Mitophagy and reactive oxygen species interplay in Parkinson’s disease

Bin Xiao 1,2,5✉, Joshua Kuruvilla 1 and Eng-King Tan 1,2,3✉

Mitophagy impairment and oxidative stress are cardinal pathological hallmarks in Parkinson’s disease (PD), a common age-related neurodegenerative condition. The specific interactions between mitophagy and reactive oxygen species (ROS) have attracted considerable attention even though their exact interplay in PD has not been fully elucidated. We highlight the interactions between ROS and mitophagy, with a focus on the signalling pathways downstream to ROS that triggers mitophagy and draw attention to potential therapeutic compounds that target these pathways in both experimental and clinical models. Identifying a combination of ROS inhibitors and mitophagy activators to provide a physiologic balance in this complex signalling pathways may lead to a more optimal outcome. Deciphering the exact temporal relationship between mitophagy and oxidative stress and their triggers early in the course of neurodegeneration can unravel mechanistic clues that potentially lead to the development of compounds for clinical drug trials focusing on prodromic PD or at-risk individuals.

npj Parkinson’s Disease (2022) 8:135; https://doi.org/10.1038/s41531-022-00402-y

INTRODUCTION

Parkinson’s disease (PD) is a progressive neurodegenerative condition identified as one of the leading causes of disability, particularly in countries with rapidly growing aging populations and with a prevalence that is predicted to double in the next 20 years1–3. A selective loss of dopaminergic (DA) neurons from the pars compacta of the substantia nigra (SN) and the formation of Lewy bodies constitute the primary pathological hallmarks of PD. Clinically, patients frequently present with tremor, rigidity, postural instability and bradykinesia accompanied by nonmotor symptoms (such as fatigue, depression, and dementia)4. The etiology of PD has been extensively investigated and is likely due to a combination of genetic, epigenetic and environmental factors. Clinical, postmortem and animal model studies suggest that mitochondrial dysfunction, oxidative stress, impaired proteasomal system, impaired autophagy/mitophagy, and neuroinflammation dysregulation4,5 contribute to the onset and development of PD. Available therapies are largely symptomatic, and disease-modifying therapies are still lacking, highlighting the necessity of therapy targeting key areas of PD pathogenesis6. There is growing evidence implicating that mitophagy impairment7,8 and oxidative stress9,10 are critical factors of neurodegeneration over time in PD.

Mitophagy

Mitophagy refers to autophagy that selectively degrades damaged mitochondria. There exists a multi-tiered, highly integrated network of mitochondrial quality control (MQC) which regulates various cellular processes to maintain the structural and functional integrity of mitochondria in response to various stressors (oxidative stress, mutagenic stress, and proteotoxicity etc). Various MQC pathways are dysregulated in PD, including mitochondrial regeneration (biogenesis)11, protein import,12 mitochondrial dynamics (fusion, fission)13,14 mitochondria-derived vesicles15 and mitochondrial removal (mitophagy)16,16. An essential component of mitochondrial homeostasis, mitophagy can be defined as the selective degradation of mitochondria, with mitochondria enveloped by autophagosomes before being degraded in the lysosome17,18. Given that this breakdown of mitochondria via autophagosome formation is crucial for neuronal health, compromised mitophagy has been implied in PD, and associated with accelerated neurodegeneration19,20. Based on their relevance to PD pathogenesis, mitophagy pathways can be categorized into Parkin-dependent and Parkin-independent pathways, with the latter largely comprising of receptor-mediated mitophagy, lipid-mediated mitophagy and ubiquitin-mediated mitophagy.

Parkin/PINK1-mediated mitophagy

Mechanisms of Parkin/PINK1-mediated mitophagy: Mutations in Parkin (a serine/threonine kinase)21 and Parkin (an E3 ubiquitin ligase)22 are the most common causes of recessive forms of PD. PINK1 and Parkin are the key components in the Parkin/PINK1-mediated mitophagy23,24. Under basal conditions, PINK1 translocates to the inner mitochondrial membrane (IMM) for cleavage by mitochondrial proteases25. Subsequently, truncated PINK1 is targeted to the proteasome for eventual degradation via an N-end rule pathway26. Mitochondrial stress or damage causes the collapse of mitochondrial membrane potential, preventing this manner of translocation of PINK1 which instead accumulates on the outer mitochondrial membrane (OMM)27,27 and undergoes autophosphorylation28. In this initiation stage, PINK1 phosphorylates OMM conjugated ubiquitins at Serine 65, driving Parkin to be recruited from cytosol to the mitochondria29,30. Phosphorylated ubiquitin may both activate Parkin and act as an anchor for Parkin on mitochondria31. Furthermore, PINK1 is also recruited from the cytosol to the mitochondria via a promiscuous E3 ligase which mediates ubiquitination of OMM

1Department of Neurology, National Neuroscience Institute, Singapore, Singapore. 2Neuroscience Academic Clinical Program, Duke-NUS Medical School, Singapore, Singapore. 3Neuroscience and Behavioral Disorders Program, Duke-NUS Medical School, Singapore, Singapore. 4email: xiao.bin@singhealth.com.sg; tan.eng.king@singhealth.com.sg

Published in partnership with the Parkinson’s Foundation
proteins non-selectively\textsuperscript{29,31–33}. Among the Parkin substrates, Miro were ubiquitinated and degraded to arrest mitochondrial motility, priming mitochondria for autophagic clearance\textsuperscript{14}. Ubiquitin attached to the substrates on the damaged mitochondria can be phosphorylated by PINK1 to recruit and activate more Parkin and form a positive feedback loop to propel the cascade of mitophagy\textsuperscript{29,31,35}. Subsequently, the ubiquitinated substrates serve as a degradation signal to be recognized by autophagy receptors, including OPTN and NDP52\textsuperscript{25}. This occurs concomitantly with Parkin/PINK1-dependent activation of TBK1 which phosphorylates and recruits OPTN and NDP52 to the depolarized mitochondria, anchoring them to the forming autophagosome coated with LC3\textsuperscript{23,35,36,37}. The process is achieved via the development of an isolation membrane surrounding the mitochondria via an ATG8-dependent positive feedback loop\textsuperscript{17}.

The damaged mitochondria are engulfed by the autophagosome which subsequently fuses with lysosome containing acidic hydrolases\textsuperscript{37–39}. Mitophagy is completed as the autophagosome-lysosome fusion leads to the degradation of mitochondria\textsuperscript{38,40} (Fig. 1). In addition, several Parkin-independent mitophagy pathways have been identified, which will be discussed later, having the potential to be utilized to compensate for the dysfunction of Parkin-mediated mitophagy.

**Genetic and pathological evidence of Parkin/PINK1-mediated mitophagy:** Parkin and PINK1 mutations disrupt their physiologic functions on mitophagy. Extensive studies in cultured cells have not only unravelled the molecular mechanism underlying Parkin/PINK1 pathway discussed above, but also revealed that over half of tested PD-related mutations in Parkin and PINK1 are associated with defect in mitophagy\textsuperscript{27,41–43}. Furthermore, there are in vivo evidence showing that mitophagy dysfunction impacts DA neuronal health and contributes to PD pathogenesis. Phosphorylation of ubiquitin at Ser65, a hallmark of PINK1 activation during mitophagy\textsuperscript{27}, is increased in a Mutator mouse with mitochondrial dysfunction, and loss of endogenous Parkin leads to specific DA neurodegeneration and L-DOPA reversible motor deficit\textsuperscript{44}. Studies using Drosophila model expressing mitophagy probes demonstrated Parkin/PINK1-dependent mitophagy activation in DA neurons in an age-dependent manner\textsuperscript{45}. Another study demonstrated that depletion of deubiquitinase UPS30 in fly may rescue Parkin/PINK1-dependent mitophagy and counteract PD-like symptoms of paraquat-induced decreased dopamine level and motor dysfunction\textsuperscript{46}. In addition to Parkin and PINK1 which play a fundamental role in mitophagy, other PD causative genes, including α-synuclein, LRRK2 and GBA, may contribute to PD pathogenesis also through their impact on mitophagy which will be discussed below.

Abnormality in PINK1-dependent mitophagy induced by aging can be inferred with the accumulation of PINK1 activity biomarker, pS65-UB found in elderly human subjects\textsuperscript{47}. Further pathological evidence of altered mitophagy was found in aging PD brain samples where there were increased mitochondrial matrix protein, ATP synthase subunit β (ATPSβ), and OMM protein, Miro. Upregulation of Miro may increase mitochondrial motility and compromise the initiation of mitophagy. Therefore, aberrant accumulation of ATPSβ and Miro in postmortem brain indicates that dysfunctional mitophagy is associated with PD\textsuperscript{16}. PD patient platelets, compared to aged-matched controls, exhibited downregulated mitophagy accompanied by reduced LC3II and MsrB2, which affects Parkin methionine oxidation\textsuperscript{48}. These pathological and clinical findings are indicative of defective mitophagy in PD.

