MITOPHAGY IN HEALTH AND DISEASE

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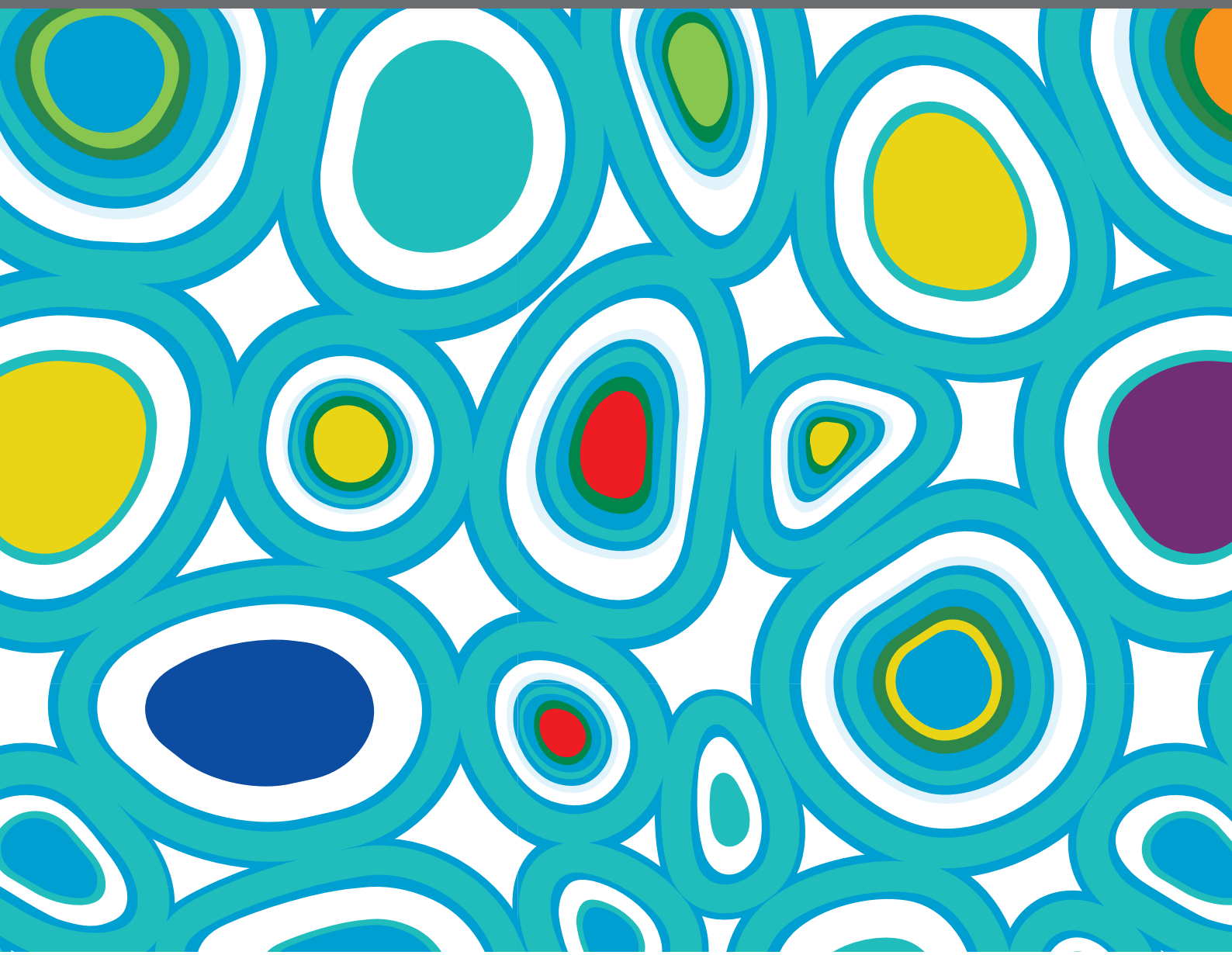

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MITOPHAGY IN HEALTH AND DISEASE

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EDITORIAL
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Editorial: Mitophagy in Health and Disease

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Editorial on the Research Topic

Mitophagy in Health and Disease

Mitophagy is the major degradation pathway, by which cells regulate mitochondrial number and integrity, in response to metabolic and physiological state. Research in mitophagy has grown exponentially over the last decade, revealing the intricate signaling pathways regulating mitophagy and the complexities of the molecular machinery involved in carrying out mitochondrial elimination. A diverse repertoire of mitophagy-related proteins has been discovered, highlighting an elaborate regulatory network of mitochondrial homeostasis that responds differentially to developmental, hormonal, and/or environmental signals. Moreover, the multi-layered crosstalk between mitophagy signaling pathways sustains energy metabolism, which is critical for tissues and organs homeostasis. Indeed, defective mitophagy causes accrual of dysfunctional mitochondria leading to bioenergetic stress, elevated ROS levels and pronounced inflammation that is accompanied by cellular and tissue degeneration. Thus, mitophagy is a pivotal contributor to cellular physiology, and tissue integrity, in addition to organismal development, healthspan, and survival.

The Research Topic on "Mitophagy in Health and Disease" in *Frontiers in Cell and Developmental Biology* includes a series of 11 articles that discuss recent advances in the field of mitophagy research and highlight challenges and outstanding questions, that need to be addressed before mitophagy modulation can be considered for the development of effective therapeutic interventions.

Several molecular mechanisms have been identified that mediate mitochondrial removal in a cell type- and tissue-specific manner. In their review, Ravanelli et al. discuss the critical role of the ubiquitin/proteasome system (UPS) in mitochondrial quality control. Alterations in the ubiquitination status of mitochondrial proteins contribute to the remodeling of the mitochondrial proteome in response to stress conditions. The authors discuss the tight crosstalk between the UPS and mitochondria that contributes to prevent proteostasis collapse and promote energy metabolism.

The article by Wang et al. surveys the regulation of mitochondrial removal by the phosphatase and tensin homolog (PTEN) isoforms. The authors introduce the molecular function of PTEN-short and PTEN-long proteins and their association with the mitophagic machinery. In addition, the authors highlight post-translational modifications as a central node of mitophagy, suggesting that their modulation can be used for the development of novel intervention approaches toward tackling mitochondrial-related disorders.

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In their review, Ravanidis and Doxakis address the role of RNA-binding proteins (RBPs) in mitochondrial homeostasis. The authors describe the vital role of RBPs in the maintenance of mitochondrial metabolism through the regulation of mRNA splicing, stability, targeting to mitochondria and translation. Importantly, substantial evidence indicates that impaired expression, or mutations in RBPs, contribute to mitochondrial dysfunction that has been implicated in the development and progression of several age-associated neurodegenerative disorders.

Although several components of the mitophagic machinery have been uncovered, the origin of mitoautophagosomal membranes remains elusive. In their review, Zachari and Ktistakis survey the molecular mechanisms that govern mitochondrial degradation, with a particular focus on the early signaling events of mitophagy initiation and mitoautophagosome formation.

A growing body of evidence suggests an intricate communication between mitophagy and cell death pathways. In their review, Ma et al. focus on how excessive mitochondrial damage can trigger innate immune responses and apoptotic cell death via BCL2 protein family members. The authors explore the molecular mechanisms that uphold mitochondrial homeostasis, including mitochondrial dynamics, mitochondrial biogenesis, and mitophagy among others, as well as, how these cellular events interfere with cell fate. Moreover, a relevant review by Joaquim and Escobar-Henriques discusses the pro-survival and pro-apoptotic role of mitophagy. The authors describe the involvement of mitofusins in mitophagy, through the modulation of mitochondrial morphology and endoplasmic reticulum (ER)-mitochondria contact sites. Finally, they summarize emerging findings, suggesting that impaired mitochondrial dynamics and mitophagy contribute to the pathogenesis of non-alcoholic liver disease.

Several studies have revealed a progressive, age-related decline of mitophagic flux in multiple tissues, including as heart, kidney, liver, and brain. In their article, Luo et al. discuss the contribution of mitophagy deregulation during aging to the homeostasis and viability of post-mitotic neurons and cardiomyocytes. Furthermore, the authors discuss recent studies that link impaired mitophagy to the development of neurodegenerative and cardiovascular pathologies.

The article by Xie C. et al. survey the role of defective mitophagy in the pathogenesis of Alzheimer disease (AD). Increasing evidence indicates that accumulation of damaged mitochondria, due to mitophagy impairment contributes to Aβ/Tau proteinopathies and stimulates persistent inflammation, causing neuronal loss, and cognitive decline. In addition, the authors discuss the potential use of mitophagy inducers, such as NAD^+ precursor molecules and urolithin A, toward ameliorating aging, and AD pathological features.

The review by Xie Y. et al. surveys the molecular pathways that govern mitochondrial elimination, focusing on the essential role of mitophagy receptors. Furthermore, the authors discuss the involvement of mitophagy receptors in tumorigenesis, and highlight the therapeutic potential of mitophagy modulation in cancer therapies.

The last two articles discuss emerging findings that highlight the anti-aging properties of mitophagy. In their review, Chen et al. delineate the molecular pathways and mechanisms of mitophagy in several model organisms, and discuss the significant contribution of mitophagy defects in age-related pathologies. In their article, Bakula and Scheibye-Knudsen introduce the term "mitophaging" pointing to the fundamental role of mitochondrial integrity in the maintenance of cellular fitness and organismal health. Both articles discuss interventions that target different steps in the process of mitophagy, by utilizing small molecular compounds as a means toward the development of novel, effective treatments against currently incurable pathologies.

In closing this Editorial piece, we would like to thank all the authors and referees, for their valuable contributions, toward compiling this up-to-date and timely Research Topic on mitophagy in health and disease. Moreover, we hope that the collection of articles included in the topic will provide a useful point of reference and a stimulus for further research aiming to ultimately decipher the complex contributions of mitophagy to cellular and organismal homeostasis.

AUTHOR CONTRIBUTIONS

KP and NT wrote the manuscript. KP, EF, and NT read and edited the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Culprit or Bystander: Defective Mitophagy in Alzheimer's Disease

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Mitophagy is a selective engulfment and degradation of damaged mitochondria through the cellular autophagy machinery, a major mechanism responsible for mitochondrial quality control. Increased accumulation of damaged mitochondria in the Alzheimer's disease (AD) human brain are evident, although underlying mechanisms largely elusive. Recent studies indicate impaired mitophagy may contribute to the accumulation of damaged mitochondria in cross-species AD animal models and in AD patient iPSC-derived neurons. Studies from AD highlight feed-forward vicious cycles between defective mitophagy, and the principal AD pathological hallmarks, including amyloid-β plaques, tau tangles, and inflammation. The concomitant and intertwined connections among those hallmarks of AD and the absence of a real humanized AD rodent model present a challenge on how to determine if defective mitophagy is an early event preceding and causal of Tau/Aβ proteinopathies. Whilst further studies are required to understand these relationships, targeting defective mitophagy holds promise as a new therapeutic strategy for AD.

Keywords: Alzheimer's disease, mitophagy, aging, neuroprotection, memory

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MOLECULAR MECHANISMS OF MITOPHAGY AND ITS ROLES IN NEUROPLASTICITY

Mitophagy is a highly conserved cellular process of removing damaged or superfluous mitochondria to maintain mitochondrial homeostasis (Pickrell and Youle, 2015; Scheibye-Knudsen et al., 2015; Fang et al., 2016b; McWilliams et al., 2016; Fivenson et al., 2017). In neurons, accumulation of damaged mitochondria is noxious to cellular function and survival. Mitophagy, at physiological level, maintains neuroplasticity and the functions of glial cells (Gustafsson and Dorn, 2019). Recent findings in human cell lines and multiple animal models have extended our knowledge in the molecular mechanisms of mitophagy from the PINK1-Parkin pathway, to the PINK1-independent pathways, including pathways that depend on NIP3-like protein X (NIX), B-cell lymphoma 2 interacting protein 3 (BNIP3), B-cell lymphoma 2-like 13 (BCL2L13), FK506 binding protein 8 (FKBP8), prohibitin (PHB2), breast cancer gene 1 protein (NBR1), optineurin (OPTN), calcium binding and coiled-coil domain 2 (NDP52), Autophagy and Beclin 1 Regulator 1 (AMBRA1), Tax1 binding protein 1 (TAX1BP1), FUN14 domain-containing protein 1 (FUNDC1),

PGAM family member 5 (PGAM5), Nipsnap Homolog 1 (NIPSNAP1), NIPSNAP2, among others (Fivenson et al., 2017; Kerr et al., 2017; Palikaras et al., 2018; Lou et al., 2019; Princely Abudu et al., 2019).

The PINK-1-dependent mitophagy is one of the well-characterized mitophagy pathways, with mutations of *PINK1* associated to familial Parkinson's disease (PD) (Plun-Favreau and Hardy, 2008; Gandhi et al., 2009; Burchell et al., 2013; Pickrell and Youle, 2015). Under physiological conditions, mitochondrial membrane potential (MMP) drives mitochondrial import of the 63 kDa full length PINK1. Presenilin-associated rhomboid-like protein (PARL) is an inner mitochondrial membrane (IMM) protease. PARL cuts the mitochondrial targeting sequence (MTS) and trans-membrane domain of PINK1, leading to the cytosolic release of the N-terminal-deleted PINK1 (ΔN-PINK1) (Deas et al., 2011). The N-terminal-deleted PINK1 (ΔN-PINK1) is degraded by the N-end rule pathway and the ubiquitin proteasome system (Pickrell and Youle, 2015). However, under various stressors or MMP fluctuations, PINK1 is shunted and retained on the outer mitochondrial membrane (OMM), promoting Parkin recruitment to the defective mitochondrial surface with the help of PINK1 autophosphorylation (Hasson et al., 2013; Lazarou et al., 2015). Parkin, an E3 ubiquitin ligase, ubiquitinates several OMM proteins, including voltage-dependent anion-selective channel protein (VDAC), mitofusin 2 (Mfn2), and dynamin-1-like protein (DRP1), leading to their recognition by autophagic adaptors: OPTN, NDP52, sequestosome 1 (SQSTM1/p62), TAX1BP1, or NBR1 (Sarraf et al., 2013; Lazarou et al., 2015; Ordureau et al., 2018).

Growing evidence indicates the existence and importance of PINK1- and/or Parkin-independent pathways. In addition to Parkin, other E3 ubiquitin ligases, such as mitochondrial ubiquitin ligase activator of NF-kB1 (MUL1), seven *in absentia* homolog 1 (SIAH1), Gp78, SMAD ubiquitin regulatory factor 1 (SMURF1), and Ariadne RBR E3 ubiquitin protein ligase 1 (ARIH1) participate in mitophagy. These E3 ubiquitin ligases are localized on OMM to generate ubiquitin chains, in order to direct coupling to the autophagy protein LC3, enabling the engulfment of the ubiquitin chain-tagged mitochondria by phagosomes, and finally fusion with the acidic lysosome to degrade the damaged mitochondria (Szargel et al., 2016; Villa et al., 2017). In addition to ubiquitin ligase-dependent mitophagy, OMM proteins can act as mitophagy receptors, targeting damaged mitochondria directly for mitophagy-mediated degradation. Examples include: BNIP3, NIX/BNIP3L, and FUNDC1 that mediate mitochondrial elimination via display of the N-terminus LIR domain into the cytosol which interact with LC3 or gamma-aminobutyric acid receptor-associated protein (GABARAP) (Sandoval et al., 2008; Liu et al., 2012; Zhang et al., 2016; Palikaras et al., 2018; Villa et al., 2018; Lou et al., 2019). Additionally, PHB2 and cardiolipin are amongst the recently discovered mitophagy proteins, which can externalized to OMM and couple with LC3 following mitochondrial membrane depolarization (Shen et al., 2017; Wei et al., 2017). In summary, while the PINK1/Parkin-dependent mitophagy pathway is well-characterized, the molecular mechanisms of multiple new mitophagy pathways are still not fully understood (**Figure 1**).

DEFECTIVE MITOPHAGY IN AD

Whilst accumulated extracellular Aβ plaques and intraneuronal Tau tangles are the disease-defining pathological features of Alzheimer's disease (AD), inflammation is now widely recognized as a key additional hallmark of AD. Relationships between mitophagy and each of the hallmarks of AD are summarized below.

Mitophagy and Amyloid-β (Aβ)

Neurons affected in AD models undergo defective mitophagy that contribute to the disease-defining Aβ pathologies, while Aβ accumulation may exacerbate impaired mitophagy and *vice versa* (Du et al., 2017; Kerr et al., 2017; Fang, 2019; Fang et al., 2019). Impaired mitochondrial proteostasis, including impaired mitochondrial unfolded protein response (UPR^{mt}), may link to Aβ proteotoxicity (Sorrentino et al., 2017). The activating transcription factor-associated with stress (ATFS-1) protein plays a fundamental role in the maintenance of UPR^{mt} and mitochondrial function, especially in stress conditions (Nargund et al., 2012). RNAi knockdown of *atfs-1* in an Aβ *Caenorhabditis elegans* model (GMC101) repressed mitophagy as well as basal and maximal respiration, and exacerbated Aβ toxicity; However, restoration of UPR^{mt} diminished AD pathology in both *C. elegans* and mouse models of AD (Sorrentino et al., 2017). Mechanistically, ATFS-1 transfers into and is degraded within mitochondrial matrix, which negatively impacts UPR^{mt}, at physiological condition (Melber and Haynes, 2018). Under the condition of mitochondrial stress, ATFS-1 favors importation into the nucleus, whereby it promotes the expression of genes with encoded proteins involved in the protection of mitochondrial function and the elimination of AD pathology (Melber and Haynes, 2018). In support of this model, mutations that cause amino acid substitutions within the MTS of ATFS-1 prevent the protein from being imported into the mitochondrial matrix, and result in constitutive UPR^{mt} activation (Rauthan et al., 2013). Abnormal mitochondrial homeostasis was reported in the mutant APP-HT22 cells relative to non-transfected HT22 cells, including increased levels of mitochondrial fission proteins (Drp1 and Fis1) and decreased levels of fusion proteins (Mfn1, Mfn2, and Opa1) (Manczak et al., 2018; Reddy et al., 2018). In addition to impaired UPR^{mt}, defective mitophagy is another major cause of impaired mitochondrial proteostasis and Aβ proteinopathy in AD. On one hand, defective mitophagy in post-mortem brain tissues from AD patients as well as in AD iPSC-derived neurons and cross-species Aβ-based AD animal models have been demonstrated (Fang et al., 2019). On the other, restoration of neuronal and microglial mitophagy ameliorated Aβ proteinopathy and rescued memory loss in the APP/PS1 mouse models of AD, highlighting the important contribution of defective mitophagy in AD (Fang et al., 2019). Disrupted−in−schizophrenia−1 (DISC1), an LC3-binding mitophagy protein, has been shown

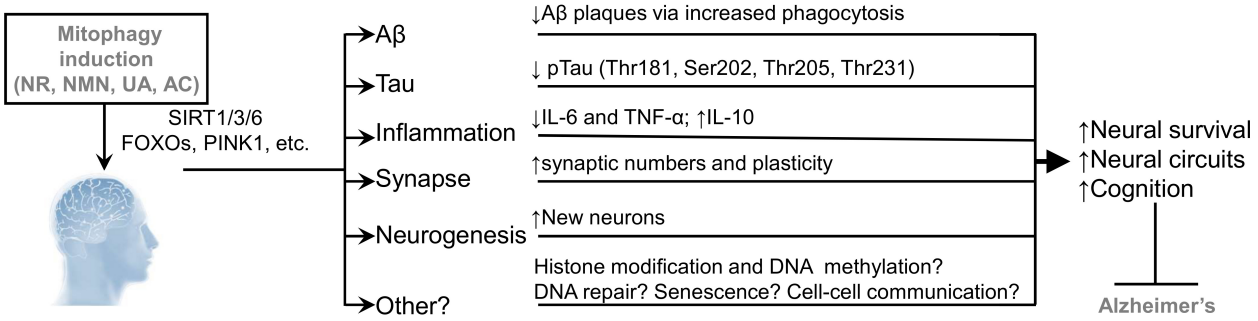

FIGURE 1 | A summary of possible molecular mechanisms on how mitophagy induction ameliorates AD pathologies. Experimental studies from *C. elegans* and mouse models of AD and from AD iPSC-derived neurons indicate genetic or pharmacological up-regulation of mitophagy inhibits Aβ/Tau proteinopathies and inflammation, as well as promotes synaptic plasticity and neurogenesis. Robust health-benefit mitophagy inducers include the NAD^+ precursors, nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), urolithin A (UA), and actinonin (AC). NAD^+ augmentation activates the NAD^+-dependent SIRT1/3/6 activities, and increases the expression and/or activities of autophagic/mitophagic proteins, including LC3-II and PINK1 and NIX, among others. Whether mitophagy induction improves histone modification and DNA methylation, neuronal DNA repair, cell-to-cell communication, and limits senescence remain to be determined. See text for more details as well as references.

to be reduced in human AD brain samples and in the APP/PS1 mice. In fact, Aβ−induced mitochondrial dysfunction, loss of spines, and impaired long−term potentiation (LTP) were rescued upon DISC1 overexpression in the APP/PS1 mice (Wang et al., 2019). Collectively, the current studies implicate that impaired mitochondrial proteostasis as a contributor to Aβ-based neurotoxicity via impaired UPR^{mt} and compromised mitophagy. However, the detailed molecular mechanisms remain to be determined.

