Mitophagy Modulators

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Introduction

Mitochondria, the semi-autonomous "powerhouses" of the cell, have a vital role in cellular homeostasis. Mitochondria have α-proteobacterial ancestors and during the course of evolution they assimilated into the eukaryotic cell. The merge between these two very different single cell organisms was possible because the prokaryotic cell presented a source for excess amount of energy for the host cell (Pickles et al., 2018). Since then this organelle has evolved not only to produce ATP through oxidative phosphorylation, but it is also indispensable for precursor synthesis of fatty acids, amino acids and ribonucleotides. Moreover, mitochondria are in the center of several signaling pathways regulating calcium homeostasis, innate immunity and cell death (Aon and Camara, 2015). However, functionally impaired mitochondria are a source of ROS (reactive oxygen species) production and heteroplasmy, due to mutations in the mitochondrial DNA (mtDNA) pool. These discrepancies can be the reason behind numerous diseases, like diabetes, cancer and neurodegenerative disorders (Silzer and Phillips, 2018; Vyas et al., 2016; Zsurka and Kunz, 2015). Therefore, several processes have evolved to ensure the proper surveillance of this organelle.

The first line of defense against mitochondrial damage accumulation is the fusion of individual mitochondria into an interconnected network, in order to "dilute" smaller, singular defects. When the damage is expanding, the cell will activate a nuclear encoded stress response, termed mitochondrial unfolded protein response (UPR^{mt}). The last attempt to protect the cell and promote its survival is the removal of irreparable mitochondria through a specific, mitochondria targeted autophagy, known as mitophagy (Tian et al., 2016; Twig and Shirihai, 2011). Autophagy is a conserved, "self-eating" process, which ensures the clearance of damaged macromolecules and organelles from the cell. Three major types of autophagy have been characterized so far: macroautophagy (hereafter referred to as autophagy), microautophagy and chaperone-mediated autophagy; mitophagy belongs to the first category, macroautophagy. The mechanism of autophagy has been reviewed in detail elsewhere (Dikic, 2017). Briefly, it requires the formation of a double membrane, called phagophore, which engulfs part of the cytoplasm or whole organelles and by closing in itself creates the autophagosome. This autophagosome will have to fuse with the lysosome for degradation to take place in the now called autolysosome. The building blocks of degraded macromolecules will be recycled by the cell (Dikic, 2017). Selective types of autophagy, like mitophagy, require receptor proteins that mark organelles for recognition by the autophagic machinery. These receptors interact with LC3 (microtubule-associated protein 1A/1B light chain 3) and GABARAP

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(gamma-aminobutyric acid receptor-associated protein) proteins, which are located on the autophagosome membrane. In this review we summarize the molecular mechanisms regulating mitophagy and our recent understanding about pharmacological modulators of the process to tackle diseases that arise from mitochondrial homeostasis failure.

Molecular Mechanisms of Mitophagy

The PINK1/Parkin Pathway

Among the several mitophagy governing pathways, we have accumulated the most comprehensive knowledge regarding the PINK1 (phosphatase and tensin homolog (PTEN)-induced putative kinase 1) and Parkin mediated pathway, which is specifically important for the clearance of damaged mitochondria from the cell. Mutations occurring in this signaling cascade have been associated with neurodegenerative disorders, like Parkinson's disease, providing another reason why this specific mitochondrial surveillance mechanism is in the focus of basic and pharmacological research.

Under normal conditions PINK1, driven by its MTS (mitochondrial targeting signal), is transported to the mitochondria where it spans across the outer (OMM) and inner mitochondrial membrane (IMM). Once in the inner membrane, PINK1 goes through two catalytic cleavages by the matrix processing peptidase (MPP) and the presenilin associated rhomboid-like protease (PARL). As a result, PINK1 is no longer anchored to the mitochondria and is degraded in the cytoplasm, following the N-end rule pathway (Sekine and Youle, 2018). However, studies on this truncated, cytoplasmic form of PINK1, called PINK1-53, yielded controversial results. On one hand, it was shown to bind to Parkin and inhibit its function, therefore to be a suppressor of mitophagy (Fedorowicz et al., 2014). On the other hand, it was demonstrated that PINK1-53 can be stabilized through K63 linked ubiquitination and promote mitophagy of healthy mitochondria, thereby clearing mitochondria before any damage could accumulate (Lim et al., 2015). Notably, these observations were made by using immortal cell lines and overexpression of PINK1-53, raising the possibility that in physiological conditions PINK1-53 has another, yet undetermined function.

Changes in mitochondrial membrane potential are perturbing the localization of PINK1, causing its accumulation in the OMM. Here, PINK1 undergoes dimerization and self-phosphorylation which is crucial for its activation (Sekine and Youle, 2018). Damaged mitochondria will also lead to the stalling of translation on the OMM. Consequently, co-translational quality control proteins are recruited to the OMM to activate PINK1 and trigger mitophagy (Wu et al., 2018). Activated PINK1 recruits an E3 ubiquitin ligase, Parkin, which resides in an auto-inhibitory state in the cytoplasm under normal conditions. Parkin induction requires phosphorylation of the protein on Ser65 and additionally the binding of phosphorylated ubiquitin (Harper et al., 2018). Recently, crystal structure and mass spectrometry analyses revealed that phospho-ubiquitin binds to the core of Parkin, causing large scale domain rearrangements and catalyzing the activation of the protein (Gladkova et al., 2018). Importantly, this study also showed that mutations in the binding site for phospho-ubiquitin are associated with the onset of Parkinson's disease. Targeting this binding site to achieve Parkin activation could lead to the development of new therapeutic drugs (Gladkova et al., 2018). Activated Parkin generates polyubiquitin chains on OMM proteins, which are important for the function of mitochondria (e.g. mitofusin 1/2, TOM 20/40/70, DRP1, Miro) (Harper et al., 2018). Parkin is able to produce polyubiquitin chains linked via K6, K11, K27, K48 and K63. While the role of each ubiquitin linkage is not clearly defined up to date, we know that all the aforementioned linkage type is required for a successful mitophagy induction (Zimmermann and Reichert, 2017).

As a consequence of ubiquitin chain accumulation on the surface of dysfunctional mitochondria, proteasomes and autophagy adaptor proteins are locally recruited (Fig. 1). Whether proteasome activation is necessary for the effective execution of mitophagy still remains elusive (Chan et al., 2011; Yoshii et al., 2011). Nonetheless, a recent study supports the notion that proteasome function and the degradation of outer mitochondrial membrane proteins is required for the rupture of the OMM and the exposure of prohibitin 2. Prohibitin 2 was characterized as a new mitophagy receptor, which interacts with LC3 via its LIR (LC3 interacting region) motif and facilitates mitophagy. However, the PINK1/Parkin pathway is essential for the initiation of this mode of elimination (Wei et al., 2017).

The role of adaptor proteins is to bridge the gap between the ubiquitinated mitochondrial surface and LC3 or GABARAP. There are several known adaptor proteins to date, such as p62/SQSTM-1 (sequestosome 1), TAX1BP1 (Tax1-binding protein 1), NDP52 (nuclear dot protein 52), OPTN (optineurin) and NBR1 (neighbor of BRCA1 gene 1). p62 is widely used by the cell as an adaptor protein, however, its role in mitophagy is questionable (Narendra et al., 2010). On the other hand, p62 expression was recently shown to be upregulated through the Nrf2 (nuclear factor E2-related factor 2) and TFEB (transcription factor EB) pathway during mitophagy, raising more questions about its exact function in this process (Ivankovic et al., 2016). Another study identified two adaptor proteins to be essential for mitophagy: OPTN and NDP52 (Fig. 1). These two proteins bind to LC3 in a Parkin independent manner and their role is partially redundant (Lazarou et al., 2015). Mitophagy can be further enhanced by TBK1 (TANK binding kinase 1). TBK1 is able to phosphorylate OPTN, p62, NDP52 and TAX1BP1 promoting their ubiquitin binding and mitophagy (Harper et al., 2018). This signal enhancement ensures the proper removal of harmful, damaged mitochondria from the cell.

Mitophagy can be inhibited by particular deubiquitylating (DUB) enzymes, called ubiquitin-specific proteases (USPs). USP8 is responsible for the deubiquitination of Parkin, so it represses mitophagy in the early stages. Another three USPs namely, USP15, USP30 and USP35 antagonize the mitophagy process through the removal of Parkin-generated polyubiquitin chains from the OMM (Harper et al., 2018). Recently, another player has been identified in the regulation of PINK1/Parkin pathway. Notably, a longer isoform of PTEN, PTEN-L, localizes on the OMM and its function is to counteract the activation of mitophagy. PTEN-L dephosphorylates ubiquitin as well as Parkin, thereby preventing its mitochondrial translocation and accumulation, causing the

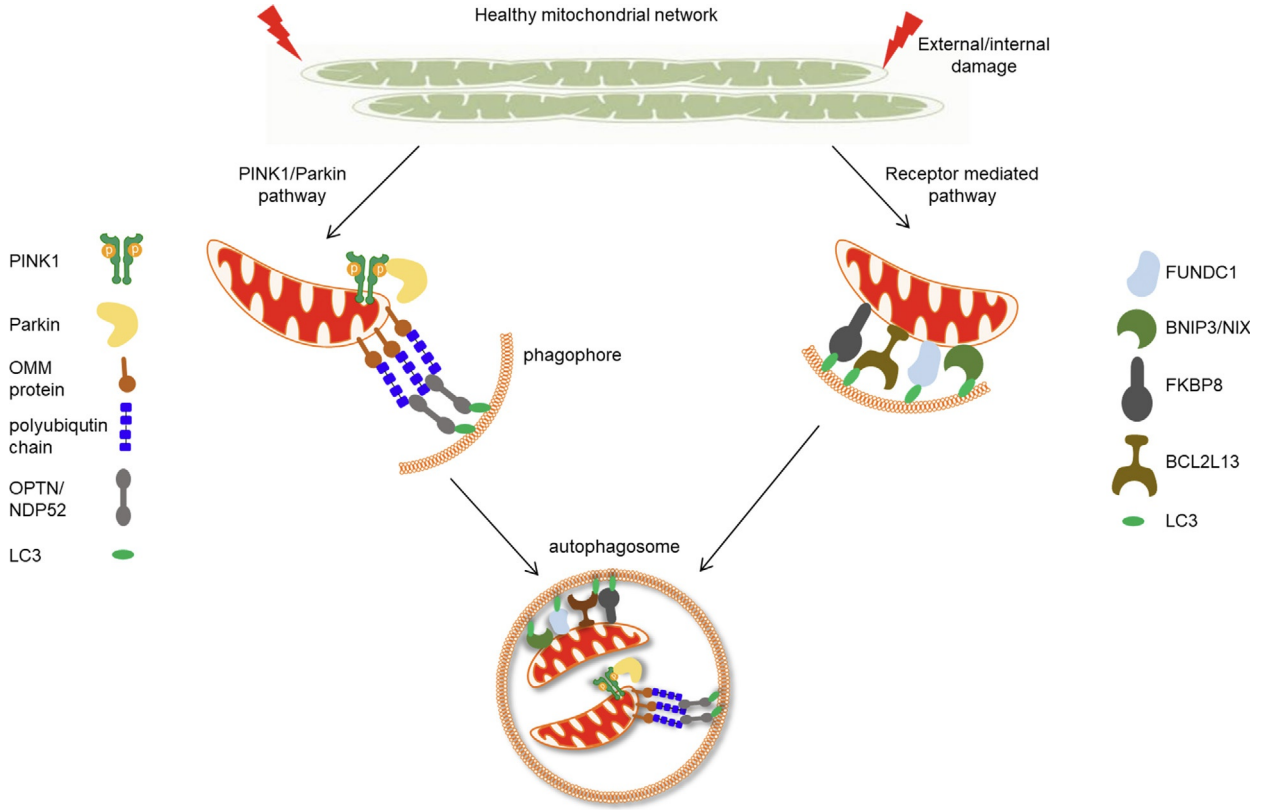

Fig. 1 Overview of mitophagy pathways. Damaged mitochondria are cleared from the cell via two major mitophagy pathways, the PINK1/Parkin and the receptor-mediated pathway. During the PINK1/Parkin pathway, PINK1 is stabilized in the outer mitochondrial membrane (OMM) and recruits the E3 ubiquitin ligase, Parkin. In turn, Parkin polyubiquitinates several OMM proteins. The polyubiquitin chains are recognized by adapter proteins, like OPTN and NDP52, which are interacting with LC3, ensuring the proper sequestration of damaged mitochondrion into the phagophore. Mitophagy receptors, like FUNDC1 or BNIP3/NIX harbor LIR motifs (LC3 interacting region), making them capable to have direct interaction with LC3 and the phagophore. Damaged mitochondria will end up in a closed double membrane, called autophagosome, and after fusing with lysosomes, they will be eliminated from the cell.

suppression of mitochondrial specific autophagy (Wang et al., 2018). These studies highlight the importance of tight regulation of the labeling of mitochondria for lysosomal degradation.

Receptor Mediated Mitophagy

BNIP3/NIX (BNIP3L) mitophagy receptor pathway

BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3) and NIX (Nip3 like protein X) were originally described as regulators of cell death. Both proteins localize on the OMM and harbor LIR motif through which they interact with LC3 and GABARAP (Hamacher-Brady and Brady, 2016).

BNIP3 was shown to be involved specifically in hypoxia induced mitophagy. Furthermore, it initiates the mitochondrial translocation of DRP1 and Parkin in cardiomyocytes, which is required for mitochondrial elimination (Lee et al., 2011). BNIP3 can also interact with PINK1 to stabilize it in the OMM and facilitate mitophagy, proving a tight connection between receptor mediated and the PINK1/Parkin pathway during mitochondrial degradation. The same way, NIX also intertwines the two aforementioned signaling pathways: it promotes the mitochondrial localization of Parkin and in the same time it is also a substrate of Parkin (Zimmermann and Reichert, 2017). While NIX was initially associated to mitophagy in reticulocytes, where its deficiency caused anemia due to uncontrolled mitochondrial accumulation and poor survival of red blood cells; recently its crucial role in retinal ganglion cell development and macrophage polarization have been discovered (Sandoval et al., 2008; Schweers et al., 2007). NIX induced mitophagy was a prerequisite for a hypoxia induced glycolytic switch during the maturation of retinal ganglion cells and macrophage stimulation (Esteban-Martinez et al., 2017).

FUNDC1-mediated mitophagy

FUNDC1 (FUN14 domain containing 1) is another OMM protein, acting as a mitophagy receptor by interacting with LC3 specifically upon hypoxic conditions (Fig. 1). Under normal conditions FUNDC1 is phosphorylated on Ser13 by the kinase CK2 (casein kinase II) and this modification serves as an inhibitory signal. The dephosphorylation of Ser13 by PGAM5

(phosphoglycerate mutase 5) allows for the recruitment of LC3 and induction of mitophagy (Hamacher-Brady and Brady, 2016). FUNDC1 also controls mitochondrial dynamics during hypoxia through the interaction with DRP1 (dynamin-related protein 1) and OPA1 (optic atrophy protein 1). Consequently, ULK1 (Unc-51 like autophagy activating kinase 1) is recruited to globular, fragmented mitochondria where it phosphorylates FUNDC1 at another residue, Ser17 to induce mitophagy (Zimmermann and Reichert, 2017). The structural consequences of these two phosphorylation events were shown in a recent work. Although the two serine residue is located close to each other, they are causing significantly different, large scale rearrangements in the structure of the protein (Lv et al., 2017).

Recently identified mitophagy receptor proteins

There are few not well studied and recently described mitophagy receptors, such as prohibitin 2 (as discussed earlier), BCL2L13, FKBP8 and AMBRA1 (Fig. 1). The very first mitophagy receptor, Atg32, was identified in yeast and until recently there was no knowledge about its mammalian ortholog (Murakawa et al., 2015). A very recent study successfully characterized BCL2L13 (Bcl2 like protein 13) as the mammalian counterpart of Atg32. Similar to other mitophagy receptors, BCL2L13 is an OMM protein and possesses a LIR motif. Overexpression of BCL2L13 caused mitochondrial fragmentation and subsequently mitophagy induction. This pathway acts at least partly independent from the PINK1/Parkin pathway since mitochondrial stress failed to induce mitophagy in cells lacking BCL2L13. Furthermore, BCL2L13 was able to complement for the loss of Atg32 in yeast, showing the functional conservation of the protein (Murakawa et al., 2015).

FKBP8 (FK 506 binding protein 8) was characterized as a noncanonical FKBP and peptidyl-prolyl-cis-trans isomerase (PPIase) with an antiapoptotic role. Upon overexpression of FKBP8, mitophagy was induced independently of Parkin. Contrarily to other mitophagy receptors, FKBP8 seems not to be regulated by phosphorylation due to the lack of appropriate residue near its LIR motif (Bhujabal et al., 2017).

AMBRA1 contains a LIR motif and is able to induce PINK1/Parkin-mediated mitophagy. Moreover, an artificially mitochondria localized AMBRA1 induced mitophagy under nonstress conditions independently of PINK1 or Parkin function. Under these conditions, OMM proteins were ubiquitinated and p62 was not required for mitophagy (Strappazzon et al., 2015). Furthermore, it was demonstrated that in SH-SY5Y neuroblastoma cells, the mitochondria-anchored AMBRA1 plays a protective role against oxidative stress, ROS generation and promotes mitochondrial clearance (Di Rita et al., 2018). Although these results were obtained using an engineered protein, specifically targeted to mitochondria, it still indicates the therapeutic potential of this mitophagy receptor. However, its exact mechanism of action needs to be further investigated under normal conditions.

Pharmacological Modulation of Mitophagy

Impaired mitochondrial homeostasis and uncontrolled accumulation of dysfunctional organelles are common denominators of diverse age-associated pathologies (Palikaras et al., 2017). Recently, pharmacological screenings are taking place to identify novel chemical modulators that might be used to promote the efficient removal of damaged mitochondria and restore the energetic status of the cell. Thus, targeting the mitophagy process selectively could be a potential therapeutic strategy to counteract age-related disorders, which are characterized by defective energy metabolism. To this direction, several synthetic chemical agents and natural occurring compounds have been identified to regulate mitochondrial elimination and subsequently improve cellular and organismal fitness (Palikaras et al., 2017; Georgakopoulos et al., 2017).

General Mitochondrial Toxicants

Proton ionophores are the most commonly used mitophagy inducing agents to date. These chemical compounds are able to cross the inner mitochondrial membrane (IMM) and influence dramatically mitochondrial metabolism. Specifically, they disrupt the electrochemical proton gradient interfering with the electron transport chain (ETC) components and subsequently leading to the impairment of oxidative phosphorylation. Thus, mitochondrial elimination is promoted since the majority of the organelles are extensively damaged.

Mitochondrial uncouplers, such as carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) and carbonyl cyanide-*p*-(trifluoromethoxy)phenylhydrazone (FCCP), have been widely used to induce mitochondrial removal via the activation of the PINK1/Parkin pathway (Narendra et al., 2010; Gatliff et al., 2014; Narendra et al., 2008). Both CCCP and FCCP have been extensively applied to stimulate mitochondrial degradation in mammalian cells and/or living organisms resulting in the identification and the characterization of several factors that participate in the process of mitophagy (Narendra et al., 2008; Palikaras et al., 2015; Whitworth and Pallanck, 2017).

Although there is widespread application of proton ionophores in biological research, several unwanted properties of these chemical agents highlight the importance to limit their use. Particularly, their effect on the entire mitochondrial population leading to dissipation of mitochondrial membrane potential, cellular energetic crisis and eventually to cell death (Wang et al., 2012). Thus, their chronic supplementation could even result in the complete degradation of cellular mitochondrial content (Narendra et al., 2008). Furthermore, their protonophoric activity is not specific to mitochondrial membrane leading to several off-target effects due to their perturbations in plasma membrane, lysosomal function, ion channel stimulation and cytoskeleton homeostasis (Georgakopoulos et al., 2017). These undesired characteristics interfere with the evaluation of mitochondrial metabolism and

limit the therapeutic index of ionophores against mitochondrial related diseases. A recent small molecule chemical library screening identified (2-fluorophenyl) {6-[(2-fluorophenyl) amino] (1,2,5-oxadiazolo[3,4-*e*]pyrazin-5-yl)} amine, called BAM15, as a novel mitochondrial uncoupling agent (Kenwood et al., 2014). Interestingly, BAM15 supplementation increases mitochondrial respiration rate exhibiting equal potency to FCCP and presents enhanced selectivity for mitochondria without depolarizing plasma membrane (Kenwood et al., 2014). Moreover, the reduced cytotoxicity and the resistance to acute renal ischemic-reperfusion injury in mice upon BAM15 treatment underline the therapeutic capacity of this novel chemical compound against mitochondrial dysfunction. However, BAM15 mitophagy-inducing abilities have not yet been monitored and further investigation is needed toward this direction.

In addition to proton ionophores, several oxidative stress inducers have been reported as potent mitophagy mediators, including paraquat, rotenone, 6-hydroxyldopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) among others (Georgakopoulos et al., 2017; Palikaras et al., 2015; Chu et al., 2013; Dagda et al., 2008; Zhu et al., 2012). All these chemical compounds trigger the excessive generation of reactive oxygen species (ROS) leading to mitochondrial damage and eventually to organelle degradation via mitophagy. Although they share common mitophagic response, these stressors stimulate mitochondrial elimination through diverse molecular mechanisms. Paraquat triggers increased complex I-dependent superoxide generation promoting mitochondrial depolarization and subsequent activation of the PINK1/Parkin pathway (Fig. 2) (Narendra et al., 2010; Narendra et al., 2008; Palikaras et al., 2015). On the contrary, PINK1-mediated mitophagy could not be induced in neuronal cells supplemented with rotenone or 6-OHDA due to the mild effect of these agents on mitochondrial membrane potential (Chu et al., 2013). Interestingly, both rotenone and 6-OHDA stimulate cardiolipin release from mitochondrial intermembrane space, thereby recruiting autophagic machinery through its direct association with LC3 autophagosomal membrane protein (Fig. 2) (Chu et al., 2013). Furthermore, extracellular-signal regulated kinase 2 (ERK2) is phosphorylated and recruited on mitochondrial surface facilitating autophagosomal sequestration and removal of defective organelles upon 6-OHDA and MPTP treatment (Dagda et al., 2008; Zhu et al., 2012).

Iron Chelators

Proper mitochondrial function assures cellular iron homeostasis since the three major iron utilization pathways, iron storage, heme and iron sulfur cluster biosynthesis, are taking place in mitochondria (Chen and Paw, 2012). Recent studies suggest that iron-depleted conditions result in mitophagy stimulation in several model organisms, including yeast, nematodes and mice (Allen et al., 2013; Schiavi et al., 2015; McWilliams et al., 2018; Nagi et al., 2016).

The iron chelator deferiprone (DFP) is reported to promote mitochondrial removal without influencing mitochondrial membrane potential. Interestingly, DFP-mediated mitophagy does not require the activation of Parkin indicating that it could be used to promote mitochondrial turnover in cells with defective PINK1/Parkin pathway (Fig. 2) (Allen et al., 2013; Kondapalli et al., 2012). In contrast to DFP, 1,10′-phenanthroline (Phen) and ciclopirox olamine, which are siderophore-like chemical agents, trigger dissipation of mitochondrial membrane and DRP1-dependent mitochondrial fragmentation leading eventually to mitophagy induction (Kirienko et al., 2015; Park et al., 2012). Furthermore, supplementation of 2′,2-bipyridyl (BP) siderophore shown to promote longevity in *Caenorhabditis elegans* through mitophagy stimulation. BP treatment triggers a hypoxic-like response

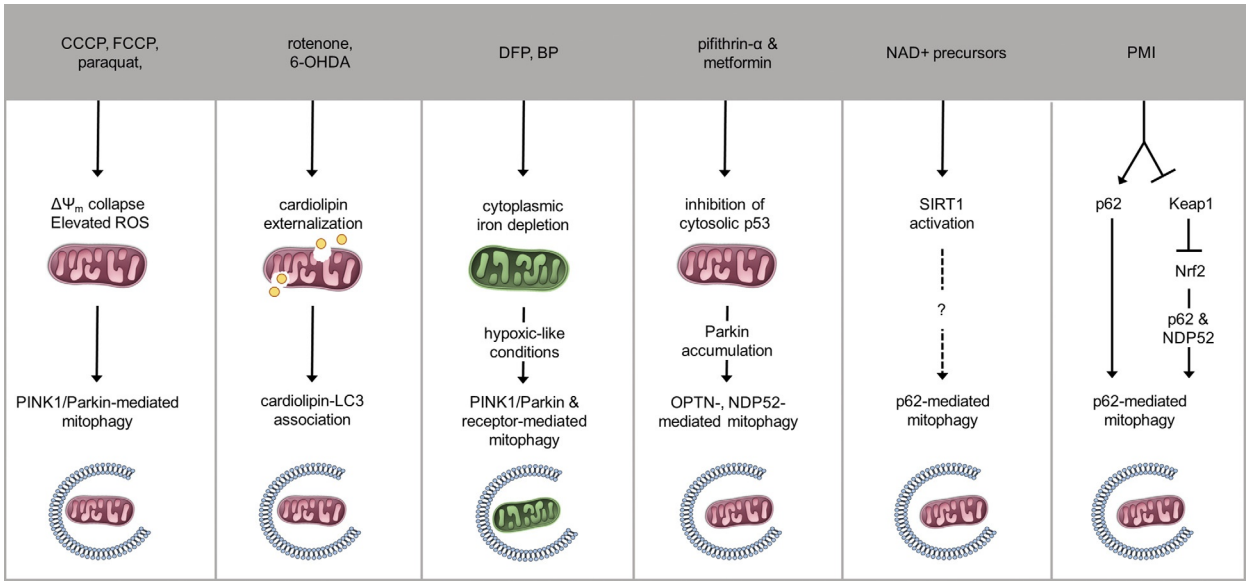

Fig. 2 Overview of synthetic chemical compounds that serves as mitophagy inducers. The molecular mechanisms and mitophagy-inducing abilities of several synthetic chemicals, such as CCCP, FCCP, paraquat, rotenone, 6-OHDA, DFP, BP, NAD^+ precursors and PMI, are presented.

promoting mitochondrial degradation via a PINK-1, PDR-1 and DCT-1 (the homologs of the mammalian PINK1, Parkin and NIX/BNIP3, respectively) dependent mechanism in nematodes (Fig. 2) (Schiavi et al., 2015).

Although several experimental evidence highlight the mitophagy-inducing capacities of iron chelating compounds, further studies are required to dissect the molecular mechanisms of their action. Moreover, the majority of the current knowledge on iron chelators is mainly based on in vitro artificial systems, thus their therapeutic potential and effects on tissue and organismal physiology remain relatively elusive.

Modulating the Activity of the PINK1/Parkin Pathway

Many aspects of mitochondrial homeostasis, including mitochondrial dynamics, biogenesis, bioenergetics, transport and assembly of autophagy apparatus, converge on the PINK1/Parkin pathway to promote the degradation of impaired organelles (Pickles et al., 2018; Harper et al., 2018). Genetic mutations in *PINK1* and *Parkin* genes alter the enzymatic activities of their respective proteins leading to mitophagy defects and eventually to the development and progression of several pathological conditions (Palikaras et al., 2017). Although recent studies have provided novel mechanistic insights into the regulation of the PINK1/Parkin pathway, the list of its pharmacological modulators is very limited.

Kinetin triphosphate (KTP) treatment is the first reported pharmacological approach that was able to enhance PINK1 activity promoting mitophagy upon mitochondrial stress (Hertz et al., 2013). KTP is a N^6 modified ATP analog that binds with higher affinity to PINK1 than its native substrate. Notably, the neosubstrate KTP amplifies PINK1 enzymatic activity both in vitro and in vivo. Moreover, supplementation of KTP triggers Parkin recruitment to damaged mitochondria, decreases mitochondrial motility in neuronal processes and reduces oxidative stress-induced apoptosis in a PINK1-dependent manner (Hertz et al., 2013). Interestingly, KTP was also demonstrated to enhance the kinase activity of the $PINK1^{G309D}$ mutant protein, which is associated with Parkinson's disease (PD) pathogenesis. Kinetin has been successfully used in clinical trials and is shown to cross the blood-brain barrier (Axelrod et al., 2011; Shetty et al., 2011). Despite the fact that these results underline the therapeutic potential of KTP in pathological conditions with impaired PINK1 function, KTP does not influence PINK1 stabilization under normal conditions highlighting the requirement of an external stimulus to initiate mitochondrial removal. Thus, KTP should be further examined in vivo in combination with disease animal models to evaluate its mitophagy-inducing capacities and potent therapeutic activity.

Recent studies revealed an intricate crosstalk between the tumor suppressor p53 protein and the PINK1/Parkin pathway in the regulation of mitophagy (Hoshino et al., 2014; Hoshino et al., 2013; Viotti et al., 2014). It is reported that *Parkin* gene is transcriptionally regulated directly by p53 (Zhang et al., 2011). Additionally, Parkin binds to the promoter sequence of *p53* repressing its expression (Viotti et al., 2014; da Costa et al., 2009). Hence, it seems that there is a complex interplay between Parkin and p53 that is stimulated, at least, in specific cell types to affect mitophagy. Indeed, p53 deficient mice display elevated basal levels of mitophagy compared to wild type animals indicating that p53 serves as a negative regulator of mitochondrial removal (Hoshino et al., 2013). Emerging evidence suggests that cytosolic p53 physically interacts with Parkin disturbing its translocation to mitochondria upon energetic stress (Hoshino et al., 2013). Supplementation of pifithrin-α, a well-known p53 inhibitor, induces Parkin-mediated mitophagy diminishing defective organelles and protecting against glucose tolerance and heart dysfunction (Hoshino et al., 2014; Hoshino et al., 2013). Interestingly, p53 protein levels are increased in multiple brain regions in human PD patients strongly associated with defective mitochondrial homeostasis (Mogi et al., 2007). Notably, pifithrin-α treatment protects against dopaminergic neuronal loss, ameliorates dopamine depletion and improves motor function in PD mouse model (Duan et al., 2002). Similar to pifithrin-α, metformin, the most widely prescribed antidiabetic drug, was also shown to mediate Parkin-dependent mitochondrial clearance through p53 inhibition and mitofusins degradation (Fig. 2) (Song et al., 2016).

Deubiquitinating enzymes (DUBs), such as USP8, USP15, USP30 and USP35, have been recently demonstrated to counteract mitophagy by promoting the hydrolysis of the Parkin-generated ubiquitin chains from the mitochondrial surface (Dikic, 2017; Harper et al., 2018; Bingol et al., 2014). Depletion of USP30 is sufficient to promote mitophagy in Parkin-deficient neuronal cells in mammals and restore motor function and mitochondrial metabolism in *Drosophila melanogaster* models of PD (Bingol et al., 2014). A very recent study presents MF-094 as a novel highly selective inhibitor of USP30 (Kluge et al., 2018). Supplementation of MF-094 in C2C12 myotubes results in decreased levels of mtDNA indicating mitophagy induction. Moreover, 15-oxospiramilactone, a diterpenoid derivative, was identified as a potent inhibitor of USP30 enzymatic activity. Interestingly, 15-oxospiramilactone administration does not affect the total mtDNA and promotes mitochondrial fusion through OPA1, MFN1 and MFN2 dependent mechanism in mouse embryonic fibroblasts (Yue et al., 2014). The mitophagic capacity of both MF-094 and 15-oxospiramilactone has not been evaluated per se in vitro or in vivo demanding further investigation. Since ubiquitination and deubiquitination events are fine-tuned processes and regulate several aspects of cellular homeostasis, their pharmacological modulation could result in off-target effects unrelated to mitophagy. Therefore, the use of DUBs inhibitors as mitophagy-inducing agents is not considered as a promising interventional strategy.

Mitophagy Induction Through the Modulation of NAD^+ Metabolism

Nicotinamide adenine dinucleotide (NAD^+) is characterized as an essential metabolite that regulates cellular metabolism and mitochondrial function by modulating the enzymatic activity of several proteins, including sirtuins and poly ADP-ribose polymerase-1 (PARP-1) among others. Notably, NAD^+/NADH ratio is reduced during aging in multiple organs, such as brain, liver, muscles and adipose tissue (Yoshino et al., 2011; Zhang et al., 2016; Zhu et al., 2015). The imbalance of NAD^+ metabolism has been associated with premature aging and many age-dependent pathologies, such as metabolic disorders and degeneration of

muscular, renal, cardiac and neuronal systems (Katsyuba and Auwerx, 2017; Fang et al., 2017). Thus, the therapeutic potential of NAD^+ boosting interventions has been explored in a wide spectrum of genetic disorders associated with mitochondrial defects (Katsyuba and Auwerx, 2017; Fang et al., 2017).

Supplementation of the NAD^+ precursor molecules, nicotinamide (NAM) and nicotinamide riboside (NR), augments the intracellular NAD^+ levels resulting in lifespan extension and enhanced mitochondrial metabolism (Mouchiroud et al., 2013). Interestingly, NAM is recently demonstrated to trigger mitophagy in mammalian cells (Jang et al., 2012). The beneficial effects of NAD^+ on cellular metabolism and organismal fitness are mediated, at least in part, by sirtuins (Fang et al., 2017). Sirtuins are NAD^+-dependent deacetylases and their enzymatic activity influences multiple cellular processes, including autophagy, mitochondrial metabolism, DNA damage responses and aging among others (Fang et al., 2017). SIRT1 is the most well studied member of the sirtuins family. Depletion of SIRT1 results in accumulation of p62 adaptor protein, excessive mitochondrial damage and increased level of lipid oxidation in multiple tissues, suggesting that SIRT1 could be a potential regulator of mitophagy (Lee et al., 2008). Indeed, NAM administration leads to SIRT1 activation, which in turn promotes mitochondrial removal (Fig. 2) (Jang et al., 2012). Furthermore, genetic inhibition of SIR-2.1 (the SIRT1 mammalian homolog) abolishes the lifespan-extending capacity of NAM in *C. elegans* (Mouchiroud et al., 2013).

In addition to NAD^+ precursor molecules, chemical inhibitors of NAD^+-consuming enzymes, such as PARPs and cyclic ADP-ribose (cADPR) synthases, modulate indirectly SIRT1 activity and could be potential mitophagy inducers. Notably, olaparib, a very well-studied PARP-1 inhibitor, is shown to mediate mitochondrial elimination (Fang et al., 2017; Fang et al., 2016). Although, PARP-1 hyperactivation mediates DNA repair and maintenance of genome integrity during genotoxic stress, its persistent activation diminishes intracellular NAD^+ levels and blocks SIRT1 function, resulting in mitophagy and mitochondrial biogenesis impairment (Fang et al., 2016). Congruently, PME-1 deficiency (the nematode homolog of PARP-1) sustains mitochondrial metabolism and promotes longevity by increasing intracellular NAD^+ concentration and sirtuins activity (Mouchiroud et al., 2013). Furthermore, mitochondrial metabolism and quality control are enhanced upon in vitro and in vivo supplementation of PARP-1 inhibitor, PJ-34 (Bai et al., 2011). Several monoclonal antibodies and chemical agents targeting the cADPR synthase activities of CD38 have been designed and used successfully to increase intracellular NAD^+ levels (Katsyuba and Auwerx, 2017). However, the mitophagic abilities of CD38 inhibitors have not yet been investigated.

Altogether, these results suggest that pharmacological and genetic modulation of intracellular NAD^+ concentration could stimulate the NAD^+−SIRT1 axis, leading to mitophagy induction and subsequent adjustment of mitochondrial content. Although the beneficial effects of NAD^+ boosting approaches have been demonstrated in several organisms and in vivo disease models, their selectivity is questioned, given the pivotal and differential role of NAD^+ in multiple cellular processes, including mitochondrial biogenesis, general autophagy and tumorigenesis among others (Katsyuba and Auwerx, 2017; Fang et al., 2017; Mouchiroud et al., 2013; Lee et al., 2008; Fang et al., 2016).

p62/SQSTM1-Mediated Mitophagy Inducer (PMI)

p62/SQSTM1-mediated mitophagy inducer (PMI) is a synthetic chemical compound that triggers p62-dependent mitochondrial elimination without influencing membrane potential or morphology of mitochondrial network (East et al., 2014). Mechanistically, PMI enhances the expression and signaling of p62/SQSTM1 adaptor molecule promoting engulfment of mitochondria by autophagosomes. Importantly, PMI supplementation could also stimulate mitophagy in PINK1 and Parkin deficient cells, highlighting its therapeutic abilities in conditions associated with defective PINK1/Parkin pathway.

Nrf2 is a very well-studied transcription factor known to orchestrate the regulation of several cytoprotective genes ensuring cellular viability in response to oxidative stress (Holmstrom et al., 2016). In addition to mitophagy, PMI also stabilizes Nrf2 by disrupting its inhibitory association with Keap1 (kelch-like ECH-associated protein 1) (Georgakopoulos et al., 2017). Keap1 serves as a linker mediating the formation of CUL3 (cullin 3)/RBX1 E3 ubiquitin ligase complex, which drives Nrf2 ubiquitination and its subsequent proteasomal degradation (Georgakopoulos et al., 2017). Notably, the mitophagy adaptor proteins, p62 and NDP52, are transcriptionally regulated by Nrf2. PMI-induced mitophagy depends on both p62/SQSTM1 and Nrf2, since mitochondrial sequestration by autophagosomal membranes is diminished in Nrf2 and p62 depleted cells (Fig. 2) (East et al., 2014).

Nrf2 activation has a beneficial effect on mitochondrial function, since it also regulates multiple genes associated with mitochondrial biogenesis (Holmstrom et al., 2016). Thus, Nrf2 stabilization and stimulation could sustain energy metabolism by regulating both mitochondrial removal and generation of newly synthesized organelles. Indeed, SKN-1 (the mammalian homolog of Nrf2) is shown to coordinate a bipartite response promoting both mitophagy and mitochondrial biogenesis in nematodes during oxidative stress (Palikaras et al., 2015). Despite the fact that PMI is a potent mitophagy stimulator, its mitochondrial biogenic capacity has not been evaluated.

Natural Small-Molecules With Mitophagy-Inducing Properties

Maintenance of Mitochondrial Homeostasis by Spermidine-Induced Mitophagy

Polyamines are small organic molecules that influence cellular viability through the modulation of several processes, such as cell growth, proliferation, energy homeostasis and innate immune responses. Thus, the biosynthetic pathways and metabolism of

polyamines are tightly associated with organismal physiology and pathology. Experimental evidence highlights and correlates the gradual decline of polyamines concentration with age and the development of pathological conditions (Markaki et al., 2018). Particularly, the cellular levels of spermidine, which is a polyamine synthesized from putrescine and acts as a precursor molecule of spermine generation, are reduced in many tissues, including thymus, heart, kidney and liver among others, during aging in mammals (Markaki et al., 2018). Hence, the reduced intracellular content of spermidine could be detrimental for organismal homeostasis. Indeed, chronic dietary supplementation of spermidine promotes longevity in several model organisms, including yeast, nematodes, flies and mice (Eisenberg et al., 2009). Interestingly, spermidine stimulates autophagy, which thereby serves as a cytoprotective mechanism to mediate lifespan extension (Eisenberg et al., 2009).

Recently, it was demonstrated that chronic administration of spermidine elicits cardioprotective effects, including reduced cardiac hypertrophy, enhanced diastolic function, diminished arterial stiffness among others, in aged rodents (Eisenberg et al., 2016). Notably, spermidine affects organismal homeostasis in a multi-systemic level, as it triggers autophagy and mitophagy in cardiac muscles, decreases the plasma levels of TNF-α pro-inflammatory cytokine, enhances the intrinsic elasticity of cardiomyocytes as indicated by the increased phosphorylation status of titin (TTN) and improves mitochondrial metabolism in cardiac tissue. Genetic inhibition of autophagy abolishes the beneficial effects of spermidine underlining the pivotal role of autophagy in cardioprotection (Eisenberg et al., 2016). Importantly, ATM (ataxia telangiectasia mutated) kinase was shown to trigger the PINK1/Parkin pathway mediating mitochondrial elimination in response to spermidine in human fibroblasts (Qi et al., 2016). ATM is a master regulator of DNA damage signaling pathway modulating mitochondrial function and promoting mitophagy to sustain cellular homeostasis (Fang et al., 2016). Spermidine elicits mitochondrial membrane dissipation resulting in ATM activation and subsequently recruitment of Parkin on mitochondrial surface (Fig. 3). Conversely, ATM inhibitors reduce PINK1 expression levels, prevent Parkin recruitment and eventually suppress mitophagy in spermidine-treated mammalian cells (Qi et al., 2016). However, it is not investigated, whether the PINK1/Parkin pathway is required for spermidine-mediated cardioprotection.

Altogether, these results suggest that dietary supplementation of spermidine could subsequently improve and/or prolong healthspan in aged individuals through autophagy and mitophagy induction. Several epidemiological studies demonstrate that spermidine-rich diets could protect against age-associated pathologies in humans corroborating the therapeutic potential of spermidine (Eisenberg et al., 2016).

Resveratrol: A Potent Modulator of Mitochondrial Metabolism

Resveratrol is a stilbenoid, a type of natural phenol compound, which is derived from red grapes skin. Resveratrol attracted the attention of scientific community due to its emerging therapeutic properties against multiple human pathologies (Berman et al., 2017). Resveratrol triggers the activation of AMP-protein kinase (AMPK) and subsequently increases the intracellular NAD^+ levels, which in turn stimulate SIRT1 deacetylase activity (Fig. 3) (Canto and Auwerx, 2009).

Resveratrol affects mitochondrial homeostasis by promoting mitochondrial biogenesis and energy metabolism through AMPK-SIRT1-PGC-1α signaling cascade, which restores muscular function and healthspan in obese mice (Palikaras et al., 2017; Markaki et al., 2018; Canto and Auwerx, 2009). Furthermore, mitochondrial bioenergetics and biogenic defects were diminished in neuronal progenitor cells of the Down syndrome mouse model upon resveratrol treatment (Valenti et al., 2016). Similar to spermidine,

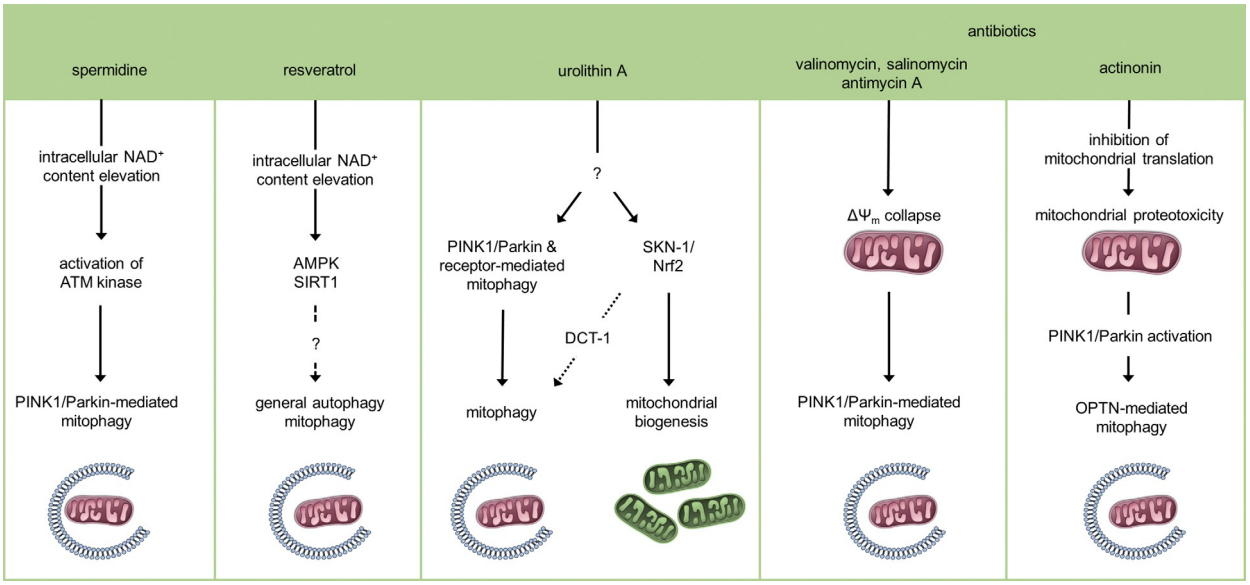

Fig. 3 Natural occurring chemical agents that promote mitochondrial removal. The molecular mechanisms and the mitophagic/biogenic capacities of several natural small-molecules, such as spermidine, resveratrol urolithin A, valinomycin, salinomycin and actinonin, are depicted.

resveratrol also promotes autophagy enhancing longevity and organismal healthspan (Morselli et al., 2010). Interestingly, both natural chemical compounds influence cellular acetylproteome in a similar fashion, whereas they trigger distinct molecular mechanisms to alter mitochondrial activity (Morselli et al., 2011). Although, autophagy is also induced in a SIRT1-dependent manner upon resveratrol supplementation, it seems that mitochondrial removal is a consequence of general autophagy induction rather than a selective cellular response (Morselli et al., 2010; Morselli et al., 2011; Wu et al., 2016).

Urolithin A: First-in Class Mitophagy Enhancer

Urolithins are ellagitannin-derivatives, which are generated by gut microbiota upon their consumption (Espin et al., 2017). Although the mechanistic insights of urolithins activity are not well-defined, several studies demonstrate their benefits on carcinogenesis, inflammation, lipid metabolism and cardiovascular diseases (Palikaras et al., 2017). However, the biosynthetic abilities of these final metabolites from ellagitannins catabolism and their beneficial effects on human health differ considerably among individuals and are associated with the composition of gut microbiota (Espin et al., 2017).

A very recent study demonstrated the pivotal role of urolithins on organismal homeostasis and longevity (Ryu et al., 2016). Interestingly, urolithin A (UA), which is the most abundant metabolite of ellagitannins in the human body, was shown to trigger specifically mitophagy in nematodes and rodents (Ryu et al., 2016). UA-mediated mitophagy sustains mitochondrial respiratory capacity leading eventually to enhanced muscular function. Likewise, chronic supplementation of UA diminishes the age-dependent mobility defects and pharyngeal pumping decline in *C. elegans*, while improving energy metabolism (Ryu et al., 2016). The beneficial effects of UA on muscle cells physiology are independent of dietary conditions and age (Ryu et al., 2016). These results underline the therapeutic potential of UA, which could be used as a novel intervention approach to restore energy metabolism improving muscular function and mobility in the elderly (Palikaras et al., 2017). Furthermore, lifespan-extending properties of UA depend on BEC-1, PINK-1, SQST-1 and DCT-1 components (the mammalian homologs of Beclin 1, PINK1, p62/SQSTM1 and BNIP3/NIX, respectively) indicating that several regulatory mechanisms of mitophagy are interconnected in the maintenance of mitochondrial function and organismal fitness in response to UA treatment (Ryu et al., 2016). In addition to mitochondrial elimination, UA could also stimulate mitochondrial biogenesis probably through SKN-1 activation. Indeed, long term UA supplementation promotes longevity in a SKN-1-dependent manner and boosts mitochondrial biogenesis to preserve mitochondrial population and activity (Ryu et al., 2016). Therefore, UA might coordinate mitochondrial biogenesis and mitophagy in a dose- and time-dependent manner to surveil and maintain energy metabolism (Fig. 3).

The Use of Antibiotics in Mitophagy Induction

Billion years ago mitochondria were generated as a result of an endosymbiotic event. Although during evolution most of the genome of endosymbiont was transferred to the nucleus of the host cell, mitochondrion still contains remnants of its ancestral genome. Consequently, mitochondria share several morphological, molecular and biochemical features with their prokaryotic ancestors (Richter et al., 2013). Thus, mitochondrial function could be deregulated by antibiotics overuse resulting in mitonuclear imbalance, energy metabolism collapse and eventually disease development and progression (Moullan et al., 2015; Stefano et al., 2017).

Several antibiotics have been used in certain concentrations to mediate mitophagy. Valinomycin and salinomycin disturb differentially mitochondrial potassium (K^+) homeostasis leading to organelle dysfunction and initiation of the PINK1/Parkin pathway (Fig. 3) (Georgakopoulos et al., 2017; Allen et al., 2013). Furthermore, antimycin A, which acts as an inhibitor of the respiratory complex III, promotes excessive generation of mitochondrial ROS leading eventually to mild membrane depolarization. It is demonstrated that the activation of F_1F_0-ATP synthase restores membrane potential upon antimycin A treatment (Georgakopoulos et al., 2017). Therefore, oligomycin, a F_1F_0-ATP synthase inhibitor, is often used in combination with antimycin A to enhance mitochondrial defects and thereby promote mitophagy via the PINK1/Parkin pathway (Lazarou et al., 2015; Allen et al., 2013). Actinonin is an antibacterial compound that blocks cancer cells proliferation by interfering with mitochondrial translation machinery. Interestingly, actinonin supplementation gradually depletes the ribosomes, ribosomal RNA and mRNAs of mitochondria resulting in organelle proteotoxicity (Richter et al., 2013). Recent evidence indicates that mitochondrial elimination is triggered in response to actinonin (Burman et al., 2017; Sun et al., 2015). Indeed, PINK1 is stabilized and recruits Parkin onto the mitochondrial surface, which in turn interacts with ubiquitin, optineurin and LC3 autophagosomal protein to mediate organelle degradation in response to actinonin (Fig. 3) (Burman et al., 2017). Tetracycline and/or doxycycline, a second-generation tetracycline derivative, have been extensively used in the development of Tet-on/Tet-off expression systems in several model organisms (Moullan et al., 2015). Surprisingly, both antibiotics trigger mitonuclear imbalance through their inhibitory effect on mitochondrial translation. Low concentrations of tetracycline influences nuclear gene expression levels, mitochondrial morphology and proteostasis in nematodes, flies, plants and mice (Moullan et al., 2015). Moreover, a very recent study demonstrated that mitophagy is also induced upon doxycycline administration (Xing et al., 2017).

Although scientists should be cautioned against the broad use of antibiotics in their experimental set-ups due to potential off-target effects, controlled use of antibacterial agents could preserve mitochondrial population health and elicit beneficial effects for cellular and tissue homeostasis. Furthermore, the aforementioned results provide the rationale for future examination of antibiotics in clinical trials as potent mitophagy regulators to establish novel therapeutic strategies against mitochondrial-associated disorders.

Pharmacological Inhibition of Mitophagy

Although, the role of mitophagy in energy metabolism and cellular homeostasis is essential, uncontrolled mitochondrial elimination results in shrinkage of mitochondrial population overstressing the remaining organelles and triggering cell death pathways (Kubli and Gustafsson, 2012; Palikaras and Tavernarakis, 2014). Therefore, several chemical agents have been utilized to block the process of mitophagy.

The most widely established approach to prevent mitochondrial degradation system relies on the use of lysosomal inhibitors, including bafilomycin and chloroquine, which could disturb lysosomal acidification and fusion of lysosomes with autophagosomal membranes (Georgakopoulos et al., 2017). Furthermore, general autophagy inhibitors, such as 3-methyladenine, are successfully used to suppress mitophagy. Despite their efficiency, these chemical compounds could lead to off-target effects due to their nonselective function.

Fragmentation of mitochondrial network is a prerequisite event for mitophagy execution (Kubli and Gustafsson, 2012). Thus, an alternative indirect method to prevent mitochondrial removal is through the modulation of mitochondrial fission machinery. Mitochondrial division inhibitor 1 (mdivi-1) is a quinazolinone derivative found to perturb mitochondrial morphology by inhibiting organelles division in yeast and mammalian cells (Cassidy-Stone et al., 2008). Interestingly, mdivi-1 impairs DRP1 enzymatic activity attenuating apoptosis and cell death both in vitro and in vivo (Cassidy-Stone et al., 2008; Grohm et al., 2012). Although the beneficial impact of mdivi-1 treatment is well established in several pathologic conditions, including heart failure, brain injury and neurodegeneration, its inhibitory effect on mitophagy per se has not been evaluated yet (Grohm et al., 2012; Rappold et al., 2014; Wu et al., 1630; Ong et al., 2010).

A recent study demonstrated a novel approach to target mitophagy selectively by using a peptide inhibitor. This specific peptide was engineered to interact directly with the LIR motif of FUNDC1 mitophagy receptor suppressing FCCP-induced mitophagy (Chen et al., 2014). Interestingly, general autophagy was unaffected indicating the selectivity of the current method. Indeed, this LIR mimetic peptide inhibits mitochondrial removal via disrupting the association between FUNDC1 and LC3 autophagosomal membrane protein reinforcing the rationale for investigating mimetic peptides to manipulate mitophagy (Chen et al., 2014).

Concluding Remarks

The investigation of mitophagy has been promoted to the forefront of scientific research in the last decade. Impaired elimination of dysfunctional mitochondria has been identified as a hallmark feature of many human pathological conditions, including neurodegenerative, cardiovascular, autoimmune, metabolic disorders and carcinogenesis among others (Palikaras et al., 2017). Mounting in vitro and in vivo experimental evidence also indicates that mitophagy regulation could be a potential therapeutic intervention strategy in the treatment of several mitochondrial related diseases (Fig. 4). In this framework, pharmacological screenings and development of novel synthetic chemical agents have been in the spotlight of related research.

In spite of the recent advances in the understanding of mechanistic details of mitophagy molecular pathways, important questions still remain obscure regarding the in vivo role of mitophagy components within distinct physiological and pathological contexts. Despite the promising results obtained by the use of synthetic and natural chemical compounds on energy metabolism, mitophagy stimulation per se has not been evaluated in vivo yet. The majority of the current knowledge on mitophagy mechanisms is mainly based on in vitro artificial systems. Therefore, the use of transgenic animal models for in vivo mitophagy monitoring, such as mito-QC and mito-Keima expressing mice, mito-Rosella expressing yeast and nematodes, will revolutionize the progress in understanding the complex interplay between mitophagy pathways (Palikaras et al., 2015; McWilliams et al., 2018; Sun et al., 2015; Mijaljica et al., 2011). Unraveling the spatiotemporal regulation of mitophagy in different cellular populations utilizing primary mammalian cells and/or animal models will lead to an unprecedented understanding of mitochondrial degradation mechanisms. Furthermore, the combination of in vivo mitophagy imaging systems with disease animal models will shed light on disease etiology and progression boosting translational research. In upcoming years, the impact of mitophagy modulators on human physiology should be evaluated through clinical trials investigating their therapeutic efficiency against mitochondria-related pathologies.

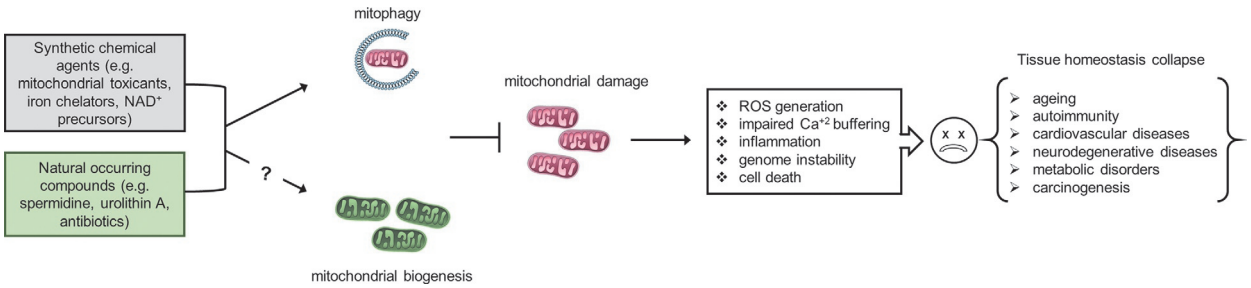

Fig. 4 Novel therapeutic intervention strategies based on chemical modulators of mitophagy. Supplementation of chemical agents, which promote mitophagy and/or mitochondrial biogenesis, preserve mitochondrial homeostasis resulting in subsequent cellular and organismal survival.

Hence, identification of novel mitophagy regulators might establish novel therapeutic intervention approaches to tackle a wide spectrum of mitochondria-associated disorders and provide critical insights with broad relevance to human health and quality of life (Fig. 4).

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References

Allen GF, Toth R, James J, and Ganley IG (2013) Loss of iron triggers PINK1/Parkin-independent mitophagy. *EMBO Reports* 14(12): 1127–1135.

Aon MA and Camara AK (2015) Mitochondria: hubs of cellular signaling, energetics and redox balance. A rich, vibrant, and diverse landscape of mitochondrial research. *Frontiers in Physiology* (6): 94.

Axelrod FB, Liebes L, Gold-Von Simson G, Mendoza S, Mull J, Leyne M, et al. (2011) Kinetin improves IKBKAP mRNA splicing in patients with familial dysautonomia. *Pediatric Research* 70(5): 480–483.

Bai P, Canto C, Oudart H, Brunyanszki A, Cen Y, Thomas C, et al. (2011) PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metabolism* 13(4): 461–468.

Berman AY, Motechin RA, Wiesenfeld MY, and Holz MK (2017) The therapeutic potential of resveratrol: A review of clinical trials. *NPJ Precision Oncology* 1: 35.

Bhujabal Z, Birgisdottir AB, Sjottem E, Brenne HB, Overvatn A, Habisov S, et al. (2017) FKBP8 recruits LC3A to mediate Parkin-independent mitophagy. *EMBO Reports* 18(6): 947–961.

Bingol B, Tea JS, Phu L, Reichelt M, Bakalarski CE, Song Q, et al. (2014) The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature* 510(7505): 370–375.

Burman JL, Pickles S, Wang C, Sekine S, Vargas JNS, Zhang Z, et al. (2017) Mitochondrial fission facilitates the selective mitophagy of protein aggregates. *The Journal of Cell Biology* 216(10): 3231–3247.

Canto C and Auwerx J (2009) PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Current Opinion in Lipidology* 20(2): 98–105.

Cassidy-Stone A, Chipuk JE, Ingerman E, Song C, Yoo C, Kuwana T, et al. (2008) Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. *Developmental Cell* 14(2): 193–204.

Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL, et al. (2011) Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Human Molecular Genetics* 20(9): 1726–1737.

Chen C and Paw BH (2012) Cellular and mitochondrial iron homeostasis in vertebrates. *Biochimica et Biophysica Acta* 1823(9): 1459–1467.

Chen G, Han Z, Feng D, Chen Y, Chen L, Wu H, et al. (2014) A regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. *Molecular Cell* 54(3): 362–377.

Chu CT, Ji J, Dagda RK, Jiang JF, Tyurina YY, Kapralov AA, et al. (2013) Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nature Cell Biology* 15(10): 1197–1205.

da Costa CA, Sunyach C, Giaime E, West A, Corti O, Brice A, et al. (2009) Transcriptional repression of p53 by parkin and impairment by mutations associated with autosomal recessive juvenile Parkinson's disease. *Nature Cell Biology* 11(11): 1370–1375.

Dagda RK, Zhu J, Kulich SM, and Chu CT (2008) Mitochondrially localized ERK2 regulates mitophagy and autophagic cell stress: Implications for Parkinson's disease. *Autophagy* 4(6): 770–782.

Di Rita A, D'Acunzo P, Simula L, Campello S, Strappazzon F, and Cecconi F (2018) AMBRA1-mediated Mitophagy counteracts oxidative stress and apoptosis induced by neurotoxicity in human neuroblastoma SH-SY5Y cells. *Frontiers in Cellular Neuroscience* 12: 92.

Dikic I (2017) Proteasomal and autophagic degradation systems. *Annual Review of Biochemistry* 86: 193–224.

Duan W, Zhu X, Ladenheim B, Yu QS, Guo Z, Oyler J, et al. (2002) p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism. *Annals of Neurology* 52(5): 597–606.

East DA, Fagiani F, Crosby J, Georgakopoulos ND, Bertrand H, Schaap M, et al. (2014) PMI: A DeltaPsim independent pharmacological regulator of mitophagy. *Chemistry and Biology* 21(11): 1585–1596.

Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, et al. (2009) Induction of autophagy by spermidine promotes longevity. *Nature Cell Biology* 11(11): 1305–1314.

Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, et al. (2016) Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nature Medicine* 22(12): 1428–1438.

Espin JC, Gonzalez-Sarrias A, and Tomas-Barberan FA (2017) The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochemical Pharmacology* 139: 82–93.

Esteban-Martinez L, Sierra-Filardi E, McGreal RS, Salazar-Roa M, Marino G, Seco E, et al. (2017) Programmed mitophagy is essential for the glycolytic switch during cell differentiation. *The EMBO Journal* 36(12): 1688–1706.

Fang EF, Kassahun H, Croteau DL, Scheibye-Knudsen M, Marosi K, Lu H, et al. (2016) NAD(+) replenishment improves lifespan and healthspan in Ataxia telangiectasia models via mitophagy and DNA repair. *Cell Metabolism* 24(4): 566–581.

Fang EF, Lautrup S, Hou Y, Demarest TG, Croteau DL, Mattson MP, et al. (2017) NAD(+) in aging: Molecular mechanisms and translational implications. *Trends in Molecular Medicine* 23(10): 899–916.

Fedorowicz MA, de Vries-Schneider RL, Rub C, Becker D, Huang Y, Zhou C, et al. (2014) Cytosolic cleaved PINK1 represses Parkin translocation to mitochondria and mitophagy. *EMBO Reports* 15(1): 86–93.

Gatliff J, East D, Crosby J, Abeti R, Harvey R, Craigen W, et al. (2014) TSPO interacts with VDAC1 and triggers a ROS-mediated inhibition of mitochondrial quality control. *Autophagy* 10(12): 2279–2296.

Georgakopoulos ND, Wells G, and Campanella M (2017) The pharmacological regulation of cellular mitophagy. *Nature Chemical Biology* 13(2): 136–146.

Gladkova C, Maslen SL, Skehel JM, and Komander D (2018) Mechanism of parkin activation by PINK1. *Nature* 559: 410–414.

Grohm J, Kim SW, Mamrak U, Tobaben S, Cassidy-Stone A, Nunnari J, et al. (2012) Inhibition of Drp1 provides neuroprotection in vitro and in vivo. *Cell Death and Differentiation* 19(9): 1446–1458.

Hamacher-Brady A and Brady NR (2016) Mitophagy programs: Mechanisms and physiological implications of mitochondrial targeting by autophagy. *Cellular and Molecular Life Sciences* 73(4): 775–795.

Harper JW, Ordureau A, and Heo JM (2018) Building and decoding ubiquitin chains for mitophagy. *Nature Reviews. Molecular Cell Biology* 19(2): 93–108.
Hertz NT, Berthet A, Sos ML, Thorn KS, Burlingame AL, Nakamura K, et al. (2013) A neo-substrate that amplifies catalytic activity of Parkinson's-disease-related kinase PINK1. *Cell* 154(4): 737–747.
Holmstrom KM, Kostov RV, and Dinkova-Kostova AT (2016) The multifaceted role of Nrf2 in mitochondrial function. *Current Opinion in Toxicology* 1: 80–91.
Hoshino A, Mita Y, Okawa Y, Ariyoshi M, Iwai-Kanai E, Ueyama T, et al. (2013) Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. *Nature Communications* 4: 2308.
Hoshino A, Ariyoshi M, Okawa Y, Kaimoto S, Uchihashi M, Fukai K, et al. (2014) Inhibition of p53 preserves Parkin-mediated mitophagy and pancreatic beta-cell function in diabetes. *Proceedings of the National Academy of Sciences of the United States of America* 111(8): 3116–3121.
Ivankovic D, Chau KY, Schapira AH, and Gegg ME (2016) Mitochondrial and lysosomal biogenesis are activated following PINK1/parkin-mediated mitophagy. *Journal of Neurochemistry* 136(2): 388–402.
Jang SY, Kang HT, and Hwang ES (2012) Nicotinamide-induced mitophagy: Event mediated by high NAD+/NADH ratio and SIRT1 protein activation. *The Journal of Biological Chemistry* 287(23): 19304–19314.
Katsyuba E and Auwerx J (2017) Modulating NAD(+) metabolism, from bench to bedside. *The EMBO Journal* 36(18): 2670–2683.
Kenwood BM, Weaver JL, Bajwa A, Poon IK, Byrne FL, Murrow BA, et al. (2014) Identification of a novel mitochondrial uncoupler that does not depolarize the plasma membrane. *Molecular Metabolism* 3(2): 114–123.
Kirienko NV, Ausubel FM, and Ruvkun G (2015) Mitophagy confers resistance to siderophore-mediated killing by Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences of the United States of America* 112(6): 1821–1826.
Kluge AF, Lagu BR, Maiti P, Jaleel M, Webb M, Malhotra J, et al. (2018) Novel highly selective inhibitors of ubiquitin specific protease 30 (USP30) accelerate mitophagy. *Bioorganic and Medicinal Chemistry Letters* 28(15): 2655–2659.
Kondapalli C, Kazlauskaite A, Zhang N, Woodroof HI, Campbell DG, Gourlay R, et al. (2012) PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating serine 65. *Open Biology* 2(5): 120080.
Kubli DA and Gustafsson AB (2012) Mitochondria and mitophagy: The yin and yang of cell death control. *Circulation Research* 111(9): 1208–1221.
Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, et al. (2015) The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* 524(7565): 309–314.
Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, et al. (2008) A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proceedings of the National Academy of Sciences of the United States of America* 105(9): 3374–3379.
Lee Y, Lee HY, Hanna RA, and Gustafsson AB (2011) Mitochondrial autophagy by Bnip3 involves Drp1-mediated mitochondrial fission and recruitment of Parkin in cardiac myocytes. *American Journal of Physiology. Heart and Circulatory Physiology* 301(5): H1924–H1931.
Lim GG, Chua DS, Basil AH, Chan HY, Chai C, Arumugam T, et al. (2015) Cytosolic PTEN-induced putative kinase 1 is stabilized by the NF-kappaB pathway and promotes non-selective mitophagy. *The Journal of Biological Chemistry* 290(27): 16882–16893.
Lv M, Wang C, Li F, Peng J, Wen B, Gong Q, et al. (2017) Structural insights into the recognition of phosphorylated FUNDC1 by LC3B in mitophagy. *Protein and Cell* 8(1): 25–38.
Markaki M, Palikaras K, and Tavernarakis N (2018) Novel insights into the anti-aging role of Mitophagy. *International Review of Cell and Molecular Biology* 340: 169–208.
McWilliams TG, Prescott AR, Montava-Garriga L, Ball G, Singh F, Barini E, et al. (2018) Basal mitophagy occurs independently of PINK1 in mouse tissues of high metabolic demand. *Cell Metabolism* 27(2): 439–449. e5.
Mijaljica D, Prescott M, and Devenish RJ (2011) A fluorescence microscopy assay for monitoring mitophagy in the yeast *Saccharomyces cerevisiae*. *Journal of Visualized Experiments* 53: 2779.
Mogi M, Kondo T, Mizuno Y, and Nagatsu T (2007) p53 protein, interferon-gamma, and NF-kappaB levels are elevated in the parkinsonian brain. *Neuroscience Letters* 414(1): 94–97.
Morselli E, Maiuri MC, Markaki M, Megalou E, Pasparaki A, Palikaras K, et al. (2010) Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy. *Cell Death and Disease* 1: e10.
Morselli E, Marino G, Bennetzen MV, Eisenberg T, Megalou E, Schroeder S, et al. (2011) Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. *The Journal of Cell Biology* 192(4): 615–629.
Mouchiroud L, Houtkooper RH, Moullan N, Katsyuba E, Ryu D, Canto C, et al. (2013) The NAD(+)/Sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. *Cell* 154(2): 430–441.
Moullan N, Mouchiroud L, Wang X, Ryu D, Williams EG, Mottis A, et al. (2015) Tetracyclines disturb mitochondrial function across eukaryotic models: A call for caution in biomedical research. *Cell Reports* 10: 1681–1691.
Murakawa T, Yamaguchi O, Hashimoto A, Hikoso S, Takeda T, Oka T, et al. (2015) Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. *Nature Communications* 6: 7527.
Nagi M, Tanabe K, Nakayama H, Ueno K, Yamagoe S, Umeyama T, et al. (2016) Iron-depletion promotes mitophagy to maintain mitochondrial integrity in pathogenic yeast *Candida glabrata*. *Autophagy* 12(8): 1259–1271.
Narendra D, Tanaka A, Suen DF, and Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *The Journal of Cell Biology* 183(5): 795–803.
Narendra D, Kane LA, Hauser DN, Fearnley IM, and Youle RJ (2010) p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy* 6(8): 1090–1106.
Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, and Hausenloy DJ (2010) Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. *Circulation* 121(18): 2012–2022.
Palikaras K and Tavernarakis N (2014) Mitochondrial homeostasis: The interplay between mitophagy and mitochondrial biogenesis. *Experimental Gerontology* 56: 182–188.
Palikaras K, Lionaki E, and Tavernarakis N (2015) Coordination of mitophagy and mitochondrial biogenesis during ageing in *C. elegans*. *Nature* 521(7553): 525–528.
Palikaras K, Daskalaki I, Markaki M, and Tavernarakis N (2017) Mitophagy and age-related pathologies: Development of new therapeutics by targeting mitochondrial turnover. *Pharmacology and Therapeutics*. 178: 157–174.
Park SJ, Shin JH, Kim ES, Jo YK, Kim JH, Hwang JJ, et al. (2012) Mitochondrial fragmentation caused by phenanthroline promotes mitophagy. *FEBS Letters* 586(24): 4303–4310.
Pickles S, Vigie P, and Youle RJ (2018) Mitophagy and quality control mechanisms in mitochondrial maintenance. *Current Biology* 28(4): R170–R185.
Qi Y, Qiu Q, Gu X, Tian Y, and Zhang Y (2016) ATM mediates spermidine-induced mitophagy via PINK1 and Parkin regulation in human fibroblasts. *Scientific Reports* 6: 24700.
Rappold PM, Cui M, Grima JC, Fan RZ, de Mesy-Bentley KL, Chen L, et al. (2014) Drp1 inhibition attenuates neurotoxicity and dopamine release deficits in vivo. *Nature Communications* 5: 5244.
Richter U, Lahtinen T, Marttinen P, Myohanen M, Greco D, Cannino G, et al. (2013) A mitochondrial ribosomal and RNA decay pathway blocks cell proliferation. *Current Biology* 23(6): 535–541.
Ryu D, Mouchiroud L, Andreux PA, Katsyuba E, Moullan N, Nicolet-Dit-Felix AA, et al. (2016) Urolithin a induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents. *Nature Medicine* 22(8): 879–888.
Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, et al. (2008) Essential role for Nix in autophagic maturation of erythroid cells. *Nature* 454(7201): 232–235.
Schiavi A, Maglioni S, Palikaras K, Shaik A, Strappazzon F, Brinkmann V, et al. (2015) Iron-starvation-induced Mitophagy mediates lifespan extension upon mitochondrial stress in *C. elegans*. *Current Biology* 25(14): 1810–1822.

Schweers RL, Zhang J, Randall MS, Loyd MR, Li W, Dorsey FC, et al. (2007) NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proceedings of the National Academy of Sciences of the United States of America* 104(49): 19500–19505.
Sekine S and Youle RJ (2018) PINK1 import regulation; a fine system to convey mitochondrial stress to the cytosol. *BMC Biology* 16(1): 2.
Shetty RS, Gallagher CS, Chen YT, Hims MM, Mull J, Leyne M, et al. (2011) Specific correction of a splice defect in brain by nutritional supplementation. *Human Molecular Genetics* 20(21): 4093–4101.
Silzer TK and Phillips NR (2018) Etiology of type 2 diabetes and Alzheimer's disease: Exploring the mitochondria. *Mitochondrion* pii: S1567-7249(17)30339-2.
Song YM, Lee WK, Lee YH, Kang ES, Cha BS, and Lee BW (2016) Metformin restores parkin-mediated mitophagy, suppressed by cytosolic p53. *International Journal of Molecular Sciences* 17(1): 122.
Stefano GB, Samuel J, and Kream RM (2017) Antibiotics may trigger mitochondrial dysfunction inducing psychiatric disorders. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* 23: 101–106.
Strappazzon F, Nazio F, Corrado M, Cianfanelli V, Romagnoli A, Fimia GM, et al. (2015) AMBRA1 is able to induce mitophagy via LC3 binding, regardless of PARKIN and p62/SQSTM1. *Cell Death and Differentiation* 22(3): 517.
Sun N, Yun J, Liu J, Malide D, Liu C, Rovira II, et al. (2015) Measuring in vivo mitophagy. *Molecular Cell* 60(4): 685–696.
Tian Y, Merkwirth C, and Dillin A (2016) Mitochondrial UPR: A double-edged sword. *Trends in Cell Biology* 26(8): 563–565.
Twig G and Shirihai OS (2011) The interplay between mitochondrial dynamics and mitophagy. *Antioxidants and Redox Signaling* 14(10): 1939–1951.
Valenti D, de Bari L, de Rasmo D, Signorile A, Henrion-Caude A, Contestabile A, et al. (2016) The polyphenols resveratrol and epigallocatechin-3-gallate restore the severe impairment of mitochondria in hippocampal progenitor cells from a down syndrome mouse model. *Biochimica et Biophysica Acta* 1862(6): 1093–1104.
Viotti J, Duplan E, Caillava C, Condat J, Goiran T, Giordano C, et al. (2014) Glioma tumor grade correlates with parkin depletion in mutant p53-linked tumors and results from loss of function of p53 transcriptional activity. *Oncogene* 33(14): 1764–1775.
Vyas S, Zaganjor E, and Haigis MC (2016) Mitochondria and cancer. *Cell* 166(3): 555–566.
Wang Y, Nartiss Y, Steipe B, McQuibban GA, and Kim PK (2012) ROS-induced mitochondrial depolarization initiates PARK2/PARKIN-dependent mitochondrial degradation by autophagy. *Autophagy* 8(10): 1462–1476.
Wang L, Cho YL, Tang Y, Wang J, Park JE, Wu Y, et al. (2018) PTEN-L is a novel protein phosphatase for ubiquitin dephosphorylation to inhibit PINK1-Parkin-mediated mitophagy. *Cell Research*. 28(8): 787–802.
Wei Y, Chiang WC, Sumpter R Jr., Mishra P, and Levine B (2017) Prohibitin 2 is an inner mitochondrial membrane mitophagy receptor. *Cell* 168(1–2): 224–238. e10.
Whitworth AJ and Pallanck LJ (2017) PINK1/Parkin mitophagy and neurodegeneration-what do we really know in vivo? *Current Opinion in Genetics and Development* 44: 47–53.
Wu Q, Xia SX, Li QQ, Gao Y, Shen X, Ma L, et al. (1630) Mitochondrial division inhibitor 1 (Mdivi-1) offers neuroprotection through diminishing cell death and improving functional outcome in a mouse model of traumatic brain injury. *Brain Research* 2016: 134–143.
Wu J, Li X, Zhu G, Zhang Y, He M, and Zhang J (2016) The role of resveratrol-induced mitophagy/autophagy in peritoneal mesothelial cells inflammatory injury via NLRP3 inflammasome activation triggered by mitochondrial ROS. *Experimental Cell Research* 341(1): 42–53.
Wu Z, Wang Y, Lim J, Liu B, Li Y, Vartak R, et al. (2018) Ubiquitination of ABCE1 by NOT4 in response to mitochondrial damage links co-translational quality control to PINK1-directed mitophagy. *Cell Metabolism*. 28(1): 130–144. e7.
Xing Y, Liqi Z, Jian L, Qinghua Y, and Qian Y (2017) Doxycycline induces Mitophagy and suppresses production of interferon-beta in IPEC-J2 cells. *Frontiers in Cellular and Infection Microbiology* 7: 21.
Yoshii SR, Kishi C, Ishihara N, and Mizushima N (2011) Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. *The Journal of Biological Chemistry*. 286(22): 19630–19640.
Yoshino J, Mills KF, Yoon MJ, and Imai S (2011) Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metabolism* 14(4): 528–536.
Yue W, Chen Z, Liu H, Yan C, Chen M, Feng D, et al. (2014) A small natural molecule promotes mitochondrial fusion through inhibition of the deubiquitinase USP30. *Cell Research* 24(4): 482–496.
Zhang C, Lin M, Wu R, Wang X, Yang B, Levine AJ, et al. (2011) Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. *Proceedings of the National Academy of Sciences of the United States of America* 108(39): 16259–16264.
Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, et al. (2016) NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science* 352(6292): 1436–1443.
Zhu JH, Gusdon AM, Cimen H, Van Houten B, Koc E, and Chu CT (2012) Impaired mitochondrial biogenesis contributes to depletion of functional mitochondria in chronic MPP+ toxicity: Dual roles for ERK1/2. *Cell Death and Disease* 3: e312.
Zhu XH, Lu M, Lee BY, Ugurbil K, and Chen W (2015) In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. *Proceedings of the National Academy of Sciences of the United States of America* 112(9): 2876–2881.
Zimmermann M and Reichert AS (2017) How to get rid of mitochondria: Crosstalk and regulation of multiple mitophagy pathways. *Biological Chemistry* 399(1): 29–45.
Zsurka G and Kunz WS (2015) Mitochondrial dysfunction and seizures: The neuronal energy crisis. *Lancet Neurology* 14(9): 956–966.

Further Reading

Fang EF, Palikaras K, Sun N, Fivenson EM, Spangler RD, Kerr JS, et al. (2017) In vitro and in vivo detection of mitophagy in human cells, *C. elegans*, and mice. *Journal of Visualized Experiments* 129: e56301.
Gatica D, Lahiri V, and Klionsky DJ (2018) Cargo recognition and degradation by selective autophagy. *Nature Cell Biology* 20: 233–242.
Khaminets A, Behl C, and Dikic I (2016) Ubiquitin-dependent and independent signals in selective autophagy. *Trends Cell Biology* 26: 6–16.
McWilliams TG, et al. (2016) Mito-QC illuminates mitophagy and mitochondrial architecture in vivo. *Journal of Cell Biology* 214: 333–345.
Mizushima N (2018) A brief history of autophagy from cell biology to physiology and disease. *Nature Cell Biology* 20: 521–527.
Palikaras K, Lionaki E, and Tavernarakis N (2018) Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. *Nature Cell Biology* 20: 1013–1022.
Sun N, Youle RJ, and Finkel T (2016) The mitochondrial basis of aging. *Molecular Cell* 61: 654–666.