and facilitates mitophagy/autophagy.	Motor neuron disease, ALS	[121]

Table 2. Compounds Targeting Mitophagy and Their Known Health Effects

Compounds	Mechanistic linkage to mitophagy	Health benefits (More than mitophagy)	Refs
NR	Precursor of NAD^+; NAD^+ enhances expression of NIX (DCT-1); induces mitochondrial fission; enhances autophagy through sirtuin-dependent deacetylation of Atg5, Atg7, and Atg8; Under starvation, the NAD^+/SIRT1 pathway deacetylates nuclear LC3 to facilitate its cytoplasmic redistribution.	Inhibits cognitive deficits, inflammation, and Tau pathology in AD; inhibits hearing loss; promotes healthspan and lifespan in mice and worms.	[4,5,18,68,122,123]
NMN	Precursor of NAD^+; NAD^+ enhances expression of NIX (DCT-1); induces mitochondrial fission; enhances autophagy through sirtuin-dependent deacetylation of Atg5, Atg7, and Atg8; Under starvation, the NAD^+/SIRT1 pathway deacetylates nuclear LC3 to facilitate its cytoplasmic redistribution.	Inhibits cognitive deficits, inflammation, and Tau pathology in AD; inhibits hearing loss; promotes healthspan and lifespan in mice and worms.	[4,5,18,68,122,123]
NAM	Precursor of NAD^+ but higher level inhibits SIRT1 activity; affects SIRT1, SIRT3, PI3K-AKT, MAPK/ERK1/2, CREB	Reduces Aβ and Tau and inhibits cognitive deficits in 3xTgAD; promotes healthspan not lifespan in mice	[66,67]
Urolithin A	Induces expression of mitophagy proteins, including full-length PINK1, Parkin, OPTN; p-ULK1, LC3B-II, Beclin1, Bcl2L13, AMBRA1, and FUNDC1 in SH-SY5Y cells; induces expression of full-length PINK1 in brain tissues of mice.	Inhibits Aβ and Tau pathology and reverses cognitive deficits in worm and mouse models of AD.	[10,72]
Actinonin	Induces expression of mitophagy proteins, including full-length PINK1, Parkin, OPTN; p-ULK1, LC3B-II, Beclin1, Bcl2L13, AMBRA1, and FUNDC1 in SH-SY5Y cells; induces expression of full-length PINK1 in brain tissues of mice.	Inhibits Aβ and Tau pathology and reverses cognitive deficits in worm and mouse models of AD.	[10,23]
Rapamycin	Induces macro-autophagy by direct binding and inhibition of mTOR; stimulates AMPK; extends lifespan in mice in an ULK1-dependent manner.	Promotes healthspan and lifespan in yeast, worms, flies, and mice; shows anticancer and anti-inflammatory activities.	[64,79,80]
Spermidine	Induces mitophagy through multiple pathways, involving the ATM-PINK1-Parkin pathway, the Nrf2-SKN-1 pathway, and through activation of AMPK and inhibition of mTOR; inhibits EP300; induces BNIP3, CTSL, and ATGs.	Promotes healthspan and lifespan in yeast, worms, flies, and mice;	[62,63,75,78]

(Continued on next page)

Table 2. ***Continued***

Compounds	Mechanistic linkage to mitophagy	Health benefits (More than mitophagy)	Refs
Metformin	Induces autophagy/mitophagy via SIRT1, IGF-1 and mTORC1, or via Parkin-mediated mitophagy.	Promotes healthspan and lifespan in worms and mice.	[79,81–83]

UA as a Mitophagy Inducer

UA, an ellagitannins-derived metabolite, has been shown to induce muscular mitophagy in cultured cells and *C. elegans* [72], and neuronal mitophagy in both *C. elegans* and the mouse brain [10]. Indeed, UA and the antibiotic actinonin (AC) [23] were shown to trigger mitophagy in AD mouse and *C. elegans* models in a PINK1/Parkin/NIX-dependent manner [10]. Treatment with either compound leads to the amelioration of several AD pathological features including memory and learning deficits, Aβ-/Tau-related aggregation, and neuroinflammation [10]. UA-induced mitophagy protects against neuroinflammation by decreasing the generation of proinflammatory cytokines, such as TNF-α and IL-6, and enhancing the levels of the anti-inflammatory IL-10 [10]. A recent study demonstrates that IL-10 triggers macroautophagy/mitophagy by diminishing the **mammalian target of rapamycin (mTOR)** through **AMPK** induction, inhibiting inflammasome activation and unresolved inflammation [73]. Results from a very recent stage I clinical trial of UA on 60 healthy elderly individuals, indicated that oral administration of UA (up to 1 g/day) over a 4-week period was safe and was able to induce plasma acylcarnitines and skeletal muscle mitochondrial gene expression (NCT02655393)[vii] [74].

Spermidine as a Mitophagy Inducer

Polyamines, including spermidine, decrease with age in the human brain and animal models. Treatment with spermidine improves memory impairments and has life-prolonging effects in flies, nematodes, and mice [62,75]. These beneficial effects depend on the expression of autophagy-related genes, hence the activity of autophagy [63]. In addition to autophagy, spermidine stimulates mitophagy in cultured cell lines, including human fibroblasts and cardiomyocytes among others [76]. Spermidine induces mitophagy by inhibition of mTOR and activation of AMPK [63,77]. Additionally, the ATM-dependent PINK1/Parkin signalling also can be activated by spermidine [78]. However, the exact underlining mechanisms between spermidine and mitophagy activation remain elusive.

Rapamycin and Metformin

Rapamycin and metformin are two FDA-approved mTOR inhibitors, which exhibit both anticancer and antiageing properties [79]. Rapamycin prevents mTOR by direct binding to and inhibition of mTOR complex 1 (mTORC1) [80]. Furthermore, rapamycin supplementation promotes the activation of AMPK in several model organisms, including flies, nematodes, and mice [64]. In turn, AMPK targets and phosphorylates mTOR leading to its subsequent inhibition. Similar to rapamycin, metformin, the most widely supplemented antidiabetic drug, is shown to induce mitochondrial removal. Metformin inhibits mTOR indirectly via multiple signalling pathways that are poorly understood [81,82]. The primary mode of action of metformin may include the inhibition of mitochondrial complex I, activation of SIRT1, AMPK, and Parkin-mediated mitophagy [82,83]. Table 2 summarises the health benefits, including neuroprotection, of different mitophagy-inducing compounds.

Concluding Remarks

Efficient mitochondrial quality control is pivotal for cellular and organismal homeostasis. Despite the extensive studies focusing on the molecular mechanisms that govern mitophagy, some outstanding questions remain (see Outstanding Questions). Firstly, it is necessary to further study the interconnected interactions between the different mitophagy pathways and how they coordinate the regulation of mitochondrial removal. Secondly, the balance between mitophagy, mitochondrial biogenesis, and the mitochondrial fusion–fission processes should be examined. Thirdly, untangling of the multifaceted roles of mitophagy in cell survival and death is needed. Mitophagy stimulation might be both

Clinician's Corner

Dementia affects over 50 million individuals worldwide, and 60% of them have AD. While there is still no conclusive link between Tau/Aβ proteinopathies and cognitive symptoms, it is generally believed that AD is caused by a mix of multiple factors, including ageing, genetic, environmental, and other nongenetic factors.

There are over 15 clinical trials for the NAD^+ precursors NR and NMN on different diseases, including biological ageing, obesity, and neurodegenerative diseases. Several finished independent clinical studies show that NR is orally bioavailable and safe at a dose of 1–2 g/day/adult for 1–3 months.

Defective mitophagy is a likely a common feature of broad neurodegenerative diseases, at least based on the data from animal models of AD and PD and ALS. Clear-cut postmortem evidence from patients that demonstrates dysregulated mitophagy actually triggers multiple forms of neurodegeneration is scarce. Large clinical trials on such drug candidates are necessary.

beneficial and detrimental for tissue homeostasis depending on cellular bioenergetics under certain pathophysiological conditions. Furthermore, status of mitophagy as well as its linkages to neurodegenerative diseases in large cohort of high quality post-mortem human brain tissues from different neurodegenerative disease cases needs to be carefully determined. We should be extremely judicious in data extrapolation. Lastly, studies investigating the clinical benefits of known mitophagy inducers and to further develop specific, robust, but low toxic mitophagy inducers are lacking.

Emerging evidence of the causative linkage between defective mitophagy in AD, PD, and other neurodegenerative pathologies, propels drug development targeting damaged mitochondria, mitophagy, and other mitochondrial elimination pathways. Significant progress on using mitophagy inducers (e.g., supplementation with NAD^+ precursors) to inhibit AD/PD has been made in both *in vivo* animal studies and patient iPSC-based systems, and further clinical studies are in progress. Small compound-based mitophagy induction may also benefit healthy ageing and the reduction of neurodegeneration. Furthermore, trends of a close collaboration between academia and industry as well as the application of artificial intelligence in mechanistic studies and drug development may accelerate the efficient development of transformative molecules targeting on these currently incurable neurodegenerative diseases [84]. Importantly, lifestyle interventions, such as physical exercise and caloric restriction [85], represent promising candidates to counteract deterioration of mitochondrial metabolism and prevent neurodegeneration. We urge large population-based, randomised, double-blind, placebo-controlled clinical trials for promising mitophagy-inducing molecules on these neurodegenerative diseases.

Outstanding Questions

Have we accumulated enough solid data to support that targeting on mitophagy induction is an effective way for AD treatment, especially for sporadic AD in view of limited laboratory models?

What are the best animal models to study sporadic AD and sporadic PD, and what are the physiological levels of mitophagy in these models?

Is it possible to devise a therapeutic strategy which restores defective mitophagy without activating other detrimental cellular pathways (e.g., inflammation or neuronal death)?

Can mitophagy induction be bad to some neurodegenerative diseases? And if yes, how can we balance the good and the bad effects?

Are there any synergistic effects between different known mitophagy inducers to AD, PD, and other neurodegenerative diseases?

Verification of the clinical effects of promising small mitophagy-inducing molecules, such as the NAD^+ precursors NR and NMN, on different neurodegenerative diseases remains of prime importance.

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Resources

[i]clinicaltrials.gov NCT02191462
[ii]clinicaltrials.gov NCT02303483
[iii]clinicaltrials.gov NCT03562468
[iv]clinicaltrials.gov NCT02942888
[v]clinicaltrials.gov NCT03482167
[vi]clinicaltrials.gov NCT03642990
[vii]clinicaltrials.gov NCT02655393

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