Mitophagy and Tau

Tau binds and stabilizes microtubules, contributing in multiple physiological functions, such as neurite outgrowth, neuronal development, axonal transport, and synaptogenesis (Ballatore et al., 2007; Dixit et al., 2008). Studies in experimental AD models have provided evidence that mitochondrial dysfunction, defective mitophagy and phosphorylated-Tau (p-Tau) interact to form a vicious cycle (Kerr et al., 2017). The toxic N-terminal truncation of human Tau (NH_2-hTau) strongly affects the interplay between the mitochondria dynamics and mitophagy affecting subcellular trafficking or recruitment of both Parkin and ubiquitin-C-terminal hydrolase L1 (UCHL-1) (Amadoro et al., 2014; Corsetti et al., 2015). In *C. elegans* and neuroblastoma cells, expression of human wild-type (hTau) and frontotemporal dementia mutant tau (hP301L) completely inhibited mitophagy by blocking the recruitment of Parkin to damaged mitochondria (Cummins et al., 2019). Furthermore, APP and tau overexpression lead mitophagy impairment in human unmodified fibroblasts (Martin-Maestro et al., 2019). Furthermore, mitophagy was impaired in hippocampus tissues from 3xTgAD mice (with both Aβ and Tau proteinopathies) (Fang et al., 2019). In addition, pharmacological restoration of mitophagy, via administration of NAD^+ precursor nicotinamide mononucleotide (NMN), urolithin A (UA), or actinonin (AC), reduced the phosphorylation of pTau at several sites (such as Thr181, Ser202/Thr205, Thr231, and Ser262) (Fang et al., 2019). Collectively, emerging evidence suggests that pathological Tau inhibits mitophagy, highlighting defective mitophagy as a novel therapeutic target for AD.

Mitophagy and Inflammation

Numerous preclinical and clinical studies have shown that immune activation in AD, including microglia, and several cytokines, has the capacity to trigger and drive the pathophysiology of AD (Heppner et al., 2015). Mitochondrial stress leads to the release of damage-associated molecular patterns (DAMPs) which activate innate immunity, with the Cyclic GMP-AMP synthase (cGAS)-STING pathway as a central regulator of the type I interferon response to cytosolic DNA (Ishikawa and Barber, 2008; Ishikawa et al., 2009; Chen et al., 2016). Mitophagy mitigates inflammation through the restriction of inflammatory cytokine secretion and the regulation of immune cell homeostasis, correlating with the pathogenesis of autoimmune diseases at multiple levels (Xu et al., 2019). Multiple studies have demonstrated that PINK1 and Parkin regulate both innate and adaptive immunities. First of all, there is a strong inflammatory phenotype in both $Pink1^{-/-}$ and $Parkin^{-/-}$ mice, both of which were central regulators in the mitophagy process. Furthermore, PINK1 and Parkin mitigated STING-induced inflammation and rescued the loss of dopaminergic neurons from the substantia nigra (SN) in both $Pink1^{-/-}$ and $Parkin^{-/-}$ mice following exhaustive exercise (Sliter et al., 2018). Additionally, PINK1 and Parkin regulate immunity by repressing mitochondrial antigen presentation (MitAP) via mitochondria-derived vesicles (MDVs) (Matheoud et al., 2016). While the roles of STING and MitAP in the inflammation phenotype of AD is obscure, impairment of the PINK1/Parkin pathway in AD (Sliter et al., 2018; Fang et al., 2019), points to a possibility of an overlapping effect between PD and AD. The concomitant and intertwined molecular pathways that link defective mitophagy to Aβ and Tau proteinopathies, and inflammation need further exploration. Lastly, restoration of neuronal mitophagy (through NAD^+ supplementation, UA, and AC) reduced AD pathologies in the APP/PS1 AD mice via

enhanced microglial phagocytosis of extracellular Aβ plaques and the mitigation of pro-inflammatory cytokines released by continually activated microglia (Fang et al., 2019). Changes of mitophagy in AD astrocytes are elusive. It has been show that astrocytes play an important role in mitophagic degradation of damaged mitochondria from adjacent neurons (Davis et al., 2014), thus mitophagy induction may also improve different functions of astrocytes in AD. A recent development of a three-dimensional (3D) human AD triculture model, with neurons, astrocytes, and microglia (Park et al., 2018), may enable the studies of cell type-specific mitophagy in an environment which mimic the human brain. Collectively, while defective mitophagy plays a pivotal role in AD progression, and turning up mitophagy forestalls AD pathology, further molecular mechanisms on how mitophagy induction impacts neurons, astrocytes and microglia are necessary.

DEFECTIVE MITOPHAGY IN OTHER NEURODEGENERATIVE DISEASE

PD is a progressive neurological disorder that observably impairs patients' ability to control body balance and movements due to lack of dopaminergic neurons in the substantia nigra (SN), which exhibits abnormal accumulation of α-synuclein fibrils in their cell body and neurites (Poewe et al., 2017). Mitochondrial dysfunction and its related oxidative stress and inflammation are increasingly appreciated as common features of dopaminergic neuronal susceptibility in PD patient brain samples, PD animal models, and/or PD iPSC-derived neurons (Ryan et al., 2015; Schondorf et al., 2018). As a classical mitophagy pathway, the PINK1/Parkin pathway eliminates damaged mitochondria. Loss-of-function mutations in *PINK1* and/or *PARK2/Parkin* lead to inability of the cell to eliminate damaged mitochondria, and this has been related to early onset PD (Ryan et al., 2015). In addition, PINK1 and Parkin also suppress mitochondrial antigen presentation (MitAP) probably through inhibition of Sorting nexin 9 (Snx9)-dependent formation of MDVs (Matheoud et al., 2016). Meanwhile, *Parkin*- and *Pink1*-mutant fly models recapitulate major phenotypes of PD, including mitochondrial dysfunction, dopaminergic neuronal loss, motor disabilities and reduced lifespan (Yang et al., 2006). For mice, while the $Parkin^{-/-}$ and $Pink1^{-/-}$ animals do not show PD phenotypes at standard laboratory living condition, they do exhibit PD phenotypes (e.g., the loss of dopaminergic neurons) at stress living conditions, such as intestinal infection, exhaustive exercise, and mitochondrial stress (Perez and Palmiter, 2005; McWilliams et al., 2018; Sliter et al., 2018; Matheoud et al., 2019). These rodent data suggest compensation of the loss of PINK1-dependent mitophagy by PINK1-independent pathways under physiological conditions are sufficient; however, the PINK1-pathway is necessary at stress/pathological conditions for the function and survival of PD-related dopaminergic neurons.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease (predominately sporadic, nearly 90%) characterized by the accumulation of aggregated proteins partially resulted from mitochondria dysfunction and oxidative stress within affected motor neurons in the spinal cord, brain stem, and motor cortex (Rowland and Shneider, 2001). Genetic studies of familial ALS have identified several genes linked to ALS (Cirulli et al., 2015). Most of the genes involved in cellular quality control pathways, and more specifically to selective autophagy and mitophagy, including mitophagy receptors OPTN, RIPK1, p62/SQSTM1, as well as TBK1 (Cirulli et al., 2015; Hawk et al., 2018). In this way, mutant OPTN and TBK1 can interfere with the process of mitophagy, while mutant p62 shows a lower affinity to LC3-II which leads to impaired mitophagy (Moore and Holzbaur, 2016). These data suggest that the inefficient turnover of damaged mitochondria and also aggregates, may contribute to disease progression in ALS (Weishaupt et al., 2016). In line with the argument that impaired autophagy/mitophagy as a driver of ALS, pharmacological or genetic up-regulation of the $SIRT1/NAD^{+}$-mitophagy axis alleviates disease phenotypes in ALS mice and ALS patients (Blacher et al., 2019; de la Rubia et al., 2019; Lautrup et al., 2019). A detailed summary of defective mitophagy in AD, PD, ALS, and Huntington's disease is available (review in Lautrup et al., 2019; Lou et al., 2019). In summary, mounting evidence from animals and post-mortem human brain tissues suggests that defective mitophagy is a common feature, and likely plays a causative role in many neurodegenerative pathologies. We summarized the relationships between AD, PD, and ALS, and defective mitophagy/autophagy (**Figure 2**).

MITOPHAGY INDUCERS

Since reduced mitophagy is common in AD, and maybe a causal mechanism, up-regulating mitophagy might provide a therapeutic strategy for AD (Kingwell, 2019). Small molecules that do not have toxicity to mitochondria (mitochondrial toxicants), but can induce the expression of mitophagy proteins or enhance mitophagy machinery hold translational promise (Ryu et al., 2016; Andreux et al., 2019; Fang, 2019; Lou et al., 2019). The classical mitochondrial uncouplers, e.g., carbonyl cyanide-p-(trifluoromethoxy)phenyl hydrazine (FCCP) and carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and mitochondrial toxins that damage mitochondrial respiration (such as valinomycin, salinomycin, antimycin A and oligomycin) (Georgakopoulos et al., 2017), may have limited translational value for AD because treatment with those drugs will result in dysfunction of normal mitochondria.

In addition, multiple novel mitophagy inducers acting independently of the respiration failure without perturbing the organelle have been reported, offering new momentum to comprehend the process and underlying strategy for therapeutic revolution (Georgakopoulos et al., 2017). One example is to enhance the PINK1/Parkin-mediated mitophagy by supplementation with the ATP analog kinetin triphosphate (KTP) which can amplify catalytic activity of both PD related mutant $PINK1^{G309D}$ and $PINK1^{wt}$ (Hertz et al., 2013) or the application of a p53 inhibitor pifithrin-a, which can release Parkin from binding to the cytosolic p53 in pancreatic β-cells (Hoshino et al., 2014). Moreover, the anti-diabetic natural compound Metformin has been shown to maintain mitochondrial integrity

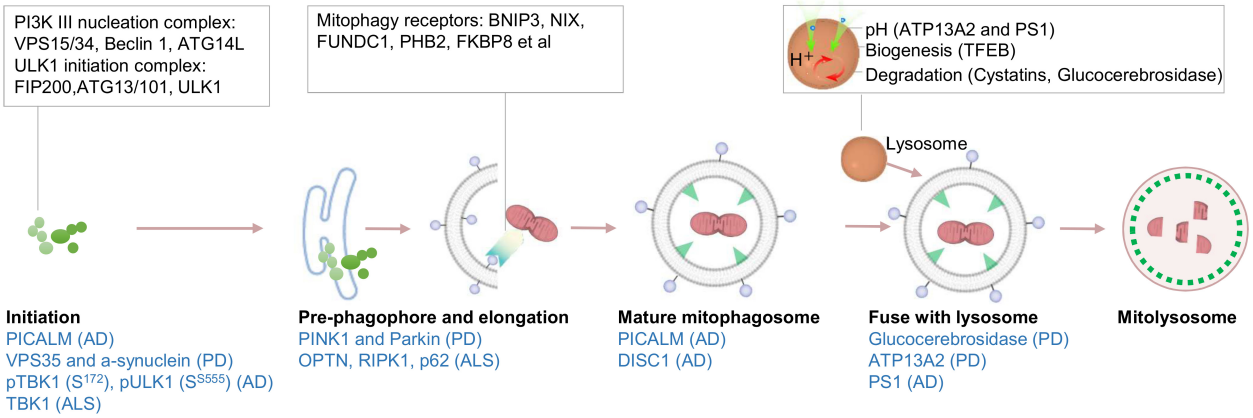

FIGURE 2 | Schematic of mitophagy pathway and its linkages to different neurodegenerative diseases. A simplified version of mitophagy is presented. Initiation of mitophagy is activated via the activity of the ULK1 complex and PI3K complex. Precursor vesicles fuse to form pre-phagophore structures that further elongate to eventually become double-membraned mitophagosomes. The completed autophagosomes are then trafficked to fuse with lysosomes to form mitolysosome. Dysfunction throughout this pathway, from initiation of mitophagosome formation to degradation in the mitolysosomes, have been suggested to be involved in AD, PD, and ALS (marked in blue).

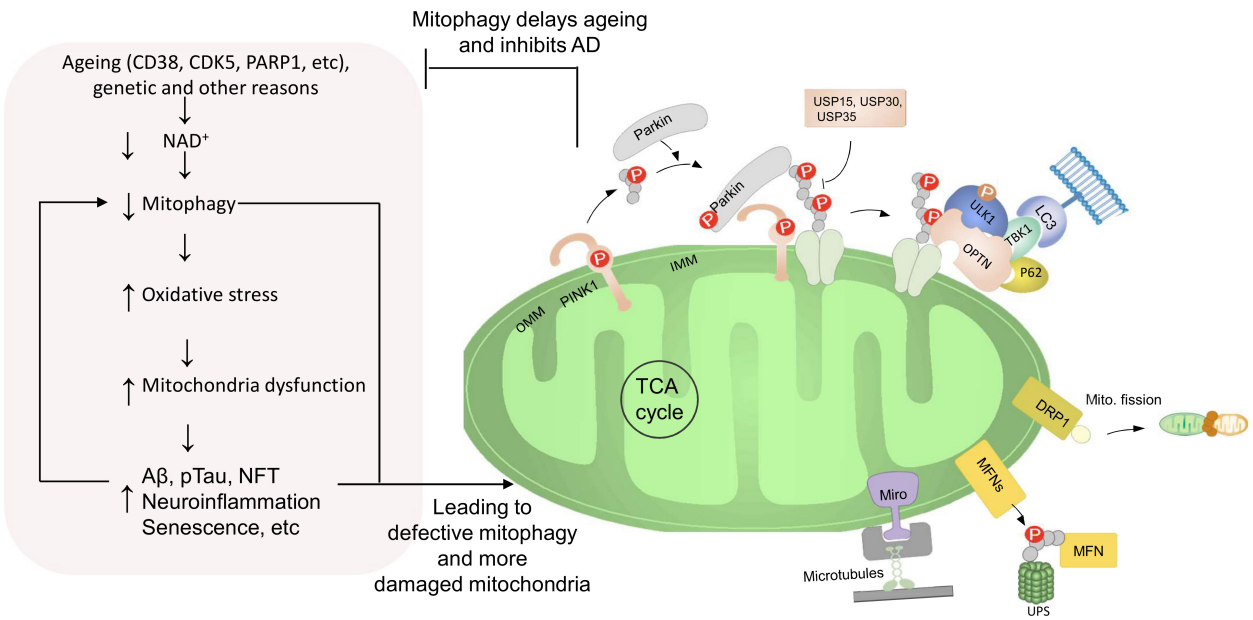

FIGURE 3 | Roles of defective mitophagy in AD with a vicious cycle between them. In normal condition, different mitophagy pathways are activated to efficiently eliminate damaged or superfluous mitochondria. E.g., the PINK1-Parkin pathway is a well-characterized mitochondrial recycling pathway. PINK1 is stabilized on the OMM of a damaged mitochondrion, promoting Parkin recruitment. Parkin ubiquitinates several outer membrane components. Poly-Ub chains are subsequently phosphorylated by PINK1 for the autophagic machinery. Adaptor proteins (e.g., p62, OPTN, NDP52, ULK1, and TBK1) recognize phosphorylated poly-Ub chains on mitochondrial proteins and initiate autophagosome formation through binding with LC3. Furthermore, the PINK1–Parkin pathway modulates mitochondrial dynamics and motility by targeting MFN and Miro for proteasomal degradation. However, aging, genetic, and non-genetic factors cause impairment of the NAD^+-mitophagy axis, which exacerbates disease defining pathologies of AD, including higher Aβ, pTau, and inflammation. These disease pathologies can further cause the damage of mitochondria and the inhibition of mitophagy, thus, generating a vicious cycle.

and boost mitochondrial biogenesis through Parkin-mediated mitophagy induction via p53 inhibition (Song et al., 2016; Palikaras et al., 2018). Targeting the up-regulation of the mammalian NF-E2 related factor 2 (Nrf2) (SKN-1, the *C. elegans* ortholog) pathway also enhances mitophagy, with molecules like the compound p62-mediated mitophagy inducer (PMI) (East et al., 2014) and the natural compound Tomatidine affluent in the green tomato (Fang et al., 2017b). NAD^+ is a fundamental molecule in human health and life since it participates in glycolysis, TCA cycle, OXPHOS, β-oxidation, and many other bioenergetic and metabolic pathways (Verdin, 2015; Fang et al., 2017a; Aman et al., 2018; Mitchell et al., 2018).

NAD^+ is reduced in biological aging, accelerated aging, and in common neurodegenerative diseases, including AD (Mouchiroud et al., 2013; Fang et al., 2014; Hou et al., 2018). Interventional studies support a causative role of NAD^+ depletion in neurodegeneration, as augmentation of tissue NAD^+, through the supplementation of nicotinamide riboside (NR) and NMN, can improve neuronal resilience and survival in both premature aging conditions and in AD, through a mitophagy-dependent manner (Fang et al., 2014, 2016a, 2019). Mechanistically, NAD^+ induces mitophagy through the NAD^+/Sirtuins-dependent pathways and several other pathways as we summarized elsewhere (Fang, 2019). In conclusion, small molecule which can induce mitophagy *in vivo*, but circumvent the cellular toxicity, hold promise for further clinical studies on AD.