**Evidence of Parkin/PINK1-mediated mitophagy from patient iPSC-derived neurons:** Although mutations in Parkin or PINK1 were found to compromise mitophagy in cultured cells and animal models, direct pathological evidence is still lacking if the same mutations would impair mitophagy and if such defect is the key factor for the loss of DA neurons in vivo or in PD patients\textsuperscript{49}. Recent advancements in stem cell and gene editing technology may help address these questions. The impact of disease-causing mutations in Parkin and PINK1 on mitophagy are being examined in more physiologically related setting with the aid of induced pluripotent stem cell (iPSC)-derived neurons from PD patients. Parkin translocation to mitochondria was arrested in DA neurons derived from PD patients carrying PINK1 mutation\textsuperscript{50}. The expression of several mitophagy-related genes was altered in DA neurons derived from Parkin knockout isogenic iPSCs\textsuperscript{51}. Aberrant accumulation of phospho-ubiquitin and aggregated mitochondria have been demonstrated in neurons differentiated from iPSCs carrying Parkin or PINK1 mutations. Of note, the majority of phospho-
ubiquitin was detected in TH-positive neurons, but not in TH-negative neurons, highlighting the cell type-specific mitophagy defects. In idiopathic PD, impaired mitochondrial clearance was also observed, but not in all the iPSC clone-derived neurons, which could be attributed to the heterogeneity of PD pathogenesis. In addition to phospho-ubiquitin, abnormalities of other mitophagy-related proteins have been recorded utilizing patient iPSC-derived neurons. It was found that Miro, a substrate of Parkin, was resistant to proteasomal degradation induced by mitochondrial depolarization, interfering mitochondrial mobility and initiation of mitophagy in PD patients with LRRK2 G2019S mutation and sporadic PD patients. Mutation in α-synuclein also causes Miro protein accumulation, inhibiting mitophagy in PD patient neurons. Consistently, co-localization of mitochondria with lysosomes and clearance of mitochondria were significantly reduced in iPSC-derived neurons carrying Miro mutation. Mt-Keima is a mitochondria-targeted fluorescent protein, which is sensitive to pH changes but resistant to degradation mediated by lysosomal proteases. When mitophagy occurs, mt-Keima is engulfed by acidic lysosomes where the excitation of mt-Keima shifts to a longer wavelength compared with mitochondrial physiological environment. The ratio change in excitation wavelengths of mt-Keima has been utilized to quantify mitophagy. With the aid of mt-Keima, mitophagy defects were confirmed in iPSC-derived neurons with Parkin/PINK1 mutations by independent groups. However, the fidelity of the iPSC-derived neuron model system to reveal the mitophagy impairment should be interpreted cautiously. Inhibition of PINK1-dependent mitophagy dramatically compromises iPSC reprogramming. In addition, mitophagy mediates the metabolic switch between glycolysis and mitochondrial oxidative phosphorylation, which is critical during cell differentiation. It is therefore possible that compensatory mechanism may have been developed during the conversion process of patient somatic cells to neurons. Alternatively, cells with substantial mitophagy defects may have failed to be transformed into neurons. Thus, the mitophagy dysfunction revealed in the patient iPSC-derived neurons may not faithfully recapitulate neuronal mitophagy status in the patient brain. Despite the limitations, studies in iPSC-derived neurons provides unequivocal evidence of mitophagy defects in PD.

Parkin-independent mitophagy. Accumulating evidence suggests that Parkin is not canonically essential for all mitophagy processes. Receptor-mediated mitophagy centers around the ability of LC3-interacting region (LIR) motif that are identified in certain protein receptors to allow binding to LC3 for mitophagy induction, regardless of the presence of Parkin. AMBRA1 utilizes LIR motif to facilitate interaction with LC3, and mitophagy induction under conditions lacking Parkin. Fundc1, initiates mitophagy independent of Parkin, whereby evidence points towards its LIR domain association with LC3 as well as March5 and Ulk1 interactions for inducing hypoaxia-related mitophagy. Bcl-2/adenovirus E1B 19K-interacting protein 3-like proteins (BNIP3L), also known as Nix is an outer mitochondrial membrane protein equipped with an LIR near its N-terminus to perform Parkin-independent mitophagy.

A study using PD patient cells identified that despite carrying non-functional Parkin, BNIP3L-Nix-mediated mitophagy had maintained mitochondrial function, suggesting the PD-relevant neuroprotection. A slew of other emerging proteins acting as receptor-mediated mitophagy interactors have been added on to this growing list, including STX17, FKBP52, Bcl2-L-13 and PHB2. Cardiolipin is a lipid that triggers mitophagy through similar mechanisms mentioned in the previous receptors, externalizing from IMM to OMM under mitochondrial stress and binding to LC3, which upon reaching a critical threshold, trigger the induction mitophagy in a neuronal setting. The sphingolipid ceramide, capable of anchoring autophagylysosomal to mitochondrial membranes via LC3 interactions has also been suggested in parkin-independent, lipid-mediated mitophagy. Ubiquitin-mediated mitophagy was identified through PINK1’s ability to trigger mitophagy through NDP52 and optineurin regardless of Parkin’s presence, rewriting Parkin’s role from an essential regulator to a signal amplifier. Other E3 ligases aside from Parkin, such as MUL1 was found to interact with mitofusin (Mfn) during mitophagy induction at damaged mitochondria, and in Parkin-independent pathway leading to mitochondrial ubiquitination and mitophagy.

Another E3 ligase, Siah1 is recruited to mitochondria by Synphilin-1 to promote PINK1-dependent mitophagy via mitochondrial ubiquitination in a Parkin-independent manner. Collectively, these observations suggest a complex mitophagy pathway involves an intricate compensatory network that responds to various homeostatic and pathological conditions. Although there is little evidence of Parkin-independent mitophagy being involved in PD, these alternative mitophagy mechanism, such as MUL1- or NIX-involved mitophagy as discussed above, may be utilized to correct the mitophagy defects to rescue neurodegeneration in PD.

Reactive oxygen species

Reactive oxygen species (ROS) are highly reactive derivatives of molecular oxygen generated as by-products of cellular respiration. Common ROS include superoxide anion radical (O2−), hydroxyl radicals (OH•), and hydrogen peroxide (H2O2). ROS are normally sustained at low levels under redox homeostasis conditions. Maintaining ROS within balance is supported by antioxidant systems, with endogenous enzymatic antioxidants, such as superoxide dismutase (SOD). Catalase (CAT), glutathione peroxidase (GPx) and thioredoxin (Trx) as well as non-enzymatic antioxidants including vitamins such as vitamin C and vitamin E which collectively counter ROS overproduction within cells directly or indirectly. ROS are predominantly the result of excessive electron slippage in the electron transport chain (ETC) exceeding the various antioxidant systems, and capable of readily oxidizing other molecules (proteins, DNA and lipids), causing a wide-range of effects. ROS oxidizing effects on proteins include protein carbonylation, the most well-documented form of protein oxidation, whereby protein side chains are targeted, resulting in loss of protein functions. ROS are the primary endogenous damaging agent of DNA, with these oxidative reactions incurred through oxidation of DNA bases. ROS compromise the structural and dynamic properties of lipid membranes by altering their fluidity and permeability. Lipid peroxidation is the common term used to describe the chain reaction induced by ROS targeting lipids lead to key breakdown molecules malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). These intermediary compounds may react with DNA and proteins to form adducts in a neurodegenerative setting.

Lipid peroxidation by-product HNE triggers AMPK/mTORC pathway and JNK signal transduction pathway subsequently leading to autophagy activation. Cardiolipin, a phospholipid and important mitophagy mediator in the inner membrane of the mitochondrial lipid bilayer, was identified to be specifically susceptible to lipid peroxidation. Oxidation of Cardiolipin leads to the generation of reactive lipid mediators, including HNE and epoxide-containing cardiolipin. The mitochondrial network as a whole is a critical target of 4-HNE, with neuronal autophagy flux identified to be modulated by 4-HNE in a concentration-dependent manner likely through direct modification of autophagy proteins. Considering the effects of lipid peroxidation on autophagy, cardiolipin and mitochondrial protein qualities, it stands to reason the possibility that ROS-induced lipid peroxidation could eventually regulate mitophagy. Despite the classic dogma of ROS and reactive nitrogen species (RNS) being widely considered as harmful to cellular structures,
their role is dynamic, serving pleiotropic intracellular signal transducers of inter-connected processes, including development processes, metabolic networks and autophagy.

Evidence of ROS in PD. ROS are signalling molecules that maintain several physiologic functions. When the physiologic compensation fails to keep ROS at appropriate levels, the resulting oxidative stress will damage cellular macromolecules and promote cell death, leading to neurodegeneration. Various in vitro and in vivo studies in PD models have shown ROS-induction are critical in the pathophysiologic mechanisms of PD genetic mutations (PINK1 and Parkin, DJ-1, LRRK2, etc) and mitochondrial dysfunction. Furthermore, dopamine auto-oxidation promotes neurotoxicity in DA neurons. In post-mortem studies, there is evidence of reduced mitochondrial electron transport chain activity, and an increase in iron in SN compared to age-matched controls. Iron is known to raise ROS production through generating hydroxyl radicals, leading to α-synuclein-mediated reactive species formation in SN via Fenton reaction and redox-active iron accumulation in neuromelanin granules in SN. DA neurons in the SN are more susceptible to excess ROS generation as a result of their distinct cellular structure (relatively increased size and complexity) and biochemical activities (unique Ca2+ pacemaking activity).

Evidence of ROS in mitophagy. There is considerable evidence suggesting a key role of ROS in mitophagy. Light-induced activation of mitochondria-targeted photosensitizer may cause selective ROS-mediated damage to a subset of mitochondria and subsequently trigger mitophagy in both cell lines and rodent neurons. In vitro studies have demonstrated that mild and transient oxidative stress can trigger mitophagy but not non-selective autophagy. Downregulation of mitochondrial fusion can spatially isolate damaged mitochondria for efficient removal by mitophagy, highlighting the selectivity of oxidative conditions in a ROS signalling cascade that dominantly triggers selective mitophagy. Our own studies found that inhibition of ROS burst attenuated mitophagy at a couple of stages, i.e. PINK1-dependent Parkin translocation to mitochondria and elimination of mitochondria through autophagy. Interestingly, chronic low-dose treatment of mitochondrial uncoupler failed to stimulate ROS upsurge and Parkin translocation to mitochondria despite bringing PINK1 protein to a comparable level as acute high-dose treatment. Given the indispensable role of PINK1 in the initiation of mitophagy, there is the possible modification of ROS on PINK1 in the process of mitophagy.

THE BRIDGES BETWEEN ROS AND MITOPHAGY

Being the primary source of cellular ROS and the central focus of mitophagy, mitochondria are the crux of a number of signalling pathways linking ROS and mitophagy. ROS are a double-edged sword, possessing bidirectional impacts on autophagic flux. Generally, excessively high levels of ROS specifically trigger general autophagy over mitophagy. In contrast, moderate levels of ROS may trigger mitophagy likely through the activation of specific signalling pathways and redox signalling with mitophagy in turn possessing neuroprotective effect on disease progression. Collectively, whilst ROS as a signalling molecule and protective mitophagy are physiologically critical, prolonged dysregulation of mitophagy flux and/or excessive ROS levels are often detrimental for neuronal status, including within PD context. Identifying the bridges linking ROS and mitophagy is crucial for establishing their roles in PD pathogenesis and postulate potential therapeutic avenues using these interplays.

Signaling pathways

NF-κB. Nuclear factor kappaB (NF-κB) represents a family of five transcription factors, including NF-κB1 (p50/p105), NF-κB2 (p100/p52), RelA (p65), RelB, and c-Rel. Critical in inflammation, NF-κB family members upon activation through canonical and/or noncanonical pathways, may rapidly translocate to the nucleus and mediate transcription of target genes by binding to κB enhancer. Substantial evidence suggests that ROS (H2O2) acting upstream of this master regulator of redox balance, NF-κB, releases its NF-κB inhibitor (IkB) via H2O2 oxidation leading to NF-κB activation. Recently, it was found that NF-κB may promote mitophagy through inducing p62 expression to attenuate mitochondrial damage triggered by NLRP3-inflammasome activator. Mitophagy death was aggraved by inhibition of mitophagy through ablation of p62, or pathologically compromised ‘NF-κB-p62-mitophagy’ pathway. The same study suggested that NF-κB, through this anti-inflammatory pathway, directly affects Parkin-mediated mitophagy. A study by Duan et al. identified several NF-κB-binding sites within the PINK1 promoter and demonstrated that NF-κB overexpression or administration of NF-κB activator upregulated PINK1 at transcription level. TRAF6, an E3 ligase that acts as a transducer of the NF-κB pathway, and its related NF-κB activation may activate PINK1 cytosolic form to promote non-selective mitophagy through stabilization by enhanced Lys-63-linked ubiquitination. In addition, NF-κB may promote RIPK1 translocation to the mitochondria where it forms a complex with PINK1 and phosphoglycerate mutase family member 5 (PGAMS) that stabilizes and activates PINK1, eventually inducing mitophagy.

p38 MAPK. p38 MAPK belongs to the family of mitogen-activated protein kinases (MAPks) and is responding to stress stimuli, including inflammatory cytokines and oxidative stress. Under the canonical pathway of activation, ROS signalling by oxidative stress specifically oxidize antioxidant protein thioredoxin (TRX) and disassociate it away from the critical component ASK-1, allowing for ASK-1 dimerization and autophosphorylation, activating p38 pathway. MAPK14, one of the four p38 isoforms, and its upstream signalling pathways are identified to be required in mammalian cells for both starvation- and hypoxia-induced mitophagy, but not macroautophagy, demonstrating the selectivity of this pathway. A recent study by Qu et al. identified that inhibition of MAPK/p38 compromised the redistribution of Parkin in Parkin/PINK1-dependent mitophagy, with ROS identified to be a critical mediator upstream of this mitophagy pathway. Additionally, our own study identified that inhibition of p38 signalling pathway halted mitophagy progression driven by ROS even after Parkin relocated to mitochondria, further corroborating the hypothesis of MAPK having a significant influence on upregulating ROS-mediated mitophagy.

mTOR. Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase, and it inhibits autophagy through a complex interplay and mTOR dysfunction can lead to apoptosis of DA neurons in PD animal models. ROS may regulate autophagy/mitophagy through the PI3K/AKT/mTOR signalling pathway. The role of MTORC1 in autophagic removal of mitophagy is confirmed with TSC1/MTORC1 identified to be essential regulators for downstream mTORC1 function. Additionally, there is a possible influence on FOXO regulated Parkin/PINK1 axis, with inhibition of mTORC1 identified to precede FOXO-dependent mitophagy upregulation.

Nrf2-related pathways. Nrf2 (nuclear factor erythroid 2-related factor 2) is a redox-regulated transcription factor, a core element in the Nrf2-Antioxidant Response Element (ARE) related pathways. Their regulation can be either Kelch-like ECH-associated protein 1-dependent or Keap1-independent (via phosphorylation...
by Casein kinase II, protein kinase C, glycogen synthase kinase 3β etc. These pathways are identified to mediate oxidative stress response and are established to be dysregulated in aging and neurodegenerative diseases, including PD. Nrf2 pathway activation in PD was observed at a systemic level, likely to counteract oxidative stress. Oxidative stress may deprive Keap1 of its ability to ubiquitinate Nrf2 by modifying key cysteinyl residues leading to nucleus transport of Nrf2. Accumulated Nrf2 subsequently activates a body of antioxidant enzymes, acting as a critical master regulator for a broad set of oxidative stress responses. Oxidative stress triggered Nrf2 binds to ARE located in the p62 promoter, with p62 possibly activating Nrf2 and driving its own transcription in a positive feedback cycle. p62-mediated mitophagy inducer (PMI) disrupts the Nrf2-Keap1 interaction and induces mitophagy independently of Parkin/PINK1. Alternatively, another study suggests that p62, in an Nrf2-dependent manner, recruits two subunits of a cullin-RING ubiquitin (Keap1 and Rbx1) to mitochondria, promoting mitochondrial ubiquitination and subsequently mitophagy in a parkin-independent manner.

SIRT. Sirtuins are a family of nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases, which respond to ROS-activated NAD+ function to regulate numerous antioxidant and redox signalling pathways through transcription factors (including Nrf2, p53, NF-κB, FOXO, PGC-1a) and several molecules of antioxidant response element (ARE). Sirtuins relevancy in PD context includes a variety of SIRTs appearing to alter mitophagy in PD models in addition to their expression dysregulated in PD context includes a variety of SIRTs appearing to alter mitophagy in mitochondrial proteins. The relationship between sirtuins (SIRT1-7) and ROS, is uniquely complex and contested, with current evidence indicating that SIRT4 conditionally upregulates or suppresses ROS; SIRT1, SIRT3 and SIRT5 function against ROS; SIRT2, SIRT6, and SIRT7 mediate critical oxidative stress genes and mechanisms. SIRT1 influences Parkin translocation to mitochondria and is closely tied to alterations NAD+/NADH ratio in studies observing SIRT1 influence on mitophagy. In addition, SIRT2-mediated mitophagy regulated via ATG32 is identified to be essential in α-synuclein toxicity in yeast samples, implicating this ROS-mitophagy interplay in PD context. SIRT3, known for downregulating ROS through modulation of several critical enzymes, was identified to be key for inducing Parkin/PINK1-mediated mitophagy, likely through SIRT3 ability to interact and deacetylate both PINK1 and Parkin directly or indirectly via FOXO3a. Interestingly, another study identified SIRT3 effect of inducing mitophagy by upregulating BNIP3 expression, this mitophagy modulating activity further tied with ERK-CREB signalling pathway.

PD-related mutations/genes

Parkin/PINK1. Mutations in the parkin and PINK1 account for the most common causes of autosomal recessive early-onset Parkinson disease (EOPD). Early studies exploring functions of Parkin and PINK1 revealed that defects in Parkin or PINK1 result in enhanced ROS production in mouse brain or patient fibroblast, suggesting that Parkin and PINK1 may confer protection to neurons by attenuating ROS-related neurotoxicity. The mechanism of this protection at least partially lies in the role of Parkin/PINK1-mediated mitophagy that is progressively unravelled and refined. Overexpressed parkin in cultured cell lines can eliminate the entire mitochondrial network in cells, eradicating detrimental factors released from damaged mitochondria. Although dramatic mitophagy is not practicable in neurons, evidence of ROS inhibition through mitophagy in a physiological context has been garnered. Parkin/PINK1-dependent mitophagy was shown to attenuate NLRP3 inflammasome activation and mitochondrial ROS production, eventually reducing apoptosis in epithelial cells and renal injury in mice. AMPKα2 may interact with phosphorylated PINK1 and trigger Parkin recruitment and subsequent mitophagy, leading to reduced ROS production and apoptosis of cardiomyocytes. Inhibition of PINK1 accumulation on mitochondria by morphine led to mitophagy defect and excessive ROS accumulation in spinal cord neurons.

α-synuclein. Mutations in the gene encoding α-synuclein (SNCA) cause autosomal-dominant PD through point mutations, including A53T, E46K, H50Q etc, as well as copy number variations (duplication or triplication). Neurodegenerative effects of α-synuclein in PD have been increasingly tied to α-synuclein...
reciprocal relationship with mitochondrial dysfunction\textsuperscript{148} with a possible link to PINK1-related autophagy/mitophagy\textsuperscript{16,149}. α-synuclein role in ROS includes its accumulation on mitochondria via TOM20 leading to an excessive generation of ROS, with pathogenic α-synuclein-TOM20 interaction confirmed in PD post-mortem samples\textsuperscript{150}. α-synuclein delays mitophagy by targeting the N-terminus of Miro, leading to excessive and abnormal Miro accumulation on the mitochondrial surface\textsuperscript{16}. Upregulated production of ROS and reactive nitrogen species (RNS) are present in iPSCs carrying a-synuclein A53T mutation relative to isogenic control lines\textsuperscript{151}. a-synuclein E46K mutation was also identified to result in oxidative stress accumulation in SN DA neurons, likely increasing neuronal vulnerability towards mitochondrial impairment by mitochondrial toxins\textsuperscript{152}. Both A53T and E46K mutations promoted mitophagy through increasing α-synuclein accumulation on mitochondria, primarily through cardiopin externalization to the OMM\textsuperscript{153}.  

**DJ-1.** DJ-1 gene has been identified to be mutated in autosomal recessive PD. DJ-1 protein acts as a neuroprotective factor by directly eliminating hydrogen peroxide, while cells expressing DJ-1 carrying PD-related mutations are sensitive to oxidative effects\textsuperscript{154,155}. Interestingly, as a sensor for oxidative stress, DJ-1 protein is susceptible to the formation of adducts with PD-relevant dopamine\textsuperscript{156}. This relationship is theorized as a key susceptibility factor within PD-relevant A9 neurons in SN region that contain dopamine susceptible to ROS-induced adduct formation by DJ-1, further supported by in vivo evidence of elevated α-synuclein aggregates in DJ-1 deficient mice leading to the increased oxidized form of DA\textsuperscript{157,158}. Additionally, a previous study identified that DJ-1 overexpression rescued DA neurons against neurotoxicity, including oxidative stress, by enhancing extracellular signal-regulated protein kinase 1/2 (ERK1/2)-dependent mitophagy\textsuperscript{159}. This is in addition to the likely reciprocal interplay with Parkin/PINK1, as DJ-1 deficiency was identified to, in a ROS-dependent manner, promote Parkin recruitment and mitophagy, while mitochondrial DJ-1 level being regulated by Parkin/PINK1\textsuperscript{160}. Hence, while the exact mechanism is yet to be fully elucidated, DJ-1 is believed to influence Parkin/PINK1 mitophagy in a ROS-dependent manner.  

**LRRK2.** LRRK2 is a multifunctional enzymatic (kinase, GTPase etc), scaffolding protein\textsuperscript{161}. LRRK2 G2019S is a common genetic cause for familial and sporadic PD in caucasian populations\textsuperscript{162}, whereas in Asian population, LRRK2 S1647T, R1628P, and G2385R variants are associated with increased risk and lowered onset age of PD\textsuperscript{163}. IPSC-derived neurons carrying G2019S mutation was shown to upregulate the expression of key genes related to oxidative stress-response and α-synuclein levels\textsuperscript{164}. Our group have shown that quenching ROS through either genetic manipulation (expressing peroxiredoxin-3)\textsuperscript{165} or administration of chemical antioxidant\textsuperscript{166} effectively rescued PD phenotypes in neuronal and Drosophila models, suggesting that ROS play a crucial role in LRRK2 pathogenesis. LRRK2 mutations possess a complicated relationship with mitophagy, as the same G2019S mutations have demonstrated various mechanisms of mitophagy alterations, from increasing mitophagy through histone deacetylase activation\textsuperscript{167}, or in contradiction, to decreasing mitophagy\textsuperscript{168} probably through compromised Miro removal\textsuperscript{169}, sirtuin activity\textsuperscript{170} or aberrant Rab10 phosphorylation\textsuperscript{171}. Regardless of the uncertainty of specific regulation, which is likely context-dependent, it can be agreed that LRRK2 mutations, particularly G2019S, result in aberrant mitophagy within the PD context, likely contributing to its increased mitochondrial vulnerability and overall neurodegeneration\textsuperscript{172}.  

GBA. Glucocerebrosidase (GBA1) gene mutation is associated with a 20- to 30-fold increased PD development risk\textsuperscript{173}. L444P mutation in GBA has been established to induce mitochondrial dysfunctions, including altered mitophagy activation, autophagy flux and ROS levels\textsuperscript{174}. Specifically, L444P mutation in GBA has been identified to inhibit two critical areas related to mitophagy, namely mitochondrial priming and autophagic removal of the organelle\textsuperscript{175}. Mitophagy induction is identified to enhance transcription factor EB (TFE2) expression leading to increased GBA1 expression. The involvement of mitophagy in lysosomal biogenesis suggests a positive feedback loop, with GBA mutations inducing dysfunctions within this system\textsuperscript{176}. In addition, dysfunctional mitophagy and excessive oxidative stress have been identified in post-mortem tissue of PD patients with GBA mutations\textsuperscript{177}, further linking the effects of GBA on ROS-mitophagy interplay in PD context.  

In our review, the selection of genes we focus on reflect the most well-documented PD-related genes with regards to how they are functionally linked to mitophagy and ROS. However, genome-wide association study (GWAS)\textsuperscript{178-180} have revealed a host of relatively novel risk loci and their genes, with limited but emerging evidence that some could be implicated in both mitophagy and ROS, including VPS13C\textsuperscript{171} and SREBF1\textsuperscript{172}. We speculate that evidence of genetic variants in PD relevant context could increase in the coming years.  

We have highlighted a few PD genes that are associated with both aberrant ROS accumulation and defective mitophagy. The body of the evidence above clearly proves that the abnormalities in these two aspects are universal and critical in PD. However, more studies are warranted to determine which factor is the major culprit for PD pathogenesis and how the entanglement of these two factors contribute to PD pathogenesis. Therefore, both factors have to be taken into account to deliver effective therapy when designing a strategy to tackle PD.  

In addition, although the overwhelming evidence supports the agonistic role of ROS in mitophagy through activating various signalling pathways, there are reports showing antioxidative molecule(s) and/or compound(s) may promote mitophagy. For instance, KH176, a chemical derivative of a water-soluble form of vitamin E, enhanced mitophagy in neurons from subjects with or without Parkin mutation, suggesting its pro-mitophagy effect in a Parkin-independent manner\textsuperscript{173}. KH176 exerts its anti-oxidative effects through direct interaction with peroxiredoxins\textsuperscript{174}, while peroxiredoxin 3\textsuperscript{175} and peroxiredoxin 6\textsuperscript{176} have been found to be involved in mitophagy. Another study showed that mitochondria-targeted antioxidant, MitoQ rescued mitophagy, mitochondrial dysfunction and apoptosis through Nrf2 and PINK1 rather than its direct antioxidative effect\textsuperscript{177}. Thus, the paradoxical pro-mitophagy effect of antioxidant may be attributed to its specific effects on mitophagy-related signalling pathways or molecules instead of its role in suppressing ROS. In addition, ROS may directly oxidize ATG3 and ATG7 to prevent LC3 lipidation, eventually impairing autophagy\textsuperscript{178}. Given that Atg3\textsuperscript{179} and Atg7\textsuperscript{180} also play indispensable roles in mitophagy, it is therefore conceivable that antioxidants may suppress the oxidation of these autophagy-related proteins to promote mitophagy in a context-dependent manner. The success of antioxidant in promoting mitophagy indicates the potential of the therapeutic strategy for PD by promoting mitophagy while inhibiting ROS.  

**THERAPEUTIC AVENUES UTILIZING THE ROS-MITOPHAGY LINK** Clinical trials throughout the decades of antioxidants (creatinine\textsuperscript{181}, vitamin E\textsuperscript{182}, coenzyme Q10\textsuperscript{183} etc) in PD have not provided conclusive evidence that they are neuroprotective. One possible reason we postulate for the failure of antioxidants could be that blockage of signalling pathway necessary for mitophagy activation by undue inhibition of ROS is unlikely to be optimal against neurodegeneration. Promoting mitophagy by direct induction of ROS may not be ideal strategy either given the high volume of evidence showing the detrimental effects of ROS on cellular...
health, on account of its ability to exacerbate oxidative stress damage in PD.

**Existing compounds/drugs with effects on ROS and mitophagy**

Targets in the downstream signalling of ROS which can simultaneously activate protective mitophagy, while minimizing the harm of ROS may be potentially utilized in PD therapeutics. Here, we highlight some promising chemical compounds/drugs that target the various signalling pathways that may be involved in mitophagy. These chemical compounds/drugs have been approved or under investigation for other diseases, so that the safety concern may be circumvented.

Melatonin (N-acetyl-5-methoxytryptamine) has been shown to increase phosphorylation of Akt and NF-κB, leading to PARK1-dependent protective mitophagy [184]. Melatonin may also upregulate NRF2-induced mitophagy to protect against neuronal apoptosis in subarachnoid haemorrhage (SAH) [188]. The ROS levels, initially increased by high glucose conditions and necessary in order to stimulate mitophagy via Akt and NF-κB pathways, were subsequently attenuated by melatonin-induced PARK1-dependent mitophagy [186]. Melatonin has been well-documented to function against ROS and RNS, but may have pro-oxidant capabilities under specific conditions [186]. Existing evidence points toward melatonin’s role as an antioxidant showing systemic therapeutic benefits in PD-relevant clinical trials (dyskinesia, sleep disorder) [187], which combined with amphiphilic nature allowing for BBB diffusion further encourages therapeutic focus revisiting melatonin as a mitophagy agonist with NF-κB and Nrf2 activating effect mentioned prior [187].

Pioglitazone, an anti-diabetic drug for Type 2 diabetes mellitus (T2DM) under the category of thiazolidinediones (TZDs), increases PARK1 expression via NF-κB activation and enhances mitophagy. It protects against mitochondrial dysfunction induced by toxins and reduces ROS in a chronic kidney disease study [186,189]. Pioglitazone also improved phenotypes (impaired locomotion, DA neurodegeneration) in rat PD model [190]. Clinical cohort studies showed a 30% reduced risk of PD among diabetes treated with TZDs [191,192].

Rapamycin, an FDA-approved compound, treated with yeast by binding and inhibiting mTORC1 [193], and has been tested as a disease-modifying agent in experimental models of PD and neurodegenerative diseases [194]. It also promotes Parkin/PARK1 mitophagy, likely via p62, to restore mitochondrial homeostasis [195,196]. Rapamycin’s effect on ROS appears to be conditional. It may downregulate intracellular ROS via Nrf2 pathway, glutathione and SOD1 [197,198]. Nevertheless, it is also able to increase ROS levels, probably through c-Jun and endoplasmic reticulum (ER) stress pathway in certain circumstances [199]. Metformin, an mTOR inhibitor, activates mitophagy via signalling pathways including AMPK-Nrf2 as well as SIRT3 pathway [200,201]. It has been shown to upregulate mitophagy in a recent randomized controlled clinical trial in type 2 diabetes [202]. Metformin has been found to downregulate ROS production [203,204]. A longitudinal cohort study in an aging population with diabetes demonstrated a lower incidence of neurodegenerative diseases in those on long-term metformin therapy [205]. Spermidine, a polyamine with anti-oxidant properties, induces mitophagy through several signalling pathways, including modulation of the mTOR axis via ATM-dependent Parkin/PARK1 pathways [206,207]. Note, PD gene PARK9 encoded protein ATR13A2 is a lysosomal exporter of polyamine (putrescine, spermidine, and spermine) [208]. Spermine is pumped by ATR13A2 into the cytosol and subsequently absorbed by mitochondria. The polyamine transport activity of ATR13A2 is responsible to counter the mitochondrial oxidative stress [209]. Mitochondrial defects observed in *C. elegans* carrying a mutation in CATP-6 (a *C. elegans* ortholog of ATR13A2) was rescued via mitophagy induction [210]. In another study, spermidine was able to rescue behavioural deficits in the PD *C. elegans* model via the Parkin/PINK1 equivalent pathways [207]. Taken together, these studies suggest that spermidine may have neuroprotective effect on PD with ATP13A2 being a key component. Despite spermidine-generated ROS being a possible upstream signal in activating the latter ATM pathway [206], spermidine appears to act as direct ROS scavengers, resulting in inhibition of mitochondrial ROS production [211,212]. Salirodine, a plant extract, has been shown to protect DA neurons in PD models through enhancing mitophagy with its bioactive effects on DJ-1/Nrf2 pathway being the likely mechanism [213]. Consistently, studies revealed neuroprotective effects of salidroside in pre-clinical trials in Alzheimers disease, stroke and PD. A number of these studies emphasized its safety and substantial bioactive effects on the regulation of oxidative stress [214].

Tomatidine, a natural compound with antiaging properties activates Nrf2-SKN-1 pathway, resulting in the *C. elegans* homologue induction of PARK1-BNIP3/Nix-mitophagy that is suspected to preserve cell function by clearing damaged or dysfunctional mitochondria [215]. Tomatidine may activate mitophagy via Nrf2-SKN-1 pathway or TRAF6. Meanwhile, it may have a negligible or mild stimulating effect on ROS production [215,216]. Although evidence is limited, Ursolic and oleanolic acids, natural triterpenoid compounds widely found in plants, have been shown to induce Parkin-independent mitophagy, possibly concurrently with activation of AKT/mTOR and Nrf2/ARE pathways, with the ROS induction suspected to be an upstream factor for mitophagy induction for both triterpenes [217].

Honokiol, an agonist of SIRT3 known for anti-inflammatory and antitumor effects, promotes mitophagy and mitochondrial dynamics in vitro in an SIRT3-dependent manner via the AMPK-PGC-1α signalling pathway, and this neuroprotection has been validated in vivo [218]. Honokiol’s effect of downregulating ROS in this study [218] is consistent with existing in vitro and in vivo evidence highlighting its inhibitory effect on ROS production, likely in an SIRT3-dependent manner [195,200]. Liraglutide, an anti-diabetic drug, is a glucagon-like peptide-1 receptor agonist that elevates the levels of SIRT1 in a dose-dependent manner and is able to trigger autophagy. It can augment Parkin expression, leading to Parkin-mediated mitophagy activation [221]. The SIRT1/Parkin/mitophagy pathway activation is protective, downregulating cellular oxidative stress [222]. Similar observations of ROS inhibition by liraglutide has been consistently observed [222,223]. Liraglutide shows good BBB permeability and neuroprotective effects in PD animal models [224]. We await the results of its ongoing trial in idiopathic PD, which should be completed soon. (ClinicalTrials.gov Identifier: NCT02953665).

We discuss here the compounds/drugs that regulate mitophagy with emphasis on ROS-related signalling pathway(s). As for the modulators of mitophagy reviewed elsewhere, most of the targets are localized on mitochondria [25,256]. Therefore, modulation of these targets may have profound impacts on mitochondrial functions, including ROS homeostasis. For instance, Ubiquitin-specific protease 30 (USP30) removes ubiquin attached by Parkin to the OMM substrates, attenuating subsequent mitophagy. On the other hand, reduction in USP30 level rescues mitophagy caused by Parkin/PINK1 mutation. Based on its influence on mitochondria, USP30 inhibitors have been developed to promote mitophagy as a potential therapeutic option for PD [227,228]. However, USP30 is also involved in the regulation of the import of intramitochondrial proteins, including subunits of electron transport chain proteins such as Complex [229]. Manipulation of USP30 may cause aberrant ROS generation by interfering the proper functions of the respiratory chain. Thus, the effects of USP30 or other mitophagy regulators on ROS production have to be assessed and the benefit of neuroprotection through mitophagy and the potential detrimental effect of ROS dysregulation has to be weighed.

Published in partnership with the Parkinson's Foundation npj Parkinson's Disease (2022) 135
Table 1. Relevant compounds for consideration of ROS-mitophagy link.

| Compound          | Target mitophagy pathway                                      | Effect on ROS levels                              | References     |
|-------------------|---------------------------------------------------------------|---------------------------------------------------|----------------|
| Melatonin         | Upregulation of NF-κB (preceded by Akt) and Nrf2 to induce mitophagy | ROS reduced                                      | 184–187        |
| Pioglitazone      | NF-κB to induce mitophagy                                     | ROS reduced                                      | 188–192        |
| Rapamycin         | mTOR inhibition to induce Parkin-mediated mitophagy           | ROS reduced in majority of studies, raised in few studies under specific conditions | 193–199        |
| Metformin         | mTOR inhibition and SIRT3 upregulation to induce Parkin-mediated mitophagy | ROS reduced                                      | 200–205        |
| Spermidine        | mTOR axis modulation to induce Parkin mitophagy               | ROS reduced overall, early ROS-generation acting as an upstream signalling factor | 206–212        |
| Tomatidine        | Activated Nrf2–SKN-1 pathway to induce PINK-1/Bnip3/Nix-mitophagy | Mild or no effect                                | 215,216        |
| Ursolic acid      | Activate AKT/mTOR and Nrf2/ARE pathways to induce Parkin-independent mitophagy | Early ROS-generation acting as an upstream signalling factor | 217            |
| Honokiol          | Trigger SIRT3 (via the AMPK-PGC-1α) pathway to induce mitophagy | ROS reduced                                      | 218–220        |
| Liraglutide       | Increase SIRT1 levels leading to Parkin-mediated mitophagy activation | ROS reduced                                      | 221–224        |

Maximizing the potential therapeutic benefits of these compounds in Table 1 requires factoring in their effects on ROS levels. Among them, a few compounds may potentially upregulate ROS, having a negative impact on cell survival. When applied in PD, these drugs should be coupled with ROS inhibitors (general antioxidants or mitochondrial-targeted antioxidants) necessary to negate any harmful oxidative effects. Most compounds listed have apparently null effects or downregulate ROS levels, whereby their use in combination with ROS inhibitors may be considered to synergistically improve therapeutic performance. We reason that mitophagy activation in PD relevant therapeutics should simultaneously avoid provoking oxidative stress, while maintaining their neuroprotective effects of downstream signalling on mitochondrial homeostasis, particularly mitochondrial turnover through autophagy-lysosomal pathway.

Some of these compounds are currently undergoing pre-clinical and clinical trials (i.e. Liraglutide, Salidroside, and Melatonin) in PD, further encouraging the efforts in drug repurposing. Therapeutic usage of these compounds could be implemented in a targeted manner or in combined multidrug regime with existing pharmacological (carbidopa-levodopa, pramipexole etc) and non-pharmacological approaches (physical, occupational, speech therapies etc).

Drug repurposing, utilizing various degrees of pre-existing clinical efforts covering efficacy and safety efforts, offsets the high cost and lengthy timeline of de novo drug discovery. However, drug repurposing comes with issues that should be taken into consideration, including but not limited to intellectual property considerations and high chance to fail in clinical trials. The ROS inhibitors as mentioned earlier (creatinine, vitamin E) have yet to be proved as effective repurposing drugs in PD treatment.

Potential target-based screening for mitophagy activator

Alternatively, potential therapeutic compounds for PD may be discovered de novo from the perspective of mitophagy. A recent study was conducted using an imaging-based drug screening in patient iPSC-derived neurons carrying Parkin or PINK1 mutation. Among the 320 compounds screened, 73 hits were found to promote mitophagy with two candidates attenuating ROS and cell death. Large-scale screenings are needed to identify more potential compounds which can be further validated and taken to clinical trials. It should be noted that neurons are usually cultured in B27 supplemented neurobasal medium, which contains antioxidant to promote neuronal survival. Potential oxidative stress caused by compounds may not be revealed in the cultured neurons. Additionally, animal model may have different tolerance and compensatory mechanism for mitophagy impairment and ROS stress. Target-based screening with stringent validation of mitophagy activator may help to efficiently identify promising drug candidate. The screening of mitophagy activator targeting ROS-related signalling pathways in the presence of certain antioxidant in mitophagy-intact or mitophagy-defective iPSC-derived neurons may yield compounds that potentially reverse the pathogenesis of PD. The compounds identified in mitophagy-defective neurons represent the potential drug candidates which may promote alternative mitophagy to rescue PD caused by mitophagy defects. The candidates that can promote mitophagy in the presence of antioxidant may trigger mitophagy by activating certain signalling pathway while eliminating potential ROS-related detrimental effects. With the efficient target-based screening, more efforts could be made to evaluate the effects of the hits on PD phenotypes in multiple model systems, including animal and human midbrain organoid PD model before taken into clinical trial together with the antioxidant (Fig. 3).

Challenges and potential solutions

Therapeutic trials studying the potential of activating ROS downstream signalling pathway(s) to boost mitophagy face some challenges (Table 2). First, it is important to pinpoint mitophagy defects in relevant biological samples. In vitro culture of DA neurons or midbrain organoid derived from patient iPSCs may provide a useful tool to study mitophagy in vitro with the aid of constantly developing fluorescence dyes to readily detect mitophagy status or single cells sequencing to identify the cell populations and signalling pathways implicated in the mitophagy. Furthermore, it is of note that pioglitazone failed to halt PD progression in trials, but lowered the risk of PD, suggesting that mitophagy activation in rescuing neurodegeneration is more likely to be useful in early stages of neurodegeneration, as opposed to clinically diagnosed PD patients whereby numerous cascades of cellular dysfunctions rendering neurodegeneration are inevitable and irreversible. Thus, mitophagy activation may be more effective in the preclinical/prodromal state. Hence clinical trials should focus on asymptomatic genetic carriers or those at risk or those with prodromic symptoms. A comprehensive analysis of genetic background (known genes related to PD) and longitudinal clinical studies of blood, urine and CSF biomarkers and clinical manifestation (including symptoms of anosmia, REM disorder)
will help to stratify clinical trial subjects and to monitor their progression.

In conclusion, the dynamic and complex interplay between mitophagy and excessive ROS plays an important pathophysiological role in both sporadic and familial PD. Identifying the relationship between these processes and their triggers early in the course of neurodegeneration will provide novel mechanistic clues that can potentially lead to the development of drugs that target specific pathways in this network. Proper selection of specific subsets of subjects for longitudinal clinical trials will enhance the chance of a favourable therapeutic outcome.

DATA AVAILABILITY
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Received: 18 May 2022; Accepted: 28 September 2022; Published online: 18 October 2022

REFERENCES
1. Jankovic, J. & Tan, E. K. Parkinson’s disease: etiopathogenesis and treatment. J. Neurol. Neurosurg. Psychiatry 8, 795–808 (2020).
2. Ou, Z. et al. Global Trends in the Incidence, Prevalence, and Years Lived With Disability of Parkinson’s Disease in 204 Countries/Territories From 1990 to 2019. Front. Public Heal. 9, 776847 (2021).
3. Abbas, M. M., Xu, Z. & Tan, L. C. S. Epidemiology of Parkinson’s Disease—East Versus West. Mov. Disord. Clin. Prac. 5, 14–28 (2018).
4. Giráldez-Pérez, R. M., Antolín-Vallespin, M., Muñoz, M. D. & Sánchez-Capelo, A. Models of α-synuclein aggregation in Parkinson’s disease. Acta Neuropathol. Commun. 2, 1–17 (2014).
5. Torres-Ocido, S. et al. Progression of pathology in PINK1-deficient mouse brain from splicing via ubiquitination, ER stress, and mitophagy changes to neuroinflammation. J. Neuroinflammation 14, 154 (2017).
6. Armstrong, M. J. & Okun, M. S. Diagnosis and Treatment of Parkinson Disease: A Review. JAMA - J. Am. Med. Assoc. 323, 548–560 (2020).
7. Hsieh, C. H. et al. Functional Impairment in Mito Degradation and Mitophagy Is a Shared Feature in Familial and Sporadic Parkinson’s Disease. Cell Stem Cell 19, 709–724 (2016).
8. Liu, H. et al. Aberrant mitochondrial morphology and function associated with impaired mitophagy and DNA1L- MAPK/ERK signaling are found in aged mutant Parkinsonian LRRK2(R1441G) mice. Autophagy 17, 3196–3220 (2021).
9. Musgrove, R. E. et al. Oxidative stress in vagal neurons promotes parkinsonian pathology and intercellular α-synuclein transfer. J. Clin. Invest. 129, 3738–3753 (2019).
10. Burbulla, L. F. et al. Dopamine oxidation mediates mitochondrial and lysosomal dysfunction in Parkinson’s disease. Science 357, 1255–1261 (2017).
11. Chen, C. et al. Investigation of mitochondrial biogenesis defects in single substantia nigra neurons using post-mortem human tissues. Neurobiol. Dis. 134, 104631 (2020).
12. Franco-Ibora, S. et al. Defective mitochondrial protein import contributes to complex I-induced mitochondrial dysfunction and neurodegeneration in Parkinson’s disease. Cell Death Dis. 9, 1122 (2018).
38. Parzych, K. R. & Klionsky, D. J. An overview of autophagy: morphology, function and pharmacological manipulation. EMBO Rep. 16, 3753–3757 (2015).
39. Murakawa, T. et al. Bcl-2-like protein 13 is a mammalian Atg32 homologue that interacts with PINK1. EMBO J. 36, 1768–1776 (2017).
40. Frank, M. et al. Mitophagy is triggered by mild oxidative stress in a mitochondrial fission dependent manner. Biochim. Biophys. Acta - Mol. Cell Res. 1823, 2297–2310 (2013).
41. Narendra, D. P. et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. Proc. Natl. Acad. Sci. 107, 10029–10034 (2010).
42. Sekine, S. et al. Reciprocal Roles of Tom7 and OMA1 during Mitochondrial Import and Activation of PINK1. Mol. Cell. 37, 1028–1043.e5 (2019).
43. Yi, W. et al. The landscape of Parkin variants reveals pathogenic mechanisms and therapeutic targets in Parkinson’s disease. Hum. Mol. Genet. 28, 2811–2825 (2019).
44. Pickrell, A. M. et al. Endogenous Parkin Preserves Dopaminergic Substantia Nigra Neurons following Mitochondrial DNA Mutagenic Stress. Neuron 87, 371–381 (2015).
45. Cornelissen, T. et al. Deficiency of parkin and PINK1 impairs age-dependent mitophagy in drosophila. Elife 7, e1–14 (2018).
46. Bingol, B. et al. The mitochondrial deubiquitinate USP30 opposes parkin-mediated mitophagy. Redox Biol. 6, 3–13 (2015).
47. Fiesel, F. C. et al. (Patho-)physiological relevance of PINK1-dependent ubiquitin phosphorylation. EMBO Rep. 16, 1114–1130 (2015).
48. Lee, S. H. et al. Mitochondrial MsrB2 serves as a switch and transducer for induced mitophagy. EMBO Mol. Med. 11, e10409 (2019).
49. McWilliams, T. G. et al. Basal Mitophagy Occurs Independently of PINK1 in Mitochondrial Diseases of High Metabolic Demand. Cell Metab. 27, 439–449.e8 (2018).
50. Seibler, P. et al. Mitochondrial Parkin recruitment is impaired in neurons derived from mutant Parkin induced pluripotent stem cells. J. Neurosci. 31, 5970–5976 (2011).
51. Shaltouki, A. et al. Mitochondrial alterations by PARKIN in dopaminergic neurons used PARK2 patient-specific and PARK2 knockout isogenic iPSC lines. Stem Cell Rep. 8, 647–859 (2015).
52. Shiba-Fukushima, K. et al. Evidence that phosphorylated ubiquitin signaling is involved in the etiology of Parkinson’s disease. Hum. Mol. Genet. 26, 3172–3185 (2017).
53. Yamaguchi, A. et al. Identifying Therapeutic Agents for Amelioration of Mitochondrial Clearance Disorder in Neurons of Familial Parkinson Disease. Stem Cell Rep. 14, 1060–1075 (2020).
54. Berenguer-Escuder, C. et al. Impaired mitochondrial-endoplasmic reticulum interaction and mitophagy in Miro1-mutant neurons in Parkinson’s disease. Hum. Mol. Genet. 29, 1353–1364 (2020).
55. Liu, Y.-T. et al. Mt-Keima detects PARK1-Parkin mitophagy in vivo with greater sensitivity than mito-Q. Autophagy 17, 3753–3762 (2021).
56. Kumar, M. et al. Defects in Mitochondrial Biogenesis Drive Mitochondrial Alterations in PARKIN-Deficient Human Dopamine Neurons. Stem Cell Rep. 15, 629–645 (2020).
57. Suzuki, S. et al. Efficient induction of dopaminergic neuron differentiation from induced pluripotent stem cells reveals impaired mitophagy in PARK2 neurons. Biochem. Biophys. Res. Commun. 483, 88–93 (2017).
58. Vazquez-Martín, A. et al. Mitophagy-driven mitochondrial rejuvenation regulates stem cell fate. Aging (Albany NY) 8, 1330–1352 (2016).
59. Esteban-Martínez, L. et al. Programmed mitophagy is essential for the glycolytic switch during cell differentiation. EMBO J. 36, 1688–1706 (2017).
60. Liu, L., Sakakibara, K., Chen, Q. & Okamoto, K. Receptor-mediated mitophagy in yeast and mammalian systems. Cell Res. 24, 787–795 (2014).
61. Strappazzon, F. et al. AMBRA1 is able to induce mitophagy via LC3 binding, regardless of PARKIN and p62/SQSTM1. Cell Death Differ. 22, 419–432 (2015).
62. Chen, Z. et al. Mitochondrial E3 ligase MARCH 5 regulates FUNDC1 to fine-tune hypoxic mitophagy. EMBO Rep. 18, 495–509 (2017).
63. Wu, W. et al.ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy. EMBO Rep. 15, 566–575 (2014).
64. Novak, I. et al. Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep. 11, 45–51 (2010).
65. Naeem, S., Qi, Y., Tian, Y. & Zhang, Y. NIX compensates lost role of parkin in cd- dependent mitophagy in HeLa cells through phosphorylation. Toxcol. Lett. 326, 1–10 (2020).
66. Koentjoro, B., Park, J. S. & Sue, C. M. Nix restores mitophagy and mitochondrial function to protect against PARK1/Parkin-related Parkinson’s disease. Sci. Rep. 7, 1–11 (2017).
67. Xian, H., Yang, Q., Xiao, L., Shen, H. M. & Liu, Y. C. STX17 dynamically regulated by Fis1 induces mitophagy via hierarchical macroautophagic mechanism. Nat. Commun. 10, 2059 (2019).
68. Bhujabal, Z. et al. FKBP8 recruits LC3A to mediate Parkin-independent mitophagy. EMBO Rep. 18, 947–961 (2017).
69. Wei, Y., Chiang, W. C., Sumpfer, R., Mishra, P. & Levine, B. Prohibitin 2 is an Inner Mitochondrial Membrane Mitophagy Receptor. Cell 168, 234–238.e10 (2017).
70. Chu, C. T. et al. Cardiolipin externalization to the outer mitochondrial membrane is an elimination signal for mitophagy in neuronal cells. Nat. Cell Biol. 15, 1197–1205 (2013).
71. Sentelle, R. D. et al. Ceramide targets autophagosomes to mitochondria and induces lethal mitochondrialopathy. Nat. Chem. Biol. 8, 831–838 (2012).
72. Yun, J. et al. MUL1 acts in parallel to the Parkin/parkin pathway in regulating mitochondrial dynamics and contributes to loss of PARKIN/parkin. Elife 4, e01958 (2014).
73. Villa, E., Marchetti, S. & Ricci, J. E. No Parkin Zone: Mitophagy without Parkin. Trends Cell Biol. 28, 882–895 (2018).
74. Szargel, R. et al. The PINK1, synphilin-1 and SIAH-1 complex constitutes a novel mitophagy pathway. Hum. Mol. Genet. 25, 3476–3490 (2016).
75. Rahal, A. et al. Oxidative stress, prooxidants, and antioxidants: The interplay. Biomed. Res. Int. 2014, 761264 (2014).
101. Sies, H. & Jones, D. P. Reactive oxygen species (ROS) as pleiotropic physiological
100. Chen, Y., Azad, M. B. & Gibson, S. B. Superoxide is the major reactive oxygen
102. Xiao, B. et al. Reactive oxygen species trigger Parkin/PINK1 pathway

Published in partnership with the Parkinson's Foundation
npj Parkinson's Disease (2022) 135

B. Xiao et al.
1. Di Sante, G. et al. Loss of sir11 promotes prostatic intraepithelial neoplasia, reduces mitophagy, and delays park2 translocation to mitochondria. Am. J. Pathol. 185, 266–279 (2015).

2. Sampaio-Marques, B. et al. SNCA (α-synuclein)-induced toxicity in yeast cells is dependent on sirtuin 2 (Sir2)-mediated mitophagy. Autophagy 8, 1494–1509 (2012).

3. Zhou, Z. D. & Tan, E. K. Oxidized nicotinamide adenine dinucleotide-dependent mitochondrial deacetylase sirtuin-3 as a potential therapeutic target of Parkinson’s disease. Ageing Res. Rev. 62, 101107 (2020).

4. Li, R. et al. Therapeutic effect of Sir2 on ameliorating nonalcoholic fatty liver disease: The role of the ERK-CREB pathway and Bnip3-mediated mitophagy. Redox Biol. 18, 229–243 (2018).

5. Palacino, J. J. et al. Mitochondrial Dysfunction and Oxidative Damage in parkin-deficient Mice. J. Biol. Chem. 279, 18614–18622 (2004).

6. Piccollo, C. et al. Mitochondrial respiratory dysfunction in familial Parkinsonism associated with PINK1 mutation. Neurochem. Res. 33, 2565–2574 (2008).

7. Narendra, D., Tanaka, A., Suen, D. F. & Youle, R. J. Parkin is recruited selectively to mitochondria in Parkinson disease. J. Mol. Cell Biol. 39, 1284–1293 (2015).

8. Kong, H. et al. Morphine induces dysfunction of PINK1/Parkin-mediated mitophagy via PINK1 phosphorylation. Autophagy 15, 1777–1790 (2019).

9. Taira, T. et al. DJ-1 has a role in antioxidative stress to prevent cell death. Exp. Neurol. 194, 232–239 (2005).

10. Di Maio, R. et al. Peroxiredoxin 6 Is a Crucial Factor in the Initial Step of Mitochondrial Clearance and is Upstream of the PINK1/Parkin Pathway. Antioxid. Redox Signal. 24, 486–501 (2016).

11. Li, J. Q., Tan, L. & Yu, J. T. The role of the LRRK2 gene in Parkinsonism. Exp. Neurol. 230, 112–122 (2011).

12. Schwartzentruber, A. et al. Oxidative switch drives mitophagy defects in dopaminergic parkin mutant patient neurons. Sci. Rep. 10, 15485 (2020).

13. Neyroud, S. C. et al. Mitochondrial dysfunction and mitophagy defect triggered by heterozygous GBA mutations. Autophagy 15, 113–130 (2019).

14. Xiong, C. et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson’s disease. Nat. Genet. 46, 989–993 (2014).

15. Bo, C. B. et al. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson’s disease. PLoS Genet. 7, e1002141 (2011).

16. Billingsley, K. J. et al. Mitochondria function associated genes contribute to Parkinson’s disease risk and later age at onset. NPJ Parkinson Dis. 5, 8 (2019).

17. Lesage, S. et al. Loss of VPS13C Function in Autosomal-Recessive Parkinsonism Causes Mitochondrial Dysfunction and Increases PINK1/Parkin-Dependent Mitophagy. Am. J. Hum. Genet. 98, 500–513 (2016).

18. Huvet, R. M. et al. Genome-wide RNA screen identifies the Parkinson disease GWAS risk locus SREBF1 as a regulator of mitophagy. Proc. Natl Acad. Sci. USA. 111, 8494–8499 (2014).

19. Schwartzenburger, A. et al. Oxidative switch drives mitophagy defects in dopaminergic parkin mutant patient neurons. Sci. Rep. 10, 15485 (2020).

20. Seyrath, J. et al. KH176 Safeguards Mitochondrial Diseased Cells from Redox Stress-Induced Cell Death by Interacting with the Thioredoxin System/Peroxiredoxin Enzyme Machinery. Sci. Rep. 8, 6577 (2018).

21. Sonn, S. K. et al. Peroxiredoxin 3 deficiency induces cardiac hypertrophy and dysfunction by impaired mitochondrial quality control. Redox Biol. 51, 10225 (2022).

22. Ma, S. et al. Peroxiredoxin 6 Is a Crucial Factor in the Initial Step of Mitochondrial Clearance and is Upstream of the PINK1/Parkin Pathway. Antioxid. Redox Signal. 24, 486–501 (2016).

23. Xiong, L. et al. The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. Redox Biol. 11, 297–311 (2017).

24. Fridd, K., Burgoyne, T. & Burgoyne, J. R. Oxidation of Atg1 and Atg7 mediates inhibition of autophagy. Nat. Commun. 9, 95 (2018).

25. Radoshevich, L. et al. ATG12 conjugation to ATG3 regulates mitochondrial homeostasis and cell death. Cell 142, 590–600 (2010).

26. Towers, C. G. et al. Mitochondrial-derived vesicles compensate for loss of LC3-mediated mitophagy. Dev. Cell 56, 2029–2042.e5 (2021).

27. Tilley, B. C. et al. Effect of Creatine Monohydrate on Clinical Progression in Patients With Parkinson Disease. JAMA 313, 584–593 (2015).

28. Shoulson, I. DATATOP: a decade of neuroprotective inquiry. Parkinson Study Group. Depenrly And Tocopherol Antioxidative Therapy Of Parkinsonism. Ann. Neurol. 44, 5160–5166 (1998).

29. Investigators, T. N. N. P. F. A randomized clinical trial of coenzyme Q 10 and GPI-1485 in early Parkinson disease. Neurology 68, 20 LP–20 (2007).

30. Onphachan, X. et al. Enhancement of high glucose-induced PINK1 expression by melatonin stimulates neuronal cell survival: Involvement of MT2/Akt/NF-kB pathway. J. Pineal Res. 63, 1–17 (2017).

31. Sun, B., Yang, S., Li, S. & Hang, C. Melatonin upregulates nuclear factor erythroid-2 related factor 2 (Nrf2) and mediates mitophagy to protect against early brain injury after subarachnoid hemorrhage. Med. Sci. Monit. 24, 6422–6430 (2018).

32. Zhang, H. M. & Zhang, Y. Melatonin: A well-documented antioxidant with conditional pro-oxidant actions. J. Pineal Res. 57, 131–146 (2014).

33. Filograna, R., Beltrami, M., Babacino, L. & Bisaglia, M. Anti-Oxidants in Parkinson’s Disease Therapy: A Critical Point of View. Curr. Neuropharmacol. 14, 260–271 (2016).

34. Yoon, Y. M. et al. Pioglitazone protects mesenchymal stem cells against P-cresol-induced mitochondrial dysfunction via up-regulation of PINK-1. Int. J. Mol. Sci. 19, 2896 (2018).

35. Palacios-Ramirez, R. et al. Pioglitazone Modulates the Vascular Contractility in Hypertension by Modulating the VEGF/VEGFR-1 Pathway. Cell Physiol. Biochem. 45, 260–283 (2017).

36. Ulusoy, G. K. et al. Effects of pioglitazone and retinoic acid in a rotenone model of Parkinson’s disease. Exp. Neurol. 231, 661–668 (2011).

37. Brakedal, B. et al. Glutamate are associated with reduced risk of Parkinson’s disease. Mov. Disorder. 32, 1594–1599 (2017).

38. Lou, G. et al. Mitophagy and Neuroprotection. Trends Mol. Med. 26, 8–20 (2020).

39. Bobe, J., Martinez-Vicente, M. & Vila, M. Fighting neurodegeneration with rapamycin: Mechanistic insights. Nat. Rev. Neurosci. 12, 437–452 (2011).

40. Wang, Y. et al. Parkin-mediated mitophagy is activated in cisplatin nephrotoxicity to protect against kidney injury. Cell Death Dis. 9, 1–14 (2018).

41. Li, Q. et al. Rapamycin Enhances Mitophagy and Attenuates Apoptosis After Spinal Ischemia-Reperfusion Injury. Front. Neurosci. 12, 865 (2018).

42. Ko, J. H., Yoon, S. O., Lee, H. J. & Oh, J. Y. Rapamycin regulates macrophage activation by inhibiting NLRP3 inflammasome in p38 MAPK-NF-kB pathways in autophagy- and p62-dependent manners. Oncotarget 8, 40817–40831 (2017).
198. Dai, C. et al. Rapamycin Confers Neuroprotection against Colistin-Induced Oxidative Stress, Mitochondria Dysfunction, and Apoptosis through the Activation of Autophagy and mTOR/Akt/CREB Signaling Pathways. ACS Chem. Neurosci. 9, 824–837 (2018).
199. Chen, W. et al. Synergistic antitumor activity of rapamycin and EF24 via increasing ROS for the treatment of gastric cancer. Redox Biol. 10, 78–89 (2016).
200. Wang, J. et al. Protective effects of metformin against osteoarthritis through upregulation of SIRT3-mediated PINK1/Parkin-dependent mitophagy in primary chondrocytes. Biosci. Trends 12, 605–612 (2018).
201. Chen, L. et al. Metformin mitigates gastrointestinal radiotoxicity and radiosensitivities P53 mutation colorectal tumours via optimising autophagy. Br. J. Pharmacol. 177, 3991–4006 (2020).
202. Bhanushali, S., Bhanushali, A., Dhutta, P., Walia, R. & Dhawan, V. Metformin upregulates mitophagy in patients with T2DM: A randomized placebo-controlled study. J. Cell. Mol. Med. 24, 2832–2846 (2020).
203. Aigle, C. et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. Cancer Prev. Res. 5, 536–543 (2012).
204. Kelly, B., Tanahill, G. M., Murphy, M. P. & O'Neill, L. A. J. Metformin inhibits the production of reactive oxygen species from NADH Ubiquinone oxidoreductase to limit induction of interleukin-1β (IL-1β) and boosts interleukin-10 (IL-10) in lipopolysaccharide (LPS)-activated macrophages. J. Biol. Chem. 290, 20348–20359 (2015).
205. Shi, Q., Liu, S., Fonseca, V. A., Theilii, T. K. & Shi, L. Effect of metformin on neurodegenerative disease among elderly adult US veterans with type 2 diabetes mellitus. BMJ Open 9, e024954 (2019).
206. Qi, Y., Qiu, Q., Gu, X., Tian, Y. & Zhang, Y. ATM mediates spermine-induced mitophagy via PINK1 and Parkin regulation in human fibroblasts. Sci. Rep. 6, 24700 (2016).
207. Wang, C. et al. Protective effects of metformin against osteoarthritis through oxidizing vascular stress resistance between the longest-living rodent, the naked mole rat, and mice. Am. J. Physiol. Heart Circ. Physiol. 291, H2698–H2704 (2006).
208. Labinskyy, N. et al. Comparison of endothelial function, O2- and H2O2 production, and vascular oxidative stress resistance between the longest-living rodent, the naked mole rat, and mice. Am. J. Physiol. Cell Physiol. 292, C708–C717 (2007).
209. Jo, J. et al. Lewy Body-like Inclusions in Human Midbrain Organoids Carrying Glucocerebrosidase and α-Synuclein Mutations. Ann. Neurol. 90, 490–505 (2021).
210. Anand, N. et al. Dysregulated iron metabolism in C. elegans catp-6/ATP13A2 deficiency disrupts lysosomal polyamine export. Nature Commun. 13, 605 (2022).
211. Vrijsen, S. et al. ATP13A2-mediated endo-lysosomal polyamine export counters mitochondrial oxidative stress. Proc. Natl Acad. Sci. USA. 117, 31198–31207 (2020).
212. Shiratori, N. et al. Dysregulated iron metabolism in C. elegans catp-6/ATP13A2 prevents heart injury in Neonatal Rats Exposed to Intracellular Hyoxia by Inhibiting Oxidative Stress and Mitochondrial Fragmentation. Oxid. Med. Cell. Longev. 2019, 5406468 (2019).
213. Wang, J. et al. Spermine alleviates cardiac aging by improving mitochondrial biogenesis and function. Aging (Albany NY) 12, 16852–16866 (2020).
214. Wang, X. et al. ATP13A2 deficiency disrupts lysosomal polyamine metabolism. Nature 587, 419–424 (2020).
215. Vrijen, S. et al. ATP13A2-mediated endo-lysosomal polyamine export counteracts mitochondrial oxidative stress. Proc. Natl Acad. Sci. USA. 117, 31198–31207 (2020).
216. Anand, N. et al. Dysregulated iron metabolism in C. elegans catp-6/ATP13A2 mutant impairs mitochondrial function. Neurobiol. Dis. 139, 104786 (2020).
217. Chai, N. et al. Spermine Prevents Heart Injury in Neonatal Rats Exposed to Intracellular Hyoxia by Inhibiting Oxidative Stress and Mitochondrial Fragmentation. Oxid. Med. Cell. Longev. 2019, 5406468 (2019).
218. Yang, X. et al. Spermine inhibits neurodegeneration and delays aging via the PINK1/Parkin-dependent mitophagy pathway in C. elegans. Aging (Albany NY). 12, 650–671 (2020).
219. Li, R. et al. Saldioroside Protects Dopaminergic Neurons by Enhancing PINK1/Parkin-Mediated Mitophagy. Oxid. Med. Cell. Longev. 2019, 9341018 (2019).
220. Zhang, Z. et al. Pharmacological activities, mechanisms of action, and safety of saldioroside in the central nervous system. Drug Des. Devel. Ther. 12, 1479–1489 (2018).
221. Fang, E. F. et al. Tomatidine enhances lifespan and healthspan in C. elegans through mitophagy induction via the SKN-1/Nrf2 pathway. Sci. Rep. 7, 1–13 (2017).
222. Hu, B. et al. Tomatidine suppresses osteoclastogenesis and mitigates estrogen deficiency-induced bone mass loss by modulating TRAF6-mediated signaling. PASEB J 33, 2574–2586 (2019).
223. Castrejón-Jíménez, N. S. et al. Urolf and oleic acids induce mitophagy in a549 human lung cancer cells. Molecules 24, 3444 (2019).
224. Wang, J. et al. Small molecule natural compound agonist of SIRT3 as a therapeutic target for the treatment of intervertebral disc degeneration. Exp. Mol. Med. 50, 144 (2018).
225. Pillai, B. & Bhansali, S. Intractable blocks and reverses cardiac hypertrophy in mice by activating mitochondrial Sirt3. Nat. Commun. 6, 6656 (2015).
226. Tang, P. et al. Honokiol alleviates the degeneration of intervertebral disc via suppressing the activation of TNXNP-NLRP3 inflammasome signalling pathway. Free Radic. Biol. Med. 120, 368–379 (2018).
227. Qiao, H. et al. Liraglutide repairs the infarcted heart: The role of the SIRT1/Parkin/mitophagy pathway. Mol. Med. Rep. 17, 3722–3734 (2018).
228. Zhu, H. et al. The neuroprotection of liraglutide against ischaemia-induced apoptosis through the activation of the PI3K/AKT and MAPK pathways. Sci. Rep. 8, 1–11 (2016).
229. Zhang, Y. et al. Liraglutide protects cardiac microvascular endothelial cells against hypoxia/reoxygenation injury through the suppression of the SR-Ca2+-XO-ROS axis via activation of the GLP-1R/PI3K/Akt/survivin pathways. Free Radic. Biol. Med. 95, 278–292 (2016).
230. Liu, W. et al. Neuroprotective effects of lissinidate and ligrudine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson’s disease. Neuroscience 303, 42–50 (2015).
231. Masaldan, S., Callegari, S. & Dewson, G. Therapeutic targeting of mitophagy in Parkinson’s disease. Biochem. Soc. Trans. 50, 783–797 (2022).