FUTURE PERSPECTIVES

Accumulating data suggest the existence of PINK1/Parkin-dependent and –independent mitophagy pathways that are critical in the maintenance of mitochondrial homeostasis as well as neuronal resilience against proteinopathies and stressors. A growing understanding of AD pathology suggests that accumulation of damaged mitochondria due to impaired mitophagy, contributes to Aβ/Tau proteinopathies and inflammation, which may ultimately lead to neuronal loss and memory impairment. Accordingly, experiments from *C. elegans* and mouse models of AD and from AD iPSC-derived neurons suggest that turning up mitophagy might mitigate AD pathologies and retain cognition (in AD animals) with possible mechanisms summarized (**Figure 3**). Some outstanding questions need to be further addressed. First, whether defective mitophagy is an early event preceding and causing Aβ/Tau proteinopathies? Second, what are the additional molecular mechanisms of defective mitophagy in AD? Cellular signaling and progresses, including histone modification and DNA methylation, DNA repair, senescence, and cell to cell communication (including neurons and glial cells) link to neural plasticity and cognitive function (Halder et al., 2016; Fang et al., 2019; Zhang et al., 2019). Possible linkages of mitophagy in these processes should be explored (**Figure 1**). Third, whether pharmacological restoration of mitophagy could rescue/delay the progression of memory loss in AD patients? Because no single strategy has been effective in treating AD, it is possible that a multi-targeted combinational approach, and even personalized treatments will be necessary to treating AD. Since long-term multidomain intervention, including intervention of diet, exercise and cognitive training, could improve or maintain cognitive functioning in at-risk elderly people from the general population, a role of mitophagy is worthy of further exploration (Ngandu et al., 2015). The availability of new experimental systems, including the AD patient-oriented neuronal and glial cells (Haenseler et al., 2017; Lin et al., 2018; Volpato et al., 2018), the 3D tri-culture system (Park et al., 2018), and the application of artificial intelligence (Aman et al., 2019) will enable mechanistic studies in models more closely resembling the human AD brain, and will propel drug development.

AUTHOR CONTRIBUTIONS

CX and EF designed the outline of the review and wrote the draft of the review. YA, BA, MC, HP-F, and JX provided scientific comments and wrote part of the review.

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REVIEW
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Mammalian Mitophagosome Formation: A Focus on the Early Signals and Steps

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Mitophagy, a conserved intracellular process by which mitochondria are eliminated via the autophagic machinery, is a quality control mechanism which facilitates maintenance of a functional mitochondrial network and cell homeostasis, making it a key process in development and longevity. Mitophagy has been linked to multiple human disorders, especially neurodegenerative diseases where the long-lived neurons are relying on clearance of old/damaged mitochondria to survive. During the past decade, the availability of novel tools to study mitophagy both *in vitro* and *in vivo* has significantly advanced our understanding of the molecular mechanisms governing this fundamental process in normal physiology and in various disease models. We here give an overview of the known mitophagy pathways and how they are induced, with a particular emphasis on the early events governing mitophagosome formation.

Keywords: autophagy, mitophagy, mTOR, endoplasmic reticulum, membrane potential

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INTRODUCTION

Macroautophagy (or simply here autophagy) is a conserved quality control pathway by which a double membrane structure, called an autophagosome, grows to engulf cytoplasmic components in order to deliver them to the lysosome for degradation. Different stimuli can induce either "bulk autophagy" which is a non-selective process degrading random portions of the cytoplasm or "selective autophagy" which is activated to specifically degrade cellular components such as whole organelles (e.g., mitochondria, ER, peroxisomes - to name a few) and protein aggregates (Kirkin, 2019). One of the best understood pathways of selective autophagy is mitophagy - the selective degradation of mitochondria via autophagy. Mitochondria are dynamic organelles whose main function is to produce energy to support the many intracellular processes our cells are constantly undertaking. Mitochondrial quality control is crucial as defects to these organelles can lead to apoptosis or tissue damage, and, unsurprisingly, such mitochondrial defects have been linked to numerous human diseases (Murphy and Hartley, 2018). Thus, elimination of damaged and potentially toxic mitochondria is of extreme importance in favor of homeostasis and survival. A major pathway for the clearance of these mitochondria - and maintenance of mitochondrial network integrity and quality control - is mitophagy. Autophagosomes engulfing mitochondria are called mitophagosomes. Mitophagy occurs in a basal level (with different cell types exhibiting different levels of basal mitophagy) but can also be induced upon stresses such as exercise and ischemia *in vivo* and by mitochondrial disrupting/damaging agents in cultured cells (Montava-Garriga and Ganley, 2019). Impaired mitophagy has been associated with aging and numerous

human disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), cancer, but also cardiovascular and liver diseases (Palikaras et al., 2017; Um and Yun, 2017; Palikaras et al., 2018). Pharmacological modulation of mitophagy has been suggested to have potential as a therapeutic strategy for the treatment of these diseases. Although there is evidence that mitophagy is involved in pathogenesis, the exact role of mitophagy and mitophagy-related genes in pathological conditions is yet unclear. Ongoing *in vivo* and *in vitro* studies are aiming to elucidate this as well as to explore whether mitophagy could make a good pharmacological target in the context of disease. Over the past two decades, key studies have significantly advanced our understanding of the molecular mechanisms governing mitophagy. Here, we will aim to review the main mitophagy pathways with a particular focus on the early signaling events.

AUTOPHAGY MACHINERY

The process of forming a double-membrane autophagosome depends on a series of hierarchical steps that bring together more than 30 proteins or protein complexes. Upon inactivation of mTOR (in pathways of non-selective autophagy) the ULK complex composed of the protein kinase ULK1 (or its homolog ULK2), and the adaptors FIP200, ATG13, and ATG101 translocates to endoplasmic reticulum (ER) tubulovesicular membranes that have been "marked" by the presence of ATG9-containing vesicles (Hara et al., 2008; Ganley et al., 2009; Hosokawa et al., 2009b,a; Karanasios et al., 2016). These membranes then recruit the VPS34 complex composed of the PI 3-kinase VPS34 [synthesizing phosphatidylinositol 3-phosphate (PI3P)] and the adaptors VPS15, ATG14, and Beclin-1 which generates PI3P on ER-associated membranes termed omegasomes (Axe et al., 2008). The PI3P-enriched omegasomes then recruit the WIPI effectors and DFCP1, with the former group responsible for bringing on site the lipidation machinery that mediates the covalent modification of ATG8 family members (LC3 and GABARAP families) with phosphatidylethanolamine (PE) (Dooley et al., 2014). These PE-modified ATG8 proteins become part of the autophagosomal membrane whereas all of the other proteins come off as the double membrane closes and travels to the lysosomes for degradation (Axe et al., 2008; Karanasios et al., 2013). One challenge specific to our topic is how this very complicated machinery for making the double membrane autophagosome co-ordinates with the machinery that selects damaged cargo during selective autophagy. We will address this question in later sections.

MAIN MITOPHAGY TRIGGERS *IN VITRO* AND *IN VIVO*

Induction of Mitophagy *in vivo*

It was recently shown that mitophagy occurs *in vivo* in multiple tissues of mice at steady state without the need of external stimuli. This so-called basal mitophagy occurs presumably to ensure quality control of mitochondria as a housekeeping mechanism (McWilliams et al., 2016; Sun et al., 2017; McWilliams et al., 2018). Apart from its basal occurrence, mitophagy is also induced to support many physiological processes *in vivo* during organismal development. For example, during early embryogenesis, mitophagy has been reported to be responsible for the degradation of paternal mitochondria from the fertilized oocyte and early embryo (Rojansky et al., 2016). Furthermore, during reticulocyte maturation, mitophagy is a key pathway in regulating elimination of mitochondria for the production of mature erythrocytes (Kundu et al., 2008; Sandoval et al., 2008). Mitophagy has been reported to trigger a metabolic switch from oxidative phosphorylation to glycolysis, which is required for retina ganglion cell (RGC) and M1 macrophage differentiation (Esteban-Martinez et al., 2017). Similarly, mitophagy is key in promoting a switch from glycolysis to oxidative phosphorylation in myoblast differentiation (Sin et al., 2016). Apart from its role during embryonic development, mitophagy induced in response to infection has been proposed to have a protective inhibitory effect on the inflammasome, to avoid an excessive immune response which can lead to tissue damage (Kim et al., 2016; Zhong et al., 2016). Multiple physiological stresses have been reported to induce mitophagy in mice, including exercise, starvation, a switch to high fat diet, ischemia and hypoxia. More specifically, acute exercise is a strong mitophagy inducer in heart and skeletal muscle to mediate mitochondrial remodeling (Moyzis et al., 2015; Laker et al., 2017; Drake et al., 2019). Starvation is well known to induce general autophagy in mice, but this stress has also been reported to induce mitophagy, and interestingly there is evidence for canonical and non-canonical mechanisms occurring during starvation-induced mitophagy (discussed below) (Mizushima et al., 2004; Nishida et al., 2009; Hirota et al., 2015; Saito et al., 2019). Cardiomyocytes from mice subjected to high fat diet were shown to exhibit elevated levels of mitophagy to prevent cytotoxicity (Tong et al., 2019), although this resulted in reduced mitophagy in liver (Sun et al., 2015). Myocardial ischemia and energy stress (48 h starvation) have been shown to induce mitophagy in cardiomyocytes of mice, whereas ischemic preconditioning as well as ischemia-reperfusion injury were shown to induce mitophagy in kidney and brain tissues (Tang et al., 2016; Livingston et al., 2019; Saito et al., 2019; Tang et al., 2019). Hypoxic conditions also result in mitophagy induction in multiple tissues in mice and in various cell lines *in vitro* (Zhang et al., 2008; Allen et al., 2013; Sun et al., 2015, Zhang W. et al., 2016).

Induction of Mitophagy *in vitro*

Apart from the above more physiological ways to trigger mitophagy, chemical inducers of mitophagy have proved to be great tools and have allowed the dissection of the molecular mechanisms of this pathway *in vitro* (mostly in tissue cultured cells). Multiple compounds have been reported to induce mitophagy and have been recently reviewed in detail (Georgakopoulos et al., 2017). For the purpose of this review we will mention a few recent and widely used mitophagy inducers for which some mechanistic detail is known. The mitochondrial uncouplers CCCP and FCCP are proton ionophores which cause

severe loss of mitochondrial membrane potential, leading to a robust activation of mitophagy. A combination of the F0F1-ATPase inhibitor oligomycin and the complex III inhibitor antimycin A (symbolized as O/A treatment) also causes membrane depolarization and is known to be a strong mitophagy inducer in cultured cells (Georgakopoulos et al., 2017). The iron chelators depheriprone (DFP) and deferoxamine (DFO) are hypoxia mimicking agents and robustly induce mitophagy in a HIF1α-dependent manner (Allen et al., 2013).

Celastrol, a plant-derived pentacyclic triterpene with reported anti-inflammatory, anti-cancer and anti-obesity effects, was shown to induce mitophagy via Nur77 in a pathway that will be described later on in more detail (Hu et al., 2017). Lastly, we recently reported that the lactone Ivermectin acutely induces a strong mitophagic response upon fragmentation of mitochondria and is the fastest mitophagy inducer to our knowledge (Zachari et al., 2019). The mechanism by which mitophagy is induced by Ivermectin is currently unknown, but it is unlikely that its action is similar to CCCP or FCCP as it reduces oxygen consumption rate (OCR) whereas CCCP and FCCP are well known to dramatically increase OCR (Georgakopoulos et al., 2017; Juarez et al., 2018; Zachari et al., 2019). On the other hand, treatment with Oligomycin/Antimycin A results in decreased OCR as Ivermectin does, although in this case the timings of the two responses are very different with Ivermectin causing a much faster induction of mitophagy. Furthermore, it is unlikely that it acts through a HIF1α-dependent pathway, as this type of mitophagy stimulation requires transcriptional activity and usually takes longer time to occur. Thus, the above compounds appear to act through different mechanisms in inducing mitophagy.

MITOPHAGY SIGNALS BEFORE ENGAGING THE AUTOPHAGY MACHINERY: UBIQUITIN DEPENDENT AND INDEPENDENT PATHWAYS

Different pathways can regulate mitophagy and one of the main differences among them is their dependency or not on ubiquitin. The best studied mitophagy pathway is regulated by the Parkinson's disease related proteins PINK1 and Parkin (Bingol and Sheng, 2016). An illustration of the PINK1/Parkin pathway is shown in **Figure 1A**. PINK1 is a serine/threonine kinase which contains a mitochondrial-targeting signal. In healthy mitochondria, PINK1 is imported into the mitochondria via the TOM and TIM complexes, following cleavage by mitochondrial proteases including PARL (Jin et al., 2010; Greene et al., 2012). Cleaved PINK1 is exported back to the cytoplasm in an unstable form which is subjected to proteasomal degradation (Yamano and Youle, 2013). Upon mitochondrial depolarization, a loss of mitochondrial membrane potential inhibits import of PINK1 triggering its stabilization on the outer mitochondrial membrane (OMM), which results in its autophosphorylation and dimerization (Okatsu et al., 2012, 2013). PINK1 then regulates E3 ligase Parkin recruitment by phosphorylating ubiquitin at serine 65 attached on multiple OMM proteins (Narendra et al., 2010; Kane et al., 2014; Kazlauskaite et al., 2014). Phosphorylated ubiquitin acts as a key signal for the recruitment and activation of Parkin (Wauer et al., 2015). Parkin activation also requires direct phosphorylation by PINK1 at serine 65 (Kondapalli et al., 2012; Shiba-Fukushima et al., 2012). Parkin then further conjugates ubiquitin on OMM proteins, marking the mitochondria for degradation by the autophagic machinery (Narendra et al., 2008). It is worth mentioning here that phosphorylation of ubiquitin by PINK1 has been reported to be sufficient to recruit mitophagy cargo receptor proteins (primarily optineurin and NDP52) independently of Parkin but this is enhanced by Parkin activity (Lazarou et al., 2015). Recent work revealed that the phosphatase PPEF2 is responsible for dephosphorylating ubiquitin at serine 65 to oppose the PINK1 effect and inhibit mitophagy (Wall et al., 2019). The deubiquitinating enzyme that reverses this process by removing ubiquitin from the OMM is USP30 (Bingol et al., 2014). Another E3 ligase that has been reported to act in parallel with Parkin, is MUL1 (Yun et al., 2014; Rojansky et al., 2016). The upstream signals that regulate MUL1 recruitment and activity require further study, as the process has been reported to be both dependent and also independent of PINK1, raising the possibility of context dependent regulation of this protein (Yun et al., 2014; Rojansky et al., 2016). Multiple other proteins have been reported to regulate PINK1/Parkin-mediated mitophagy. More specifically, choline dehydrogenase (CHCD), a mitochondrial enzyme regulating methionine synthesis, was shown to act as a ubiquitin "eat me" signal and to associate with p62 for phagophore recruitment (Park et al., 2014). Depletion of CHCD was reported to impair PINK1/Parkin mitophagy induced by CCCP (Park et al., 2014). Similarly, two mitochondrial matrix proteins required for PINK1/Parkin mitophagy, NIPSNAP1 and NIPSNAP2, were recently reported to accumulate on mitochondria upon CCCP or hypoxia treatment, to act as "eat me" signals by directly recruiting mitophagy adaptor proteins (NDP52 and p62) as well as LC3/GABARAPs (Princely Abudu et al., 2019). Apart from receptor proteins, cardiolipin, a phospholipid normally localized in the inner mitochondrial membrane (IMM), translocates to the OMM upon Rotenone (a complex I inhibitor) and FCCP/CCCP treatment to act as a receptor for LC3 and thus mediate autophagosomal engulfment of damaged mitochondria (Chu et al., 2013). Cardiolipin potentially acts downstream of PINK1/Parkin signaling, although further research is required to confirm this scenario (Chu et al., 2013). An inner membrane mitochondrial protein, prohibitin 2, was also reported to mediate mitophagy by binding to LC3 upon proteasomal-dependent OMM rupture caused by O/A treatment (Wei et al., 2017). The presence of prohibitin 2 is important for PINK1/Parkin mitophagy but also for elimination of paternal mitochondria upon oocyte fertilization in *C. elegans* (Wei et al., 2017). It was recently suggested that prohibitin 2 might be involved in PINK1/Parkin mitophagy by positively regulating PINK1 stabilization onto mitochondria (Yan et al., 2019).

We recently reported a second ubiquitin-dependent mitophagy pathway which is rapidly induced by the lactone Ivermectin (Zachari et al., 2019). Ivermectin treatment results in acute mitochondrial damage as observed by a decrease in

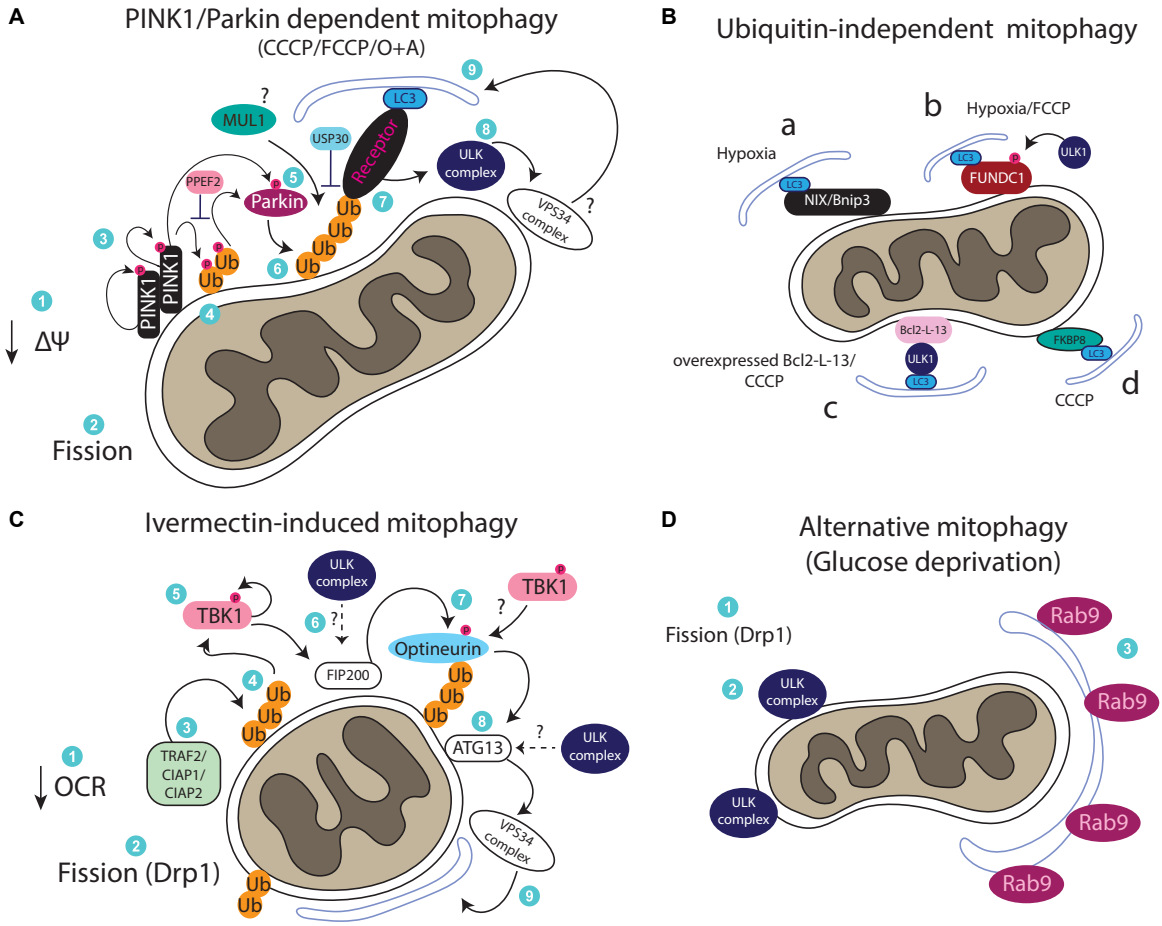

FIGURE 1 | (A) Cartoon of the PINK1/Parkin pathway. Upon loss of membrane potential (ΔΨ), PINK1 stabilizes on the OMM, dimerizes and autophosphorylates. PINK1 next phosphorylates Ubiquitin attached onto OMM proteins, leading to the recruitment of Parkin, which also gets phosphorylated and activated by PINK1. Parkin further ubiquitinates OMM proteins leading to the recruitment of receptor proteins for the generation/recruitment of an autophagosome and subsequent degradation of the mitochondrion. **(B)** Representation of mitophagy pathways which do not rely on ubiquitin. Various mitochondrial proteins can act as mitophagy receptors including: (a) NIX/Bnip3, (b) FUNDC1, (c) Bcl2-L-13, and (d) FKBP8. **(C)** Ivermectin-induced mitophagy relies on mitochondrial fragmentation and ubiquitylation via TRAF2/CIAP1/CIAP2. Upon ubiquitylation, TBK1 is required for FIP200 recruitment, which results in optineurin recruitment and downstream activation of ATG13 and the rest of the autophagic machinery for mitophagy. **(D)** An alternative autophagy pathway which does not rely on LC3 lipidation can also mediate mitophagy. This pathway requires mitochondrial fission, ULK1 and Rab9-possitive membranes.

OCR and severe fragmentation of the mitochondrial network. This leads to induction of mitophagy, independently of PINK1 and Parkin, but dependent on the E3 ligases TRAF2, CIAP1, and CIAP2 which work synergistically (and potentially in complex) to conjugate ubiquitin onto fragmented mitochondria. Upon ubiquitination, TBK1 becomes activated in order to regulate recruitment of the adaptor protein optineurin, to mediate mitophagy in a pathway which will be discussed later in more detail (Zachari et al., 2019) (**Figure 1C**). The DUB enzyme(s) reversing this process is yet to be identified. Apart from optineurin and NDP52, several other adaptor proteins are involved in mitophagy of ubiquitinated mitochondria including TAX1BP, p62, and NBR1 (Geisler et al., 2010; Moore and Holzbaur, 2016; Zachari et al., 2019). Another TRAF2/ubiquitin-dependent mitophagy pathway occurs in the presence of celastrol and the cytokine TNFa (Hu et al., 2017). More specifically, under these conditions, celastrol binds to Nur77 (a transcription factor nuclear receptor protein which can translocate to mitochondria to induce apoptosis), resulting in its translocation to mitochondria. When on mitochondria, Nur77 recruits TRAF2 resulting in mitochondrial ubiquitination and p62 recruitment for autophagic elimination in an anti-inflammatory mechanism (Hu et al., 2017).

Mitophagy can also occur independently of the presence of ubiquitin on the mitochondria (**Figure 1B**). These mitophagy pathways are regulated by adaptor proteins containing mitochondrial-targeting domains and LC3-interacting region (LIR) motifs. Whereas only one yeast mitophagy receptor has been identified so far (the protein ATG32) (Kanki et al., 2009), multiple mitochondrial receptors have been identified in mammals, namely: BNIP3, NIX (or BNIP3L), FUNDC1, and Bcl2-L-13. BNIP3 and NIX are two very similar proteins with

pro-apoptotic functions that are transcriptionally upregulated upon hypoxia in a HIF1α-dependent manner to mediate hypoxia-induced mitophagy (Zhang et al., 2008; Hanna et al., 2012, Ney, 2015; Yuan et al., 2017). NIX is also required for mitophagy to mediate mitochondrial removal during reticulocyte maturation (Schweers et al., 2007) and was recently reported to mediate ischemia-reperfusion-induced mitophagy in the brain (Yuan et al., 2017). Furthermore, NIX overexpression can rescue mitophagy induced upon CCCP treatment in cells deficient for Parkin-mediated mitophagy (Koentjoro et al., 2017). The interaction of NIX with LC3 during mitophagy has been suggested to be regulated by phosphorylation of NIX (at serine residues 34 and 35), although the kinase mediating these events is not yet identified (Rogov et al., 2017). Furthermore, NIX was recently reported to be a substrate of Parkin during mitophagy (Gao et al., 2015). Parkin-mediated ubiquitination of NIX results in the recruitment of NBR1 to mitochondria to mediate their autophagosomal engulfment and removal (Gao et al., 2015). Interestingly, BNIP3 has been reported to promote PINK1-dependent mitophagy as well, by stabilizing PINK1 levels onto mitochondria in hypoxic conditions (Zhang T. et al., 2016). Of note, overexpression of BNIP3 alone causes membrane potential loss and is sufficient to induce mitophagy, highlighting its significant role in activating this pathway (Rikka et al., 2011). The OMM protein FUNDC1 has been reported to regulate mitophagy induced upon hypoxia, but also upon mitochondrial depolarization with FCCP (Liu et al., 2012; Chen et al., 2014). Basal phosphorylation of FUNDC1 by Src (at tyrosine 18) and CK2 (at serine 13) inhibit its interaction with LC3 and concomitantly mitophagy, whereas hypoxia-induced dephosphorylation increases its interaction with LC3 to promote mitophagy (Liu et al., 2012; Chen et al., 2014). FUNDC1 has also been reported to be regulated by direct phosphorylation by ULK1 (at serine 17) to promote mitophagy both in the context of hypoxia and of FCCP treatment (Wu et al., 2014). Recently, NIX and FUNDC1 have been reported to be essential for mitophagy during cardiac progenitor cell differentiation, mediating mitochondrial network remodeling (Lampert et al., 2019). Bcl2-L-13 has been suggested to be the mammalian homolog of the yeast Atg32 due to sequence similarity and because it can rescue mitophagy in yeast upon loss of Atg32 (Murakawa et al., 2015). Bcl2-L-13 is thought to regulate mitophagy both by binding to LC3 but also by mediating mitochondrial fission (Murakawa et al., 2015). Another recently discovered mitophagy adaptor, FKBP8 with a preferred binding to LC3A, induces mitochondrial fragmentation and mitophagy when overexpressed in cells, independently of the PINK1/Parkin pathway (Bhujabal et al., 2017) (**Figure 1B**).

RECRUITMENT OF THE AUTOPHAGIC MACHINERY

Since receptor proteins are central in mediating mitophagy and can bind both mitochondria and LC3/GABARAP proteins, it was thought until recently that they mediate recruitment of the autophagic machinery by binding to LC3/GABARAP-positive forming phagophores. Recent evidence suggests that both ubiquitin dependent and independent mitophagy adaptor/receptor proteins can also act as hubs for the recruitment of proteins involved in early autophagy events and working upstream of LC3/GABARAPs such as ULK1 complex components and the PI3P-binding proteins WIPIs and DFCP1. These data reveal that mitophagy can occur not only by utilizing already forming autophagosomes but also by direct initiation of autophagosome formation via activation of early components. A study by Itakura et al. showed that during Parkin-mediated mitophagy, early autophagy proteins such as ULK1, ATG14, DFCP1, WIPI1, and ATG16L1 translocated onto damaged mitochondria even in the absence of LC3 conjugation on membranes (Itakura et al., 2012). It was later shown by the Youle laboratory that this is mediated in a receptor-dependent manner (Lazarou et al., 2015). More specifically, upon mitochondria depolarization, phospho-ubiquitin produced as a result of PINK1 activation, recruits the receptor proteins NDP52 and optineurin, which are required for the recruitment of ULK1 and the omegasome markers WIPI1 and DFCP1 (Lazarou et al., 2015). Interestingly, later work showed that ectopic targeting of NDP52 onto mitochondria is sufficient to recruit the ULK1 complex (via a direct interaction of NDP52 with FIP200), ATG14 and ATG16L1 and concomitantly induce mitophagy in both Parkin-dependent and independent mechanisms (Vargas et al., 2019). In the same study it was shown that targeting of ULK1 to mitochondria was sufficient to induce mitophagy (even when NDP52 or TBK1 are absent), supporting the notion that the function of early ubiquitin/receptor signals is to recruit the autophagy initiation machinery for the *de novo* formation of an autophagosome on the target mitochondrion. The key event regulated by NDP52-mediated recruitment of the ULK1 complex is a direct interaction between NDP52 and FIP200 which requires the presence and activation of TBK1 (Vargas et al., 2019). Binding of receptor proteins to FIP200 is not a mitophagy specific mechanism. It was recently reported to be the key event in other types of selective autophagy as well including ER-phagy, aggrephagy, xenophagy, and pexophagy (Smith et al., 2018; Ravenhill et al., 2019; Turco et al., 2019) highlighting that different selective autophagy pathways share common features. In addition, it was recently shown that LC3/GABARAP proteins can mediate recruitment of the adaptor proteins optineurin and NDP52 to mitochondria, in a positive feedback loop in order to accelerate PINK1/Parkin mediated mitophagy (Padman et al., 2019). Interestingly, LC3/GABARAPs likely play different roles during PINK1/Parkin mitophagy as GABARAPs appear to be more essential than the LC3s (Nguyen et al., 2016). Furthermore, loss of all LC3/GABARAPs did not abolish autophagosome formation (as seen by EM and recruitment of earlier autophagy markers such as ULK1 on mitochondria) but it rather caused defects in mitophagosome-lysosome fusion (Nguyen et al., 2016). Independently of PINK1 and Parkin, we recently showed that ubiquitination of fragmented mitochondria induced by

Ivermectin leads to activation of TBK1 which is required for optineurin recruitment and mitophagy (Zachari et al., 2019). In this mitophagy pathway, recruitment of optineurin required FIP200 but not ULK1/2 or ATG13, revealing that the activity of the ULK1/2 kinases is not essential for certain mitophagy pathways. [It is worth mentioning here that the FIP200 and ATG13 proteins have been previously reported to act independently of the complex during autophagy (Alers et al., 2011) although the exact mechanisms underlying these functions are poorly understood.] Furthermore, TBK1 activation was hierarchically earlier than FIP200, and was required for optineurin recruitment. Given the recently published evidence discussed above, it is possible that a direct interaction between optineurin (and possibly more adaptors) and FIP200 is key also for the progression of Ivermectin-induced mitophagy. ATG13 was required for the formation of the omegasomes, within which the LC3-positive mitophagosomes formed (Zachari et al., 2019). ATG9 vesicles - essential components of the autophagic machinery - have been involved in both PINK1/Parkin and Ivermectin-induced mitophagy, although their exact role during mitophagy remains elusive (Itakura et al., 2012; Yamano et al., 2018, Zachari et al., 2019).

Mitophagy induced via Bcl2-L-13 was also recently reported to involve recruitment of ULK1 to mitochondria, even though this was shown to occur in an indirect manner whereby Bcl2-L-13 recruits LC3B, leading to recruitment of ULK1 which binds to LC3B via a LIR motif (Murakawa et al., 2019). As mentioned earlier, FUNDC1-dependent mitophagy also involves recruitment of ULK1 to the mitochondria. In this case FUNDC1 is phosphorylated by ULK1 which causes enhanced binding to LC3 during mitophagy (Wu et al., 2014). Predictably, ULK1 kinase activity is required for this pathway (Wu et al., 2014) (**Figure 1B**).

Even though mitophagy generally is reported to occur via the canonical autophagic machinery that we described earlier, a non-canonical mitophagy pathway which does not require LC3 lipidation was reported to occur in cardiomyocytes upon ischemic stress and starvation as well as in HeLa cells upon starvation and hypoxia (Hirota et al., 2015; Saito et al., 2019). In cardiomyocytes, this pathway depends on ULK1 but not on ATG5 and does not involve LC3-positive autophagosomes; instead it is mediated by Rab9 positive membranes/vesicles which drive lysosomal degradation of mitochondria. It is currently unknown whether ubiquitin signals and receptor proteins are involved in ULK1-Rab9 dependent mitophagy (Saito et al., 2019) (**Figure 1D**). Rab9-mediated autophagy has been previously reported as a non-canonical/alternative non-selective autophagy pathway in cells lacking ATG5, where it was also suggested that it plays a role in mitochondrial clearance during erythrocyte differentiation (Nishida et al., 2009).

It is important to mention here that mitochondrial fission (the separation from the network or fragmentation), appears to be an essential (but not sufficient) step for mitophagy (Twig et al., 2008; Williams and Ding, 2018). A key regulator of mitochondrial fission is Drp1 - a member of the dynamin family of large GTPases (Labrousse et al., 1999). Although multiple reports have shown that Drp1 is required for both PINK1-dependent and independent mitophagy pathways (Lee et al., 2011; Kageyama et al., 2014; Wu et al., 2016; Li et al., 2019; Zachari et al., 2019), other studies suggest that it might be dispensable for mitophagy (Murakawa et al., 2015; Yamashita et al., 2016; Burman et al., 2017). Thus, mitophagy dependency on Drp1 could be potentially context specific and further investigation is needed to understand its role in this pathway. A generalized illustration of our current understanding of mitophagy signaling is shown in **Figure 2**.

DOES MTORC1 REGULATE MITOPHAGY?

As mentioned earlier, one of the master regulators of bulk autophagy is mTORC1, which when active, suppresses autophagy via direct phosphorylation of the ULK complex components ULK1 and ATG13 (Ganley et al., 2009; Hosokawa et al., 2009a; Kim et al., 2011; Puente et al., 2016). Even though mTORC1 has a well-established role during starvation-induced autophagy, its role during mitophagy is unclear. Inhibition of mTORC1 with rapamycin has been reported to improve mitochondrial health (Nacarelli et al., 2018). Studies with CCCP and FCCP in a non-mitophagy context, have reported that these proton uncouplers cause a reduction in mTORC1 activity, suggesting that PINK1/Parkin mitophagy might require mTOR inactivation as bulk autophagy does (Inoki et al., 2003; Kim et al., 2013; Bartolome et al., 2017). Furthermore, recent reports suggest that mTORC1 hyperactivation can have an inhibitory effect on PINK1/Parkin mediated mitophagy as well as general autophagy (Bartolome et al., 2014, 2017; Bordi et al., 2019). Importantly, hypoxia leads to a reduction in mTORC1 activity as well, meaning that mTORC1 might play a role in hypoxia-induced mitophagy (Vadysirisack and Ellisen, 2012). Ivermectin did not appear to affect mTORC1 activity in the time points and concentrations we used to induce mitophagy (although treatments of 24–48 h have been shown to have an inhibitory effect on mTORC1) (Dou et al., 2016; Liu et al., 2019; Zachari et al., 2019). This might mean that the ULK complex can mediate mitophagosome formation even if it is phosphorylated by mTORC1 or that mitophagy is mediated by a pool of ULK complex that has escaped inhibition of mTORC1. Vargas et al. (2019), showed that mitophagy-induced due to ULK1 targeting to mitochondria was unaffected by mTOR overexpression and concomitant increase in ULK1 phosphorylation. It is important to mention here that amino acid (or full nutrient) starvation has been shown to block mitophagy in multiple cell lines as a result of reduced mitochondrial fission, even though mTORC1 activity is lost in these conditions (Gomes et al., 2011; Rambold et al., 2011). Thus, mTORC1 inactivation does not necessarily lead to mitophagy induction. Most of the mitophagy studies do not evaluate mTORC1 activity and our knowledge on this is limited. In conclusion further research is required to understand its involvement during mitophagy. Of note, AMPK appears to play an important (but not fully understood) role during mitophagy in multiple contexts and it is worth mentioning that mTORC1 regulates AMPK and that both these kinases can potentially influence mitophagy via

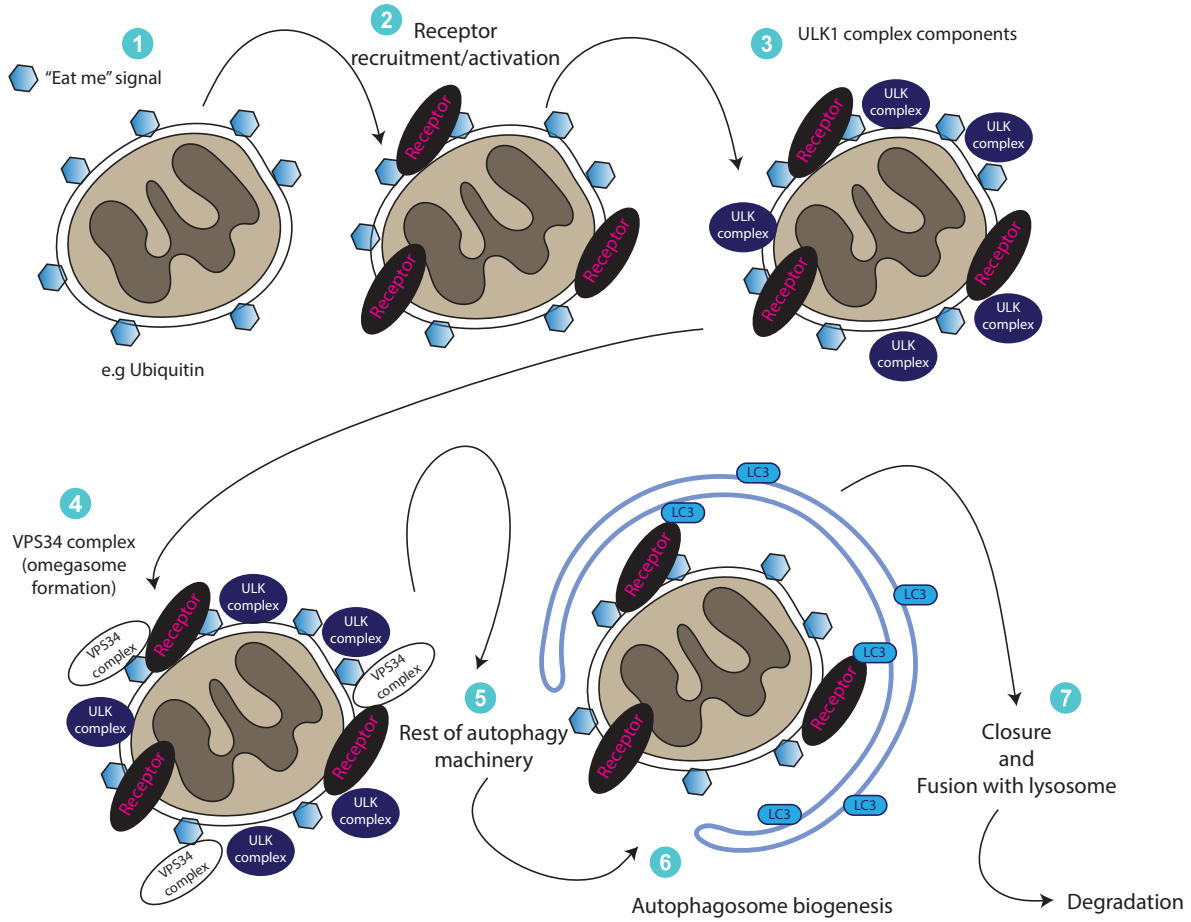

FIGURE 2 | A generalized illustration of mitophagy. Upon damage or other stimulus, mitochondria become positive for molecules resulting in an "eat me" signal. This results in recruitment (or activation of already existing) receptor proteins, which regulate the recruitment of forming autophagosomes or that of early autophagy machinery proteins (ULK complex, VPS34 complex) for the generation of a double membrane mitophagosome. Ultimately, mitophagosomes fuse with the lysosomes to degrade themselves.

ULK1. The literature on this topic has recently been reviewed (Herzig and Shaw, 2018).

IS THERE A MEMBRANE SOURCE SPECIFIC FOR MITOPHAGY?

The origin of the autophagosomal membrane during bulk autophagy induced by amino acid starvation is a long-standing question: autophagosomes form *de novo* and the membranes contributing to autophagosomal growth are devoid of protein-markers of the donor membranous compartments, making it hard to identify their provenance. Different organelles and membrane compartments have been proposed to contribute membrane to the autophagosome forming upon amino acid starvation, and these include the ER, Golgi, plasma membrane, mitochondria, and endosomes (Ktistakis and Tooze, 2016; Wei et al., 2018). As discussed earlier, during amino acid starvation autophagosomes form in ER platforms called omegasomes which are marked by the omegasome marker DFCP1 (Axe et al., 2008). Thus, a candidate membrane donor has been proposed to be the ER, with contributions from virtually all intracellular membranes in a way not entirely clear (Ktistakis, 2020). When it comes to mitophagy and other types of selective autophagy our understanding of the membrane source(s), and whether this differs from bulk autophagy, is even less clear. For Ivermectin-induced mitophagy we recently showed that at an early step of the process mitochondria became entrapped within ER strands prior to omegasome formation and mitophagosome generation (Zachari et al., 2019). In our analysis by both live imaging and electron tomography it appeared that the autophagosomal membrane engulfing the mitochondria grew as an extension of the neighboring ER strands-such close apposition of ER with targeted mitochondria was evident in some older publications studying PINK1/Parkin-induced mitophagy (discussed in Zachari et al., 2019). Since observations from other research groups support the notion that mitophagosomes (independently of induction mode) form within omegasomes (Yang and Yang, 2013; Wong and Holzbaur, 2014; Gelmetti et al., 2017; Hsieh and Yang, 2019) it is possible that the ER plays

a key role as a membrane source for mitophagy in general. It will be interesting to determine if the different ER proteins that have been reported to be involved in autophagosome biogenesis upon amino acid starvation are also important for mitophagy as well (Walker and Ktistakis, 2019; Ktistakis, 2020). It was recently reported that in yeast mitochondria-ER contact sites are crucial for mitophagy (and pexophagy) to occur (Bockler and Westermann, 2014; Liu et al., 2018). Given the importance of ER-mitochondrial contacts in autophagosome formation in general, it is likely that they are crucial for mitophagy in mammals too, although this is yet to be confirmed experimentally (Hamasaki et al., 2013).

CONCLUSION AND FUTURE PERSPECTIVES

Our understanding of mitophagy signaling process and function has significantly advanced in the past decade. It is now clear that multiple proteins are involved in the regulation of mitophagy and various physiological events or types of stress can induce different mitophagy pathways both *in vivo* and *in vitro*. The PINK1/Parkin pathway has attracted a lot of attention, partially due to the importance of these proteins in Parkinson's disease and undoubtedly as a result of the detailed mechanistic studies that have dissected it. However, it is now evident that this pathway is not required for the regulation of basal mitophagy (as well as other types of mitophagy) in mice and it might be more relevant in clearing mitochondria following particular damage/stress. Thus, more work is required to understand its physiological relevance (McWilliams et al., 2018; Drake et al., 2019). In terms of other types of mitophagy, there are still a lot of unanswered questions remaining in the field. For example, what kind of damage exactly do the mitophagy-inducing agents cause to the mitochondria? How can this relate to mitochondrial stress in humans (e.g., exposure to environmental hazards or aging)? How can this lead to disease? Can mitophagy be targeted for the development of therapeutics? Related to the last question, biochemical advances in the autophagy field, led to the generation of therapeutically promising compounds called "AUTACs" which can bind to mitochondria causing their ubiquitylation and induction of mitophagy (Takahashi et al., 2019). This way, AUTACs were shown to successfully drive mitochondria to the autophagosomal lumen for degradation, opening up an exciting new era in preclinical research on selective autophagy and its potential in treatment development.

AUTHOR CONTRIBUTIONS

MZ and NK wrote and edited the manuscript.

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REVIEW
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Mitophagy: An Emerging Role in Aging and Age-Associated Diseases

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Mitochondrial dysfunction constitutes one of the hallmarks of aging and is characterized by irregular mitochondrial morphology, insufficient ATP production, accumulation of mitochondrial DNA (mtDNA) mutations, increased production of mitochondrial reactive oxygen species (ROS) and the consequent oxidative damage to nucleic acids, proteins and lipids. Mitophagy, a mitochondrial quality control mechanism enabling the degradation of damaged and superfluous mitochondria, prevents such detrimental effects and reinstates cellular homeostasis in response to stress. To date, there is increasing evidence that mitophagy is significantly impaired in several human pathologies including aging and age-related diseases such as neurodegenerative disorders, cardiovascular pathologies and cancer. Therapeutic interventions aiming at the induction of mitophagy may have the potency to ameliorate these dysfunctions. In this review, we summarize recent findings on mechanisms controlling mitophagy and its role in aging and the development of human pathologies.

Keywords: mitophagy, aging, mitochondria, caloric restriction, ROS

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INTRODUCTION

Mitochondria are highly organized and dynamic organelles that undergo continuous fission and fusion (Chen and Chan, 2009; Pham et al., 2012). They originated from endosymbiotic proteobacteria and conferred substantial advantages for eukaryotic cells during evolution. Thus, mitochondria play a critical role in ATP synthesis via oxidative phosphorylation (OXPHOS), β-oxidation regulating fatty acid metabolism, the synthesis of intermediate metabolites through the TCA cycle, as well as calcium homeostasis. On the other hand, like a double-edged sword, mitochondria can turn into a potential threat to cellular homeostasis and survival. In the past decades it has been well documented that mitochondria are the central organelle controlling apoptotic cell death and that the permeabilization of the mitochondrial outer membrane, with the resultant release of pro-apoptotic proteins such as cytochrome c, SMAC/DIABLO, ENDOG, OMI/HTR and AIF, irrevocably leads to cellular demise (Susin et al., 1999; Du et al., 2000; van Loo et al., 2002; Green and Kroemer, 2004; Liu et al., 2009; Wang and Youle, 2009; Li et al., 2017). Moreover, mitochondria are the major source of reactive oxygen species (ROS). During OXPHOS electrons originating mostly from complexes I and III of the electron transport chain,

can generate ROS that in turn oxidizes proteins, lipids, and nucleic acids, inside (and outside) the mitochondria, leading to mitochondrial malfunction and cellular damage (Paradies et al., 2000; Hamilton et al., 2001; Short et al., 2005; Miyoshi et al., 2006; Zorov et al., 2014; Redza-Dutordoir and Averill-Bates, 2016). Furthermore, mitochondria serve as an origin of damage associated molecular patterns (DAMP) and in particular mitochondrial DNA (mtDNA), which, once released from mitochondria into the cytosol, can trigger inflammatory responses (Iyer et al., 2009, 2013; Tschopp, 2011; Nakahira et al., 2015; West et al., 2015; Contis et al., 2017).

During aging a wide spectrum of alterations in mitochondrial structure and function can occur. Thus, although cellular antioxidants and free radical scavenging enzymes eliminate most of the generated ROS, a small proportion that escapes clearance can oxidize proteins, lipids and DNA, particularly within the mitochondria. The resulting mutational damage accumulates over lifetime, in particular affecting respiratory chain complexes, which itself results in the overproduction of ROS, forming a vicious cycle that ultimately leads to mitochondrial dysfunction (Greco et al., 2003; Petersen et al., 2003; Short et al., 2005; Lee and Wei, 2012). Morphologically, aging in flies and mammalians manifests with the enlargement of mitochondria, irregular cristae shape and size as well as a decrease in mitochondrial number (Miquel et al., 1980; Terman and Brunk, 2005; Yoon et al., 2006; Leduc-Gaudet et al., 2015). Functionally, OXPHOS activity, and thus ATP synthesis declines with age while ROS production increases in aged animals (Lee and Wei, 2012). As a result, it is not surprising that mtDNA deletions and mutations are detected in tissues from aged animals and humans (Fayet et al., 2002; Eshaghian et al., 2006; Trifunovic, 2006; Lee and Wei, 2012). Consistent with these observations, mtDNA mutator mice that express a proof-reading-deficient version of the mitochondrial DNA polymerase G (POLG) show reduced lifespan and exhibit a premature onset of aging-associated phenotypes including weight loss, reduced subcutaneous fat, alopecia (hair loss), kyphosis (curvature of the spine), osteoporosis, anemia, reduced fertility, and heart enlargement (Trifunovic et al., 2004).

Macroautophagy, which is generally referred to as autophagy, is a conserved intracellular degradation mechanism that removes dangerous, unnecessary or dysfunctional cytoplasmic constituents and invading microbes (Mizushima, 2007; Schuck et al., 2014; Dou et al., 2015; Mochida et al., 2015; Chai et al., 2019). Autophagic activity declines during aging, and autophagy is required for lifespan extension by caloric restriction or caloric restriction mimetics (CRM) such as resveratrol, spermidine, and several chalcones (Eisenberg et al.; Rubinsztein et al., 2011; Lopez-Otin et al., 2016; Madeo et al., 2018; Carmona-Gutierrez et al., 2019). Although the relation between autophagy and aging has been firmly established as an important mitochondrial quality control mechanism, the role of mitophagy in aging and age-related disorders has remained elusive for a long time. However, recent studies have shown that mitophagy has a key function in delaying aging and age-related disorders such as neurodegenerative disorders, cardiovascular pathologies, and cancer. Here, we provide an update on mechanisms that control mitophagy, its role in aging and therapeutic interventions that harness mitophagy to treat age-related disorders.

MOLECULAR MECHANISMS OF MITOPHAGY

Mitophagy shares the core molecular machinery with general macroautophagy and can occur in an either selective or non-selective fashion (Levine and Kroemer, 2019). Thus, during nutrient starvation mitochondria were found in autophagosomes together with cytosolic proteins and organelles such as ER and peroxisomes indicative for non-selective mitophagy (Kopitz et al., 1990; Takeshige et al., 1992; Scott and Klionsky, 1998; Kim et al., 2007; **Figure 1**). Studies in yeast revealed that mitochondria can be selectively degraded by mitophagy, a process that involves the outer mitochondrial membrane protein SUN family protein Uth1 (Uth1), and type 2C protein phosphatase Ptc6 (Ptc6, better known as Aup1), a phosphatase localizing in the mitochondrial intermembrane space (Petros et al., 1991; Kissova et al., 2004). Mitophagy has been shown to occur under a series of potentially harmful conditions, such as oxidative stress, hypoxia, mitochondrial transmembrane potential loss, the accumulation of unfolded proteins and iron starvation. Moreover, impaired mitophagy and dysfunctional mitophagic mechanisms were associated with numerous physiological and pathological processes including development, differentiation, aging, neurodegenerative disorders, cardiovascular pathologies and cancer.

PINK1 AND PARKIN-REGULATED MITOPHAGY

Mutations in PTEN-induced putative kinase 1 (PINK1) and parkin RBR E3 ubiquitin protein ligase (PRKN, better known as Parkin) are associated with autosomal recessive juvenile parkinsonism characterized by motor disturbances and dopaminergic neurodegeneration. Later, genetic analysis showed the molecular mechanism which links PINK1 and Parkin in a common pathway regulating mitophagy, with PINK1 sensing mitochondrial transmembrane potential loss, followed by the recruitment of the E3 ubiquitin ligase Parkin to damaged organelles (Clark et al., 2006; Park et al., 2006). In healthy state, PINK1 led by an N-terminal targeting sequence is imported into mitochondria through the translocase of the outer mitochondrial membrane (TOM) and the translocase of the inner mitochondrial membrane (TIM) complexes, where it is cleaved by matrix processing peptidase (MPP) and presenilins-associated rhomboid-like protein (PARL) (Jin et al., 2010; Deas et al., 2011; Meissner et al., 2011; Greene et al., 2012). Cleaved PINK1 is retro-translocated and released into the cytosol for proteasomal degradation (Yamano and Youle, 2013). However, the loss of mitochondrial transmembrane potential in damaged mitochondria abolishes cleavage, and stabilizes PINK1 on the outer mitochondrial membrane. Recently, the adenine nucleotide translocator (ANT) complex was reported to stabilize PINK1 by

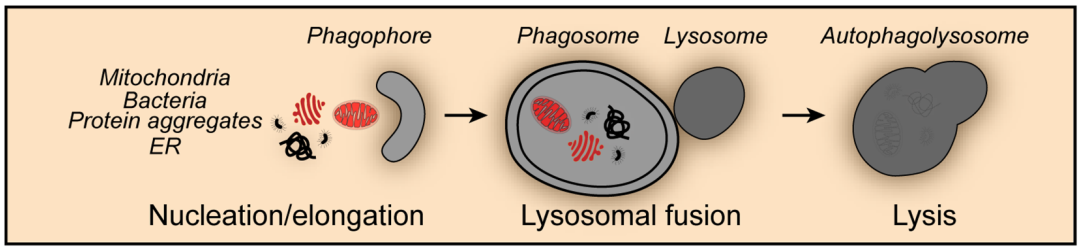

FIGURE 1 | Non-selective mitophagy. Mitophagy shares the core molecular machinery with general macroautophagy and can occur in a non-selective fashion. Thus, mitochondria are engulfed during the nucleation and elongation phase into the forming phagophore together with other cellular content such as protein aggregates, endoplasmic reticulum (ER) derived structures and invasive bacteria. The fusion of the phagosome with lysosomes leads to the formation of the autophagolysosome and the degradation of its content.

inhibiting the pre-sequence translocase TIM23 independently of its nucleotide translocase catalytic activity (Hoshino et al., 2019).

The accumulation of full length PINK1 leads to the phosphorylation (on serine 65) of pre-existing ubiquitin molecules, which are already attached to the outer mitochondrial membrane. Phosphorylated ubiquitin in turn recruits cytosolic Parkin to the mitochondrial membrane and triggers the activation of its ubiquitin ligase activity (Koyano et al., 2014; Wauer et al., 2015). Furthermore, PINK1-dependent phosphorylation of the ubiquitin-like domain of Parkin (Kondapalli et al., 2012; Shiba-Fukushima et al., 2012; Iguchi et al., 2013; Kane et al., 2014) leads to the release of the catalytic RING2 domain and locks Parkin in a functionally active state. Activated Parkin exhibits low substrate specificity and ubiquitylates outer mitochondrial membrane proteins including voltage-dependent anion-selective channel (VDAC) and mitochondrial Rho GTPase (MIRO) proteins (Sarraf et al., 2013; Ordureau et al., 2014; Gladkova et al., 2018).

Studies in cardiomyocytes demonstrated that PINK1 phosphorylates (at serine 442 and threonine 111) mitofusin 2 (MFN2), a GTPase that mediates mitochondrial fusion, which in turn mediates the recruitment of Parkin to damaged mitochondria for mitophagy initiation (Chen and Dorn, 2013; Xiong et al., 2019). Furthermore, it has been suggested that mitochondrial fission might be yet another prerequisite for the initiation of mitophagy. Thus, it was reported that Parkin, among other substrates, ubiquitylates mitofusin 1 (MFN1) and MFN2, leading to their proteasomal degradation, and subsequent mitochondrial fission preceding mitophagy, while the inhibition of mitochondrial fission prevented Parkin-induced mitophagy (Tanaka et al., 2010). Parkin-mediated poly-ubiquitination of outer mitochondrial membrane proteins triggers the recruitment of autophagy receptors such as optineurin (OPTN), calcium binding and coiled-coil domain 2 (CALCOCO2, better known as NDP52) and Tax1 binding protein 1 (TAX1BP1), concomitantly with the activation of the TANK binding kinase 1 (TBK1) that phosphorylates OPTN (at serine 177, 473, and 513) further enhancing its ubiquitin chain binding ability (Wild et al., 2011; Wong and Holzbaur, 2014; Heo et al., 2015; Lazarou et al., 2015). Once recruited to the mitochondria, autophagy receptors can employ initiator proteins from the autophagic machinery such as unc-51 like autophagy activating kinase 1 (ULK1), zinc finger FYVE-type containing 1 (ZFYVE1, better known as DFCP1) and WD repeat domain, phosphoinositide interacting 1 (WIPI1, also known as ATG18) to assemble the autophagosome (Wong and Holzbaur, 2014; Lazarou et al., 2015; Ravenhill et al., 2019; Turco et al., 2019; Vargas et al., 2019) and ATG8s, which could further recruit autophagy receptors to amplify mitophagy signals (Padman et al., 2019). The key function of the ULK1-containing complex for selective autophagy has been recently discussed elsewhere (Turco et al., 2020). Additionally, independently of Parkin, PINK1 may recruit NDP52 and optineurin to mitochondria to directly stimulate mitophagy (Lazarou et al., 2015). It has also been suggested that Parkin mediates the broad proteasomal degradation of outer mitochondrial membrane proteins which leads to membrane rupture and the exposure of the mitophagy receptor prohibitin 2 (PHB2) (Chan et al., 2011; Wei et al., 2017). Conversely, PHB2 can promote PINK1/Parkin-dependent mitophagy by inhibiting the function of PARL and the resultant stabilization of PINK1 on the surface of mitochondria (Yan et al., 2019). However, cells deficient of all Atg8 family members could still undergo mitophagy although the overall size of mitophagosomes is smaller (Nguyen et al., 2016).

Although mutations or deletions of Parkin or PINK1 cause Parkinson disease in humans, mice deficient in either PINK1 or Parkin do not display any related phenotype. However, accumulating evidence shows that Parkinson's disease is accompanied by immune responses that lead to an increase in serum levels of pro-inflammatory cytokines such as interleukin-6 (IL6), tumor necrosis factor alpha (TNFα), interleukin-1β (IL1B), and interferon gamma (IFNG) (Brodacki et al., 2008; Koziorowski et al., 2012; Lindqvist et al., 2012; Dzamko et al., 2015; Houser and Tansey, 2017; Caggiu et al., 2019). Consistently, the challenge of PINK1 or Parkin deficient mice with immunogenic stress leads to the onset of Parkinson disease-like symptoms (Frank-Cannon et al., 2008; Sliter et al., 2018; Matheoud et al., 2019). Thus, administration of low-dose lipopolysaccharide (LPS) can cause subtle fine-motor deficits and selective loss of dopaminergic neurons in substantia nigra in Parkin deficient mice, although LPS treatment triggered similar persistent neuroinflammation in both wild type and *Parkin*$^{-/-}$ mice (Frank-Cannon et al., 2008). The loss of dopaminergic neurons and motoric defects also occur in aged *Parkin*$^{-/-}$; *mutator* mice (Sliter et al., 2018), which accumulate mutations

in mtDNA, as well as in *Pink1*$^{-/-}$ mice that were orally infected with Gram-negative bacteria (Matheoud et al., 2019). In macrophages, dysfunctional mitochondria are marked by Parkin-dependent ubiquitylation and then recognized by sequestosome 1 (SQSTM1, better known as p62), which is transcriptionally upregulated by nuclear factor kappa B (NF-κB), followed by mitochondrial clearance via mitophagy. This NF-κB and p62-dependent mitophagy pathway prevents excessive inflammation by restraining NLRP3-inflammasome overactivation (Zhong et al., 2016). Moreover, mtDNA released from damaged mitochondria can promote stimulator of interferon response cGAMP interactor 1 (STING1)-dependent interferon regulatory factor 3 (IRF3)-mediated signaling triggering inflammatory response (West et al., 2015), while Parkin-mediated mitophagy prevents inflammation by mitophagic mtDNA clearance (Sliter et al., 2018). Additionally, PINK1 and Parkin signaling can suppress inflammation by repressing mitochondrial antigen presentation delivered by mitochondrial derived vesicles (Matheoud et al., 2016).

The roles of PINK1 and Parkin in heart function have been extensively studied. PINK1 protein levels significantly decrease in humans with end-stage heart failure. PINK1 deficient mice develop left ventricular dysfunction and pathological cardiac hypertrophy, characterized by an increase in oxidative stress and impaired mitochondrial function (Billia et al., 2011). Different from PINK1, Parkin deficiency sensitizes mice to myocardial infarction resulting in reduced overall survival. Morphologically, Parkin deficiency manifests with a disorganized mitochondrial network and a significant decrease in mitochondrial size. Nevertheless, Parkin-deficient mice exhibit normal cardiac function for up to 12 months of age (Kubli et al., 2013). In response to cardiac ischemia, Parkin-mediated mitophagy is induced to mitigate detrimental effects of a prolonged lack of oxygen supply in the heart of wild type mice, indicating the important role of mitophagy for heart homeostasis (Kubli et al., 2013). Simvastatin, an HMG CoA reductase inhibitor used to lower low-density lipoprotein (LDL) and triglycerides levels and thus to prevent heart attack, can stimulate Parkin-dependent mitophagy. Simavastin has the ability to reduce the size of the infarction caused by ischemia/reperfusion in wild-type mice but not in Parkin-deficient animals (Andres et al., 2014). Interestingly, mtDNA released from damaged mitochondria triggers inflammatory responses in cardiomyocytes that culminate in myocarditis and dilated cardiomyopathy (Oka et al., 2012). Moreover, Parkin mediated mitophagy turns over fetal cardiomyocyte mitochondria to facilitate the replacement of mature adult mitochondria, an effect that likely contributes to the perinatal maturation of cardiac metabolism (Kageyama et al., 2014; Gong et al., 2015; Lampert et al., 2019).

FUNDC1-MEDIATED MITOPHAGY

FUN14 domain containing 1 (FUNDC1) is an outer mitochondrial membrane protein with three transmembrane domains, which serves as a mitophagy receptor in mitochondrial uncoupling-, and hypoxia-mediated mitophagy as well as paternal mitochondrial clearance in *C. elegans* (Liu et al., 2012; Chen et al., 2014; Lim et al., 2019). FUNDC1 contains a conserved microtubule associated protein 1 light chain 3 beta (MAP1LC3B better known as LC3)-interacting region (LIR) domain facing the cytosol, which is necessary for its interaction with LC3, a key regulator of autophagy (Liu et al., 2012). FUNDC1-deficiency blocks hypoxia-induced mitophagy, which can be rescued by re-expressing wild-type FUNDC1 but not with a LIR-mutated protein, indicating a key role of LIR-mediated LC3 interaction in FUNDC1 activity (Liu et al., 2012). Indeed, FUNDC1 is constitutively phosphorylated (at tyrosine 18 and serine 13) by the protein kinases SRC proto-oncogene, non-receptor tyrosine kinase (SRC) and casein kinase 2 (CK2), respectively, which reduces its interaction with LC3 (Liu et al., 2012; Chen et al., 2014). Upon hypoxia or loss of mitochondrial transmembrane potential, dephosphorylation (of tyrosine 18 and serine 13) mediated by the mitochondrial phosphatase PGAM family member 5 (PGAM5) and concomitant phosphorylation (of serine 17) by ULK1 enhances the interaction of FUNDC1 with LC3 to promote mitophagy (Liu et al., 2012; Chen et al., 2014; Wu W. et al., 2014). However, the phosphatase responsible for (tyrosine 18) dephosphorylation remains elusive.

The activity of PGAM5 is fine-tuned to regulate FUNDC1-mediated mitophagy, thus during homeostasis PGAM5 activity is inhibited by BCL2-like 1 (BCL2L1 better known as BCL-XL), and the degradation of BCL-XL induced by hypoxia leads to the dephosphorylation of FUNDC1 and the induction of mitophagy (Wu H. et al., 2014). Under oxidative stress conditions, PGAM5 forms multimers to release BCL-XL, which in turn is followed by an increase in BCL-XL phosphorylation and ultimately leads to apoptosis. Once liberated from BCL-XL sequestration, multimeric PGAM5 is able to dephosphorylate FUNDC1, to augment mitochondrial fission and induce mitophagy. Thus, the reciprocal interaction between PGAM5 with BCL-XL and FUNDC1 may serve as a molecular switch between mitophagy and apoptosis under oxidative stress conditions (Ma et al., 2019). Recent studies suggested additional factors such as syntaxin 17 (STX17), a SNARE protein located in the mitochondria-associated membranes (MAM) and mitochondria is also required for PGAM5 to dephosphorylate FUNDC1 during mitophagy (Sugo et al., 2018).

Moreover, in addition to this tight control, mitochondrial dynamics participate in FUNDC1-mediated mitophagy. Thus, it was reported that FUNDC1 interacts with both the mitochondrial fission key factor dynamin 1 like (DNM1L, better known as DRP1) and inner membrane fusion regulator OPA1 mitochondrial dynamin like GTPase (OPA1) to coordinate mitochondrial dynamics and mitophagy. Mitophagic stress stimulates the disassembly of the FUNDC1-OPA1 complex, while enhancing the association of FUNDC1 with DRP1, leading to mitochondrial fission, thus fostering mitophagy (Chen et al., 2016). FUNDC1 was described to associate with the ER protein calnexin (CANX) in mitochondria-associated ER membranes (MAMs). During hypoxia, the association between FUNDC1 and CANX is decreased, thereby liberating FUNDC1 for its interaction with DRP1, triggering mitochondrial fission and mitophagy (Wu W. et al., 2016). Interestingly,

membrane associated ring-CH-type finger 5 (MARCHF5) can ubiquitylate FUNDC1 for proteasomal degradation, and desensitize mitochondria to hypoxia-induced mitophagy thus constituting a negative regulation mechanism at early stages of hypoxia (Chen et al., 2017).

The physiological role of FUNDC1 has been studied in detail and it has been shown that FUNDC1 plays an important role in liver cancer and obesity. In a mouse model of human hepatocellular carcinoma (HCC) induced by the chemical carcinogen, diethylnitrosamine (DEN), liver specific knockout of FUNDC1 facilitates the cytosolic release of mtDNA due to a defect in mitophagy, resulting in an accumulation of dysfunctional mitochondria, an elevated release of proinflammatory cytokines, such as IL1B and hyperproliferation of hepatocytes, finally culminating in the initiation and progression of DEN-induced HCC (Li et al., 2019). Furthermore, skeletal-muscle-specific knockout of FUNDC1 impairs mitochondrial energetics and negatively affects physical fitness. However, FUNDC1 deficiency decreases the susceptibility to high-fat-diet-induced obesity with improved insulin sensitivity and glucose tolerance. In fact, FUNDC1 deficiency elicits a retrograde response in muscle with an upregulation of fibroblast growth factor 21 (FGF21) expression, and thereby promotes the thermogenic remodeling of adipose tissue (Fu et al., 2018). FUNDC1 and BCL2 interacting protein 3 like (BNIP3L, better known as NIX) but not PINK1/Parkin-dependent mitophagy facilitates the removal of impaired mitochondria and thus maintains mitochondrial network reorganization during cardiac progenitor cell (CPC) differentiation. Interestingly, mice expressing a proofreading-defective mitochondrial DNA polymerase G gamma ($PolG^{D257A/D257A}$), experience premature aging and develop accelerated age-related cardiomyopathy due to the accumulation of mtDNA mutations (Lampert et al., 2019).

BNIP3 AND NIX-DEPENDENT MITOPHAGY

BCL2 interacting protein 3 (BNIP3) and NIX, belong to the BH3 only domain proteins of the BCL2 family, which localize at the outer mitochondrial membrane and are involved in stress sensing and the induction of cell death when cellular stress prevails (Zhang and Ney, 2009). More recently, the role of BNIP3 and NIX in autophagy has been extensively studied. Both BNIP3 and NIX are hypoxia-inducible genes (Bruick, 2000; Sowter et al., 2001; Kubasiak et al., 2002), and play an important role in hypoxia-induced macroautophagy and mitophagy (Zhang and Ney, 2009). An increase in BNIP3 protein levels can lead to the liberation of Beclin1 (BECN1) from BCL2 apoptosis regulator (BCL2) and/or BCL-XL sequestration to initiate mitophagy, to prevent ROS production and subsequent cell death (Zhang et al., 2008).

NIX is known for its prominent function in the mitophagy-dependent maturation of red blood cells. Mammalian erythroid cells undergo enucleation and the removal of organelles during terminal differentiation, in which the maturation process of enucleated immature reticulocytes to erythrocyte necessitates complete mitochondrial clearance depending on NIX (Schweers et al., 2007; Sandoval et al., 2008). During erythrocyte differentiation NIX expression is significantly increased, and leads to a decrease in mitochondrial transmembrane potential and the induction of mitophagy (Aerbajinai et al., 2003). Cells from Nix-deficient mice exhibit defects in the incorporation of mitochondria into autophagosomes and further autophagosomal maturation (Schweers et al., 2007; Sandoval et al., 2008). Furthermore, the elimination of mitochondria does not require the core autophagic gene ATG5, but depends on the autophagic kinase ULK1, indicating a specific function of ULK1 in mitophagy during red blood cell maturation (Kundu et al., 2008; Honda et al., 2014).

Mechanistic analysis indicated that NIX functions as a mitophagy receptor that interacts with LC3 via its LIR domain and thus recruits LC3 family proteins to damaged mitochondria. Ablation of the NIX-LC3/GABA type A receptor-associated protein (GABARAP) interaction retards mitochondrial clearance in maturing murine reticulocytes (Novak et al., 2010). Similarly, the mutation of the LIR motif within the BNIP3 gene leads to the ablation of BNIP3-LC3 interaction and impairs mitophagy and ERphagy, although it does not affect the pro-death activity of BNIP3 (Hanna et al., 2012). Interestingly, the interaction of BNIP3 and NIX with LC3 are fine-tuned by the phosphorylation state of moieties adjacent to the LIR domain. Thus, the phosphorylation of serine 17 and serine 24 flanking the BNIP3 LIR motif promotes its binding affinity to LC3 and GABA type A receptor associated protein like 2 (GABARAPL2) (Zhu et al., 2013). Likewise, phosphorylation of NIX (at serine 34 and 35) in close proximity to the LIR stabilizes the NIX-LC3 complex and enhances autophagosomal recruitment to mitochondria (Rogov et al., 2017). However, the kinases and phosphatases specific for BNIP3 and NIX phosphorylation remain elusive. Moreover, high oxidative phosphorylation activity leads to the recruitment of the small GTPase Ras homolog, mTORC1 binding (RHEB) to the mitochondrial outer membrane which promotes mitophagy through physical interaction with NIX and LC3 (Melser et al., 2013).

In several human cancer types, including hematological malignancies, lung, breast, gastric, pancreatic, and liver cancer, the epigenetic silencing of BNIP3 expression is reported to correlate with invasiveness and metastasis (Okami et al., 2004; Koop et al., 2009; Chourasia et al., 2015). Conversely, some studies indicate that BNIP3 and NIX are upregulated in human breast ductal carcinoma *in situ*, which manifest with high-grade, necrotic lesions and invasive tumors (Sowter et al., 2001, 2003). In malignant glioma cells, ceramide induces autophagic cell death via lethal mitophagy (Sentelle et al., 2012), through the activation of BNIP3 (Daido et al., 2004). However, the role of BNIP3- and NIX-mediated mitophagy in cancer has to be carefully reevaluated, considering the fact that BNIP3 and NIX are proapoptotic BH3-only proteins. Furthermore, the expression of BNIP3 is upregulated in post-natal ventricular myocytes and adult rat hearts subjected to hypoxia, and in animals that exhibit a chronic heart failure, which is associated with myocardial cell death. Both the pan-caspase inhibitor z-VAD-fmk and the mitochondrial permeability transition pore (MPTP) inhibitor bongkrekic acid prevent BNIP3-induced mitochondrial defects

and cell death (Regula et al., 2002). In yet another ischemia model, hypoxia upregulates mRNA and protein levels of BNIP3, while acidosis stabilizes the protein and increases its association with mitochondria for the induction of cell death (Kubasiak et al., 2002). In ischemia induced injury, BNIP3 is engaged in pro-death signaling, whereas its role in mitophagy in this setting needs further investigation (Hamacher-Brady et al., 2007). It has been reported that mitophagy is significantly impaired in neurodegenerative disorders such as Alzheimer's disease (AD) and Ataxia telangiectasia (A-T), while mitophagy stimulation induces beneficial effect including an increase in cognition and an extended lifespan in a NIX- or PINK1 and Parkin-dependent manner (Fang et al., 2016, 2019). Mitophagy restoration enhances the phagocytic efficacy of microglia to diminish the aggregation of insoluble amyloid-β, and thus reduces pro-inflammatory factors such as IL6 and TNFA while increasing the anti-inflammatory cytokine interleukin-10 (IL10) which has been shown to promote mitophagy in macrophages (Ip et al., 2017; Fang et al., 2019).

ATG32/BCL2L13-CONTROLLED MITOPHAGY

In yeast, mitophagy selectively occurs in post-log phase cells under respiratory conditions. Mitophagy protein Atg32 (Atg32) is a transmembrane protein imbedded in the outer mitochondrial membrane with a ubiquitin-like protein Atg8 (Atg8) interacting-motif (AIM) for the recruitment of autophagosomes (Okamoto et al., 2009). In addition, Atg32 interacts with the adaptor autophagy protein Atg11 (Atg11) to facilitate the incorporation of mitochondria into the nascent autophagic vacuole (Kanki et al., 2009). The activity of Atg32 is additionally regulated via proteolytical cleavage by the mitochondrial i-AAA protease Yme1 (Yme1), which is essential for the interaction between Atg32 and Atg11 and the induction of mitophagy (Wang et al., 2013). Atg32 activity is further fine-tuned via the phosphorylation at Ser114 and Ser119 by casein kinase 2 (CK2) downstream of the mitogen-activated protein kinases (MAPK) Hog1 and Pbs2 to promote its interaction with Atg11 (Aoki et al., 2011; Mao et al., 2011; Kanki et al., 2013). Alternatively, yet another MAPK signaling pathway implicating Slt2 can regulate both mitophagy and the selective degradation of peroxisomes (pexophagy), although the mechanism remains elusive (Mao et al., 2011). Mitochondrial dynamics appear to constitute another regulatory instance for the induction of mitophagy in yeast. Thus, Atg11 recruits the fission machinery to mitochondria via its interaction with Dnm1 to segregate degrading mitochondria from the network for mitophagy (Mao et al., 2013). The mammalian homolog of Atg32 has been identified as Bcl-2-like protein 13 (BCL2L13), which also contains a LIR domain to interact with LC3 and can induce mitophagy in mammalian cells and Atg32 deficient yeast (Murakawa et al., 2015, 2019). A recent study indicated that Atg32 might be implicated in age asymmetry between the mother and daughter cells in yeast (Jiang et al., 2019). However, the detailed roles of Atg32 and/or BCL2L13 in aging and age-related diseases need further research.

OTHER MITOPHAGY RECEPTORS

During recent years with increasing interest in the exploration of mitophagy, additional mitophagy receptors have been identified to mediate mitophagy including autophagy and beclin 1 regulator 1 (AMBRA1), which acts in a PARKIN- and p62-independent manner (Di Rita et al., 2018; Strappazzon et al., 2019), FK506 binding protein 8 (FKBP8) that specifically interacts with microtubule associated protein 1 light chain 3 alpha (MAP1LC3A better known as LC3A) and thus facilitates mitophagy (Bhujabal et al., 2017), and NLR family member X1 (NLRX1) which contains an LIR domain and is harnessed by Listeria during infection to induce mitophagy for its survival in macrophages (Zhang et al., 2019). Interestingly, upon mitochondrial depolarization, 4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (NIPSNAP1) and NIPSNAP2 translocate from the mitochondrial matrix to the surface of the organelle and recruit autophagy receptors and ATG8 proteins for mitophagy. It is worthy to note that NIPSNAP1-deficient zebrafish larvae display parkinsonian phenotypes, including the loss of tyrosine hydroxylase (Th1)-positive dopaminergic (DA) neurons, reduced motor activity, and increased oxidative stress, as well as reduced mitophagy in the brain (Princely Abudu et al., 2019).

Lipids can also function as mitophagy receptors by interacting with LC3. Thus, ceramide has been reported to target autophagolysosomes to mitochondrial membranes and provoke lethal mitophagy (Sentelle et al., 2012). However, in acute myeloid leukemia (AML) cells, ceramide synthesis is suppressed by Fms-like tyrosine kinase 3 (FLT3)-internal tandem duplication (ITD) signaling, which confers its resistance to cell death. Molecular or pharmacologic inhibition of FLT3-ITD in AML cells reactivated ceramide synthesis, mitochondrial division, mitophagy and cell death, indicating a potential application for the therapeutic induction of mitophagy in cancer (Dany et al., 2016). While cardiolipin, a phospholipid mainly localized at the inner mitochondrial membrane, can externalize to the outer membrane and serve as a mitophagy receptor in neuronal cells (Chu et al., 2013). Cardiolipin mediated mitophagy has been shown to play an important role in traumatic brain injury (TBI) by removing damaged mitochondria thus mitigating ROS overproduction and decreasing apoptosis (Chao et al., 2019).

PIECEMEAL MITOPHAGY

Besides the wholesale mitophagy described above, a piecemeal mitophagy mechanism exists to deliver small vesicles budded off from mitochondria to lysosomes for degradation, which is important for the maintenance of mitochondrial homeostasis (**Figure 2**). In a screen aiming at the identification of autophagic protein substrates, metaxin1 (MTX1) was shown to be degraded by piecemeal mitophagy, in which MTX1-containing vesicles are segregated from mitochondria and then degraded by lysosomes in a microtubule associated protein 1 light chain 3 gamma (MAP1LC3C better known as LC3C)- and p62-dependent manner (Le Guerroue et al., 2017). When mitochondria

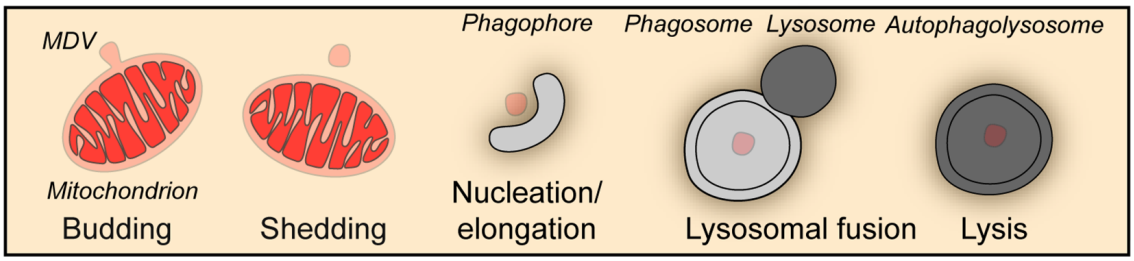

FIGURE 2 | Piecemeal mitophagy. Mitophagy can occur through the formation of mitochondria-derived vesicles (MDV), which in turn are degraded by the autophagic machinery in a piecemeal fashion.

face unfolded protein stress, PINK1 and Parkin facilitate a DRP1-dependent segregation of mitochondrial subdomains from the network for degradation by mitophagy to prevent proteotoxicity spreading (Burman et al., 2017). Furthermore, under oxidative stress, TOMM20 positive mitochondrial derived vesicles deliver oxidized proteins to lysosomes for degradation (Soubannier et al., 2012a,b). Strikingly, this process does not require ATG5 or LC3, but is driven by PINK1 and Parkin and depends on syntaxin 17 (STX17) to mediate the fusion between vesicles and endolysosomes (Soubannier et al., 2012a; McLelland et al., 2014, 2016).

THE ROLE OF MITOPHAGY IN AGING

Heteroplasmy of mtDNA is a hallmark of aging. The homogeneity of mtDNA in newborn life is ensured by the selective removal mechanism of deleterious mtDNA in the female germline (Lieber et al., 2019) and paternal mitochondrial removal after fertilization (Al Rawi et al., 2011; Sato and Sato, 2011; Politi et al., 2014; Rojansky et al., 2016; Sato et al., 2018), in both of which mitophagy is highly involved. As mtDNA mutations and deletions accumulate with age, which are associated with a variety of diseases, such as cancer, neurodegenerations, and cardiovascular diseases (Liu et al., 1996; Petros et al., 2005; Sharma et al., 2005; Wallace, 2005; Turnbull et al., 2010), mitochondrial respiration activity and mitochondrial function are damaged, which lead to decreased mitochondrial potential. It has been reported that Parkin is recruited to mitochondria with low potential and required for the mitophagic degradation of malfunctional mitochondria with mtDNA mutations (Gilkerson et al., 2012). And long-term overexpression of Parkin can increase the ratio between the mitochondria with wild type mtDNA and the ones with deleterious COXI mutations (Suen et al., 2010). Interestingly, in mice, even heteroplasmy of normal mtDNA leads to reduced activity, food intake, respiratory exchange ratio; accentuated stress response; and cognitive impairment (Sharpley et al., 2012), which might be related to the absence of mitophagy-dependent elimination of paternal mitochondria. Although mitochondria are mostly of maternal origin, resulting from the mitophagy-dependent clearance of paternal mitochondria, exceptional cases are reported in human (Luo et al., 2018), sheep (Zhao et al., 2001), mouse (Gyllensten et al., 1991), and drosophila (Nunes et al., 2013; Dokianakis and Ladoukakis, 2014) in which paternal inheritance of mtDNA and thus mtDNA heteroplasmy exist. In *C. elegans*, mitophagy-dependent paternal mitochondrial elimination has been extensively studied, and delayed clearance of paternal mitochondrial after fertilization leads to an increase in embryonic lethality (Zhou et al., 2016). However, the effect of normal mtDNA heteroplasmy on aging needs further research.

The involvement of mitophagy in aging has been extensively studied in *C. elegans*. Mitophagy mediated by dct-1, the ortholog of NIX, plays an important role during *C. elegans* aging. Mitochondria accumulate with age in wild type worms, and deficiency in *dct-1*, as well as the autophagy key gene bec-1, recapitulates the effect of aging on mitochondrial mass in young adult animals. Pronounced induction of mitophagy was observed in long-lived *daf-2* mutants, and impairment of mitophagy by knockdown of *dct-1*, *pink-1,* and *pdr-1* (the nematode Parkin homolog) significantly shortens the lifespan of *daf-2* mutants. In fact, *dct-1* is transcriptionally induced under the control of *skn-1* and *daf-16* [the nematode homolog of mammalian nuclear factor, erythroid 2 like 2 (NFE2L2, better known as NRF2) and forkhead box O3 (FOXO3), respectively] to remove dysfunctional mitochondria via mitophagy and coordinate mitochondrial biogenesis and mitophagy (Palikaras et al., 2015). Mitochondrial biogenesis and mitophagy may cooperate to antagonize the aging process (Palikaras et al., 2015; Fang et al., 2017). Interestingly, tomatidine, a natural compound abundant in unripe tomatoes, inhibits age-related skeletal muscle atrophy in mice and extends health- and lifespan in *C. elegans*. Mechanistic analysis showed that tomatidine stimulates mitochondrial biogenesis and PINK1- and DCT1-related mitophagy and increases healthspan (Fang et al., 2017). Moreover, *dct-1*, *pink-1*, and *pdr-1* are engaged in lifespan extension induced by mild mitochondrial stress achieved by frataxin depletion-induced iron-starvation in *C. elegans* (Schiavi et al., 2015). Excessive iron chelation also stimulates mitophagy in mammalian cells, which however does not require PINK1 or Parkin activation but depends on glycolysis (Allen et al., 2013).

Exercise has long been known to promote healthy aging and decrease the susceptibility to age-related diseases probably, depending on the induction of autophagy (He et al., 2012; Escobar et al., 2019). Mitophagy may also be involved in the

beneficial effects of exercise. A recent study has shown that exercise activates the AMPK-ULK1 cascade to provoke the removal of damaged mitochondria via mitophagy. Moreover, exercise improves glucose tolerance in wild type mice but not in ULK1 deficient mice (Laker et al., 2017).

Caloric restriction is yet another way to extend healthy lifespan. Similar to exercise, nutrient deprivation activates the AMPK-ULK1 cascade that is required for mitophagy to remove damaged mitochondria and promote cellular survival (Egan et al., 2011). Nutrient starvation causes the rapid depletion of cytosolic acetyl-coenzyme A, and subsequently reduces the activity of the acetyltransferase E300, which is known to acetylate ATG proteins and to inhibit their pro-autophagic function (Lee and Finkel, 2009; Marino et al., 2014). The depletion of general control of amino acid synthesis 5-like 1 (GCN5L1), a component of the mitochondrial acetyltransferase machinery that counteracts deacetylation mediated by SIRT3 (Scott et al., 2012), results in p62 and Atg5-mediated mitochondrial autophagy (Webster et al., 2013). Furthermore, the depletion of GCN5L activates both the transcription factor EB (TFEB), which is a master regulator of autophagy, and PPARγ coactivator 1α (PGC-1α), which controls mitochondrial biogenesis, coordinating the turnover and biogenesis of mitochondria (Scott et al., 2014).

Due to the difficulties to maintain long-term caloric restriction, the concept of caloric restriction mimicry has been developed (Madeo et al., 2019). The intracellular concentration of spermidine, a natural polyamine and prototype caloric restriction mimetic (CRM), declines during aging, and the administration of spermidine can extend the lifespan of yeast, flies and worms, and human immune cells (Eisenberg et al., 2009). Interestingly, spermidine stimulates mitophagy in cardiomyocytes of both young and aged mice, which might impinge on spermidine-mediated cardioprotection (Eisenberg et al., 2016). However, the role of mitophagy in spermidine induced lifespan extension needs further investigation. Aspirin, another CRM, induces autophagy by inhibiting EP300 and stimulates mitophagy in the heart of mice (Pietrocola et al., 2018). Additionally, *dct-1*, the *C. elegans* ortholog of the mammalian mitophagy receptor NIX and BNIP3, mediates longevity and mitophagy in nematodes (Palikaras et al., 2015), and silencing of *dct-1* abolished aspirin induced autophagy in *C. elegans* (Pietrocola et al., 2018). Different from spermidine and aspirin which stimulate autophagy by inhibiting acetylase EP300, induction of autophagy by resveratrol, a naturally occurring polyphenol (and yet another CRM), requires the nicotinamide adenine dinucleotide–dependent deacetylase sirtuin 1 (SIRT1) (Morselli et al., 2011). Apparently, resveratrol has the capacity to induce mitophagy through increasing the expression of PINK1, Parkin, and Beclin1, and AMPK activation by resveratrol participates in neurodegenerative diseases, cerebral ischemia, muscular dystrophy, and inflammation (Ferretta et al., 2014; Wu J. et al., 2016; Sebori et al., 2018; Wang et al., 2018; Cao et al., 2019; Pineda-Ramirez et al., 2019).

Additional compounds exert their lifespan extending effect via mitophagy. Thus, urolithin A, the end-products of both ellagitannins and ellagic acid, extends lifespan and improves fitness during *C. elegans* aging and improves muscle function and exercise capacity in rodents. In-depth analysis demonstrates that mitophagy is required for the beneficial effect of urolithin A (Ryu et al., 2016). Recently, it was reported that Urolithin A reverses memory impairment through PINK1-, PDR1-, or DCT1-dependent mitophagy in both amyloid-β (Aβ) and tau *C. elegans* models of Alzheimer's disease (Fang et al., 2019). A clinical investigation suggests that urolithin A improves mitochondrial and cellular health following regular oral consumption in humans (Andreux et al., 2019). However, one report suggests that urolithin A stimulates autophagy but not mitophagy to inhibit ER stress in a model of ischemic neuronal injury (Ahsan et al., 2019).

Nicotinamide adenine dinucleotide (NAD) is a critical metabolite involved in many physiological processes, including metabolism, post-translational protein modification, and DNA repair and its concentration is closely associated with aging. NAD levels decrease with age, while the upregulation or replenishment of NAD metabolism has been shown to exhibit beneficial effects against aging and age-associated diseases (Li et al., 2001; Mouchiroud et al., 2013; Yaku et al., 2018). Treatments that increase intracellular NAD^+ improve mitochondrial quality via mitophagy and thus extend health- and life-span in Ataxia Telangiectasia models and reverse cognitive deficits in models of Alzheimer's disease (Fang et al., 2016, 2019). Sirtuins, whose activity depend on NAD^+, may also participate in NAD^+ administration stimulated mitophagy, and it appear that their function declines with aging (Li et al., 2001; Mouchiroud et al., 2013; Feldman et al., 2015; Kerr et al., 2017). Interestingly, in response to oxidative stress, SIRT3, a mitochondrial sirtuin, deacetylates the transcription factor FOXO3 to regulate BNIP3, NIX and LC3 expression, thereby stimulating mitophagy as well as mitochondrial biogenesis and dynamics (Tseng et al., 2013).

Rapamycin, an allosteric inhibitor of mechanistic target of rapamycin (mTOR), prolongs life in yeast, worms, flies, and mice. Rapamycin also prevents age-related conditions in rodents, dogs, nonhuman primates, and humans (Blagosklonny, 2019). mTOR is a critical nutrient sensor and has multiple downstream effects, including protein synthesis, and autophagy. Recent studies indicate that eliminating damaged mitochondria via mitophagy may be one of the mechanisms responsible for the beneficial effects of rapamycin. Tuberous sclerosis complex 2 (TSC2) is upstream of mTOR and its inhibition leads to constitutive mTOR activation. Interestingly, TSC2 deficiency impairs mitophagic

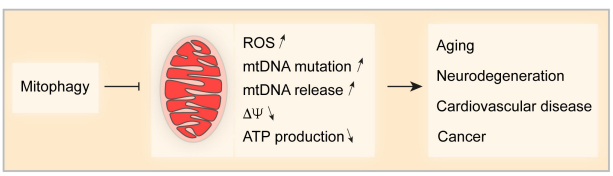

FIGURE 3 | Impact of Mitophagy on age-related pathologies. Mitophagy is a key mechanism for mitochondrial quality and quantity control. Thus, mitophagy limits the production of reactive oxygen species (ROS), the accumulation of mutations in and the release of mitochondrial DNA (mtDNA), appearance of transmembrane potential loss and the decrease in ATP production. Taken together, mitophagy controls various factors that can drive pathologies such as aging-related disorders and neurodegeneration, cardiovascular disease and cancer.

flux, as indicated by reduced expression of PINK1 and PARK2 translocation to uncoupled mitochondria, a defect that can be restored with rapamycin administration (Bartolome et al., 2017). Moreover, axonal and global mitophagy of damaged mitochondria is impaired in neuronal *in vitro* and *in vivo* models of tuberous sclerosis complex, contrasting with the fact that blocking mTORC1 or inducing mTOR-independent autophagy restores mitochondrial homeostasis (Ebrahimi-Fakhari et al., 2016). In another study, rapamycin significantly enhanced mitophagy by increasing the translocation of p62 and Parkin to the damaged mitochondria in a mouse spinal cord injury model (Li et al., 2018). Consistent with these findings, PINK1 and Parkin-dependent mitophagy is impaired and mTOR is hyperactivated in primary human fibroblasts derived from individuals with Down syndrome. In this context, inhibition of mTOR using AZD8055 restores autophagic flux, as well as mitophagy initiated by PINK1 and Parkin (Bordi et al., 2019).

PERSPECTIVES

Mitochondria are important for cellular life and death, implying that mitochondrial homeostasis must be tightly controlled and fine-tuned when cells respond to stress. Mitophagy is the primordial mechanisms for mitochondrial quality and quantity control and multiple mechanisms control this process. Some studies indicate an ample crosstalk between different mitophagy pathways that may coordinate and complement to deal with environmental challenges. Nevertheless, the detailed mechanism that link the different pathways in the complex network of mitophagy control need further investigation (Chen et al., 2014; Gao et al., 2015; Zhang et al., 2016). Dysfunction of mitochondria is one of the major characteristics of aging and age-related disease. Increasing evidence shows that mitophagy (by removing damaged mitochondria) is significantly involved in counterbalancing age-related pathological conditions (**Figure 3**). Thus, chronic stimulation of mitochondrial turnover by enhancing mitophagy is a promising approach to delay age-related diseases and to extend health- and lifespan.

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REVIEW
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MitophAging: Mitophagy in Aging and Disease

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Maintaining mitochondrial health is emerging as a keystone in aging and associated diseases. The selective degradation of mitochondria by mitophagy is of particular importance in keeping a pristine mitochondrial pool. Indeed, inherited monogenic diseases with defects in mitophagy display complex multisystem pathologies but particularly progressive neurodegeneration. Fortunately, therapies are being developed that target mitophagy allowing new hope for treatments for previously incurable diseases. Herein, we describe mitophagy and associated diseases, coin the term mitophaging and describe new small molecule interventions that target different steps in the mitophagic pathway. Consequently, several age-associated diseases may be treated by targeting mitophagy.

Keywords: autophagy, mitophagy, aging, mitophaging, monogenic disorders, interventions

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MITOCHONDRIAL INTEGRITY DEFINES ORGANISMAL HEALTH

Mitochondria, the powerhouses of eukaryotic cells, are the key organelles for energy production allowing organismal growth and survival. Besides serving as adenosine triphosphate generators, mitochondria act as signaling hubs for programmed cell death, regulate calcium homeostasis and are required for cholesterol, nucleotide and amino acid synthesis (Sun et al., 2016). To fulfil their broad range of biological roles, mitochondria contain more than 1,000 proteins that localize and function in four specialized compartments, the outer membrane, the inner membrane, the intermembrane space and the matrix. The minority of mitochondrial proteins are encoded by the circular mitochondrial genome, whereas the vast majority is encoded in the nuclear genome. However, mutations in both genomes can cause a heterogeneous group of disorders, known as mitochondrial diseases, which are characterized by severe metabolic and neurological defects. Due to their highly variable clinical features, the prevalence of mitochondrial diseases has likely been underestimated (Haas et al., 2007; Wallace, 2018). Nevertheless, advances in next generation sequencing technologies have simplified the clinical diagnosis and enabled molecular characterization of so far undescribed mitochondrial diseases (Calvo et al., 2012; Cui et al., 2013; Legati et al., 2016). Notably, computational approaches relying on phenotypic description of mitochondrial diseases can help to characterize new mitochondrial diseases of previously unknown pathogenesis (Scheibye-Knudsen et al., 2013).

Increased evidence indicates that mitochondrial integrity is disrupted during the aging process and contributes to the pathogenesis of age-related disorders in humans (Kauppila et al., 2017; Youle, 2019). In line with this, mice that carry a defective proof-reading mitochondrial DNA polymerase gamma show an accelerated aging phenotype that may be driven by the accumulation of mutations in the mitochondrial DNA (mtDNA) (Trifunovic et al., 2004). The described correlation between levels of mtDNA deletions in human brain and aging as well as the association between mtDNA haplogroups and diseases, further supports the direct influence of mitochondria on health- and

lifespan in organisms (Cortopassi and Arnheim, 1990; Corral-Debrinski et al., 1992; Hudson et al., 2014; Wallace, 2015). Indeed, dysfunctional degradation of mitochondria through the process of mitophagy is increasingly associated with degenerative diseases and aging, a phenomenon we call mitophaging. Evidently, the maintenance of functional mitochondria is necessary to sustain cellular homeostasis and organismal health.

MITOCHONDRIAL QUALITY CONTROL MECHANISMS

Mitochondria have evolved multiple mechanisms ensuring mitochondrial quality. For instance, mitochondrial chaperones and proteases are constantly preventing the accumulation of misfolded and aggregated proteins by monitoring proteostasis through the mitochondrial unfolded protein stress response (UPR^{mt}) (Melber and Haynes, 2018), a mechanism that has been shown to be critical for longevity in mammals (Houtkooper et al., 2013; Mouchiroud et al., 2013). Further, mitochondria are dynamic organelles existing in large tubular and highly dynamic networks that constantly undergo fission and fusion processes, thereby leading to the dilution of non-functional mitochondria (Youle and van der Bliek, 2012).

Nevertheless, autophagy is the only known pathway that mediates the turnover of whole mitochondria to avoid cellular damage and apoptosis. The degradation process is mediated by a double-membrane vesicle, known as the autophagosome, and it was first observed in mammalian cells by electron microscopy (De Duve and Wattiaux, 1966). For a long time, autophagy was considered a non-selective bulk degradation pathway, however, when the yeast mitochondrial protein Uth1p was found to be involved in the selective degradation of mitochondria (Kissová et al., 2004), the term "mitophagy" was subsequently introduced (Lemasters, 2005).

Herein, we discuss the role of mitophagy in impacting human disease development and the aging process itself. Further, interventions that target mitophagy will be discussed that may provide a promising strategy for the treatment of a broad spectrum of diseases.

WHAT IS MITOPHAGY?

The process of mitophagy can act either as a response to various stress stimuli including nutrient starvation and oxidative stress or as a programmed removal of mitochondria (Palikaras et al., 2018; Pickles et al., 2018). Different pathways are known to regulate mitophagy, the best-studied pathway is mediated by the phosphatase and tensin homologue (PTEN)-induced putative kinase 1 (PINK1) and the E3-ubiquitin ligase Parkin (**Figure 1A**). Mutations in both genes encoding PINK1 and Parkin (PARK2), have been reported to cause autosomal recessive forms of Parkinson's Disease (PD) (Kitada et al., 1998; Valente et al., 2004). Under un-stressed conditions, PINK1 is imported via the translocase of the outer membrane and translocase of the inner membrane (TOM/TIM) complex in a membrane potential dependent manner into mitochondria, leading to proteolytic cleavage of PINK1 (Jin et al., 2010; Deas et al., 2011; Meissner et al., 2011). The N-terminal truncated PINK1 is subsequently released to the cytosol, and degraded by the proteasome (Yamano and Youle, 2013). Loss of mitochondrial membrane potential disrupts the transport of PINK1 across the mitochondrial membrane leading to the accumulation of uncleaved PINK1 at the outer mitochondrial membrane. Subsequently, PINK1 regulates the recruitment and activation of the cytosolic Parkin via direct phosphorylation of the Parkin Ub-like (UBL) domain or via the phosphorylation of ubiquitin (Kondapalli et al., 2012; Shiba-Fukushima et al., 2012; Iguchi et al., 2013; Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014; Ordureau et al., 2014; Wauer et al., 2015). Once activated, Parkin drives the ubiquitination of multiple substrates, which leads to a positive feed forward mechanism through the generation of additional substrates for Pink1 (Ordureau et al., 2014).

In recent years, several substrates, in particular mitochondrial outer membrane proteins and autophagy receptors, have been identified to be ubiquitinated by the PINK1/Parkin-mediated signaling pathway (Sarraf et al., 2013). For instance, the mitochondrial fusion proteins mitofusin 1 and 2 (Mfn1 and Mfn2) are degraded in a PINK1/parkin dependent manner to make mitochondria accessible for degradation and to prevent fusion of damaged mitochondria with the healthy network (Gegg et al., 2010; Tanaka et al., 2010). However, conditional double-knockout of Mfn1 and Mfn2 in mice leads to mitochondrial dysfunction and, in line with this, Mfn2-depleted cardiomyocytes are deficient in Parkin recruitment to the mitochondrial outer membrane (Chen et al., 2011; Chen and Dorn, 2013). A similar priming function of mitochondria has been described for other mitochondrial proteins such as Miro1 and VDAC1 (Geisler et al., 2010; Wang et al., 2011; Sun et al., 2012; Safiulina et al., 2019). Recently, the apoptotic protein BAK has been identified as a Parkin target, further connecting Parkin-mediated mitophagy to the regulation of cellular apoptosis (Bernardini et al., 2019). The ubiquitination events driven by PINK1 and Parkin enable the recruitment of autophagy substrate receptors to the mitochondrial membrane including p62, Optineurin and NDP52, thereby promoting the engulfment of mitochondria by autophagosomes (Geisler et al., 2010; Wong and Holzbaur, 2014; Lazarou et al., 2015).

Notably, transcriptional regulation is a crucial process for functional PINK1-Parkin-mediated mitophagy. For instance, PINK1-Parkin-mediated mitophagy induction upon cellular stress such as through reactive oxygen species or ethanol exposure leads to the nuclear translocation of several transcription factors, including the transcription factor EB (TFEB) and the nuclear respiratory factors (NRFs), controlling the expression of mitochondrial, autophagy and lysosomal genes (Nezich et al., 2015; Ivankovic et al., 2016; Eid et al., 2019). Parkin expression itself has also been shown to be tightly controlled by stress pathways such as the unfolded protein response pathway and its activating transcription factor 4 (ATF4) (Bouman et al., 2011). Altogether, this highlights the great number of potential therapeutic avenues to target the PINK1-Parkin signaling pathway.

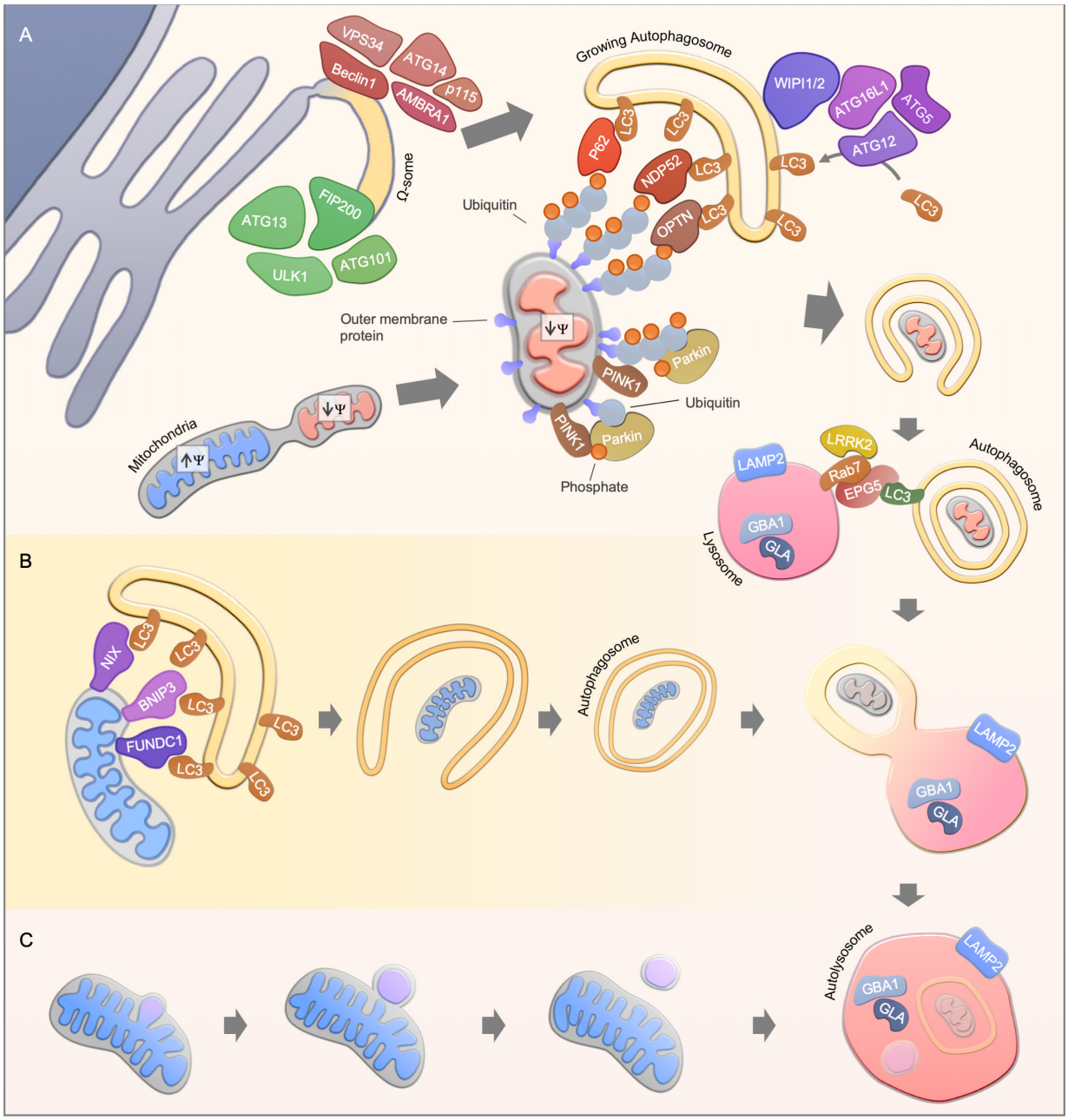

FIGURE 1 | Mitophagy pathways. **(A)** Ubiquitin-dependent PINK1/Parkin-mediated mitophagy. Upon mitochondrial damage, PINK1 is stabilized at the outer mitochondrial membrane, leading to Parkin activation and subsequent ubiquitination of mitochondrial proteins. Finally, autophagy receptors such as NDP52, OPTN, and p62 are recruited to mediate the engulfment of mitochondria by the autophagosomal membrane through the interaction with LC3. A possible source of the autophagosomal membrane is provided by the endoplasmic reticulum, where the autophagy core complexes VPS34 and ULK1 initiate the membrane formation. The membrane formation is further mediated by WIPI1 and WIPI2, leading to the recruitment of the ATG16L1-complex and LC3, thereby facilitating the formation of autophagosomes. Finally, autophagosomes fuses with acidic lysosomes, a step that is regulated by concerted action of autophagosomal and lysosomal proteins. **(B)** Ubiquitin-independent receptor-mediated mitophagy. Ubiquitin-independent receptor mediated mitophagy is mediated by the recruitment of autophagy receptor proteins such as NIX, BNIP3, and FUNDC1 to the mitochondrial membrane. The receptor proteins recruit LC3, which enables the engulfment of mitochondria by autophagosomes. **(C)** Alternative degradation pathways. Piecemeal mitophagy and mitochondrial-derived vesicle degradation are cellular pathways that mediate localized degradation of mitochondria.

Pink1/Parkin-independent mitophagy pathways mainly rely on receptor proteins which mediate the recruitment of LC3/GABARAPs for the removal of mitochondria (**Figure 1B**). For instance, the BCL2-related protein NIX (also known as BNIP3L) mediates mitophagy in mammals during reticulocyte differentiation, a process that requires the elimination of

mitochondria (Schweers et al., 2007; Sandoval et al., 2008; Novak et al., 2010). In line with this, *NIX* knockout mice develop anemia and reticulocytosis (Schweers et al., 2007; Sandoval et al., 2008). The interaction of NIX with LC3 protein members is mediated via the LC3-interacting (LIR) motif, however, re-expression of LIR-mutant NIX in NIX deficient reticulocytes partially rescued the observed phenotype, indicating LC3-independent or even autophagy-independent mechanisms for mitochondrial clearance in reticulocyte differentiation (Novak et al., 2010). Another LIR-motif containing protein, FUNDC1, regulates mitophagy under hypoxic conditions by promoting mitochondrial fission (Liu et al., 2012; Chen et al., 2016). During cardiac progenitor cell differentiation, FUNDC1 and NIX, but not Pink1 and Parkin, are upregulated to maintain a functional mitochondrial network (Lampert et al., 2019). Mitophagy is therefore also regulated in a lineage dependent fashion.

Localized removal of mitochondrial subdomains can be mediated by piecemeal mitophagy or mitochondrial-derived vesicles (**Figure 1C**). Mitochondrial-derived vesicle formation is thought to be dependent on PINK1/Parkin but independent of the canonical autophagy machinery (Soubannier et al., 2012; McLelland et al., 2014). Whereas, the accumulation of misfolded mitochondrial protein aggregates leads to localized recruitment of Parkin and autophagy proteins, thereby facilitating the degradation of mitochondrial subdomains (Burman et al., 2017). A PINK1/Parkin-independent piecemeal mitophagy has been recently reported that drives LC3C- and p62-mediated degradation of mitochondrial subregions (Le Guerroué et al., 2017). However, the protein machinery for these mitochondrial degradation pathways may overlap with the classic mitophagy pathways as well as their physiological relevance needs to be further investigated.

MITOPHAGING

A decline in mitochondrial function is a hallmark of the aging process and is connected to other aging hallmarks such as telomere dysfunction, genome instability and cellular senescence. However, it remains largely unclear how these processes are interconnected and finally provoke disruption of the cellular and tissue integrity (López-Otín et al., 2013). There is accumulating evidence that mitophagy impacts health- and lifespan in different model organisms. Using a transgenic mouse strain that expresses the fluorescent mitophagy reporter mt-Keima, a decreased mitophagy level was observed in the hippocampal dentate gyrus in 21-month old mice compared to 3-month old mice (Sun et al., 2015). A decline in mitophagy was also observed in aged mouse hearts, in line with this, altered mitophagy has been shown to influence different cardiac pathologies (Hoshino et al., 2013; Bravo-San Pedro et al., 2017). Other tissues that contribute to aging phenotypes are also characterized by defective mitophagy, as shown recently for aged skeletal muscle satellite cells isolated from humans or mice (García-Prat et al., 2016). Notably, decreased expression of mitophagy genes was observed in the skeletal muscle of physically inactive elderly women (Drummond et al., 2014).

The effect of changes in mitophagy on health- and lifespan has been particularly demonstrated by using the model organisms *C. elegans* and *D. melanogaster*. Several genetic studies in *D. melanogaster* revealed that the overexpression of mitochondrial and mitophagy genes leads to increased health- and/or lifespan. For instance, the overexpression of the mitochondrial fission protein dynamin-related protein 1 (DRP1) increased the lifespan along with a prolonged healthspan in flies (Rana et al., 2017). The importance of mitochondrial fission on drosophila lifespan was further demonstrated by the observation that lifespan extension caused by the overexpression of p62 was abrogated in DRP1 mutant flies (Aparicio et al., 2019). Lifespan extension in flies was also observed after overexpression of Parkin and Pink1, whereby, Parkin overexpression counteracted increased Mfn2 levels, which can be observed during aging (Todd and Staveley, 2012; Rana et al., 2013). These findings are consistent with studies in *C. elegans*, where mitophagy has been shown to contribute to lifespan regulation (Palikaras et al., 2015; Schiavi et al., 2015). Evidently, there is substantial data supporting a role of declining mitophagy, mitophaging, in aging.

WHAT HAPPENS WHEN MITOPHAGY GOES WRONG?

Impaired mitophagy contributes to the pathogenesis of several human diseases, in particular to age-related sporadic disorders, such as Parkinson's disease, Alzheimer's disease, cardiomyopathies and cancer (Bernardini et al., 2017; Fivenson et al., 2017; Levine and Kroemer, 2019). While these observations yield interesting correlations between certain disease states and alterations in mitophagy it is difficult to deduct causation. Here, monogenic diseases with specific defects in mitophagy may give us mechanistic understanding of pathogenesis and biology (**Table 1**). Thus, monogenic disorders may provide valuable tools for studying molecular pathomechanisms that are driven by defective mitophagy. To explore the clinical phenotype of autophagy diseases, we identified the clinical descriptions in the literature of all the diseases in **Table 1** and performed hierarchical clustering based on the prevalence of those features (**Figure 2A**; Scheibye-Knudsen et al., 2013; Andreassen et al., 2019). Although the clustering did connect clinically similar diseases (such as Charcot–Marie–Tooth 2A2 and 2B), it became immediately apparent that there is no good correlation between clinical outcome and the putative molecular function of the gene responsible for the disease. Indeed, principal component analysis also did not show any obvious separation of clinical groups based on proposed molecular functions (**Figure 2B**). This suggests that our knowledge of the pathogeneses of most of these disorders is quite limited. Nevertheless, when looking at the average prevalence of clinical features across all aging diseases there was a considerable overrepresentation of neurological features suggesting that defects in autophagy often leads to brain disease (**Figure 2C**). More specifically, the phenotype in the autophagy disorders show significant overlap with what is seen in mitochondrial diseases indicating that mitochondrial dysfunction may be driving diseases in many autophagy-related

TABLE 1 | Examples of autophagy/mitophagy-related monogenic disorders.

Disease	Gene	Protein function	Symptoms	References
Amyotrophic lateral sclerosis	OPTN (AD)	Autophagy receptor	Motor neuron degeneration	Weil et al., 2018
Alzheimer's disease	APP (AD)	Transmembrane protein	Dementia	Fang et al., 2019
Ataxia-telangiectasia	ATM (AR)	DNA-damage response	Cerebellar degeneration, Telangiectasia, Radiosensitivity	Fang et al., 2016
Autosomal dominant optic atrophy	OPA1 (AD)	Mitochondrial fusion protein	Optic atrophy	White et al., 2009; Liao et al., 2017
Barth syndrome	TAZ (XLR)	Mitochondrial protein	3-Methylglutaconic aciduria, Cardiomyopathy, Neutropenia; Muscle weakness	Hsu et al., 2015
Charcot–Marie–Tooth disease	MFN2, RAB7 (AD, AR)	Mitochondrial fusion protein, endolysosomal protein	Neuropathy, Muscle weakness	Yamano et al., 2014; Rizzo et al., 2016
Charlevoix-Saguenay spastic ataxia	SACS (AR)*	Co-chaperone	Cerebellar degeneration, Neuropathy, Spasticity	Bradshaw et al., 2016; Morani et al., 2019
Cockayne syndrome	ERCC6 (AR)	DNA damage repair	Cerebellar degeneration, Short stature, Sun sensitivity	Scheibye-Knudsen et al., 2012
Danon disease	LAMP2 (XLD)	Autolysosome formation	Cardiomyopathy, Developmental delay, Myopathy	Tanaka et al., 2000; Hashem et al., 2017
Fabry disease	GLA (XL)	Lysosomal enzyme	Nephropathy, Cardiomyopathy, Hearing loss, Neuorpathy	Chévrier et al., 2010; Ivanova et al., 2019
Fanconi anemia	FANCC (AR)	DNA damage repair	Short stature, Anemia, Skin pigmentation changes, Osteopenia	Sumpter et al., 2016
Frontotemporal dementia and/or amyotrophic lateral sclerosis	TBK1, SQSTM1 (AD)	Serine/threonine protein kinase, autophagy receptor	Dementia, Motor neuron degeneration,	Geisler et al., 2010; Richter et al., 2016
Gaucher disease	GBA1 (AR)	Lysosomal enzyme	Hepatosplenomegali, Pancytopenia, Gaucher cells	Osellame et al., 2013
Intellectual developmental disorder with short stature and variable skeletal anomalies	WIPI2 (AR)	Autophagosome formation	Mental retardation, Cerebral atrophy, Short stature	Zachari et al., 2019
Krabbe disease	GALC (AR)*	Lysosomal enzyme	Spasticity, Leukodystrophy, Myoclonus	Del Grosso et al., 2019
Lafora disease	EPM2A (AR)	Glycogen synthesis	Seizures, Mental retardation	Lahuerta et al., 2018
Microcephaly 18	WDFY3 (AD)	Selective autophagy, aggrephagy	Cognitive deficits, Microcephaly	Napoli et al., 2018
MRXST	HUWE1 (XL)	E3-ubiquitin protein ligase	Mental retardation, Macrocephaly, Macroorchidism, Seizures	Di Rita et al., 2018
Mucolipidosis II	GNPTAB (AR)*	Lysosomal enzyme	Developmental delay, Short stature, Cardiomegaly, Dysostosis multiplex	Otomo et al., 2009
Multiple sulfatase deficiency	SUMF1 (AR)*	ER-resident enzyme	Cerebellar degeneration, Mental retardation, Hepatosplenomegaly	Settembre et al., 2008
NADGP	SQSTM1 (AR)	Autophagy receptor	Cerebellar degeneration, Mental retardation, Vertical gaze palsy, Dystonia	Geisler et al., 2010
NBIA5	WDR45 (XLD)*	Autophagosome formation	Cerebellar degeneration, Developmental delay, Brain iron accumulation, Dystonia	Saitsu et al., 2013
NEDSBAS	WDR45B (AR)*	Autophagosome formation	Seizures, Developmental delay, Spasticity, Cerebral atrophy	Bakula et al., 2017; Suleiman et al., 2018

(Continued)

TABLE 1 | Continued

Disease	Gene	Protein function	Symptoms	References
Neuronal Ceroid Lipofuscinosis	PPT1 (AR)*	Lysosomal enzyme	Mental retardation, Seizures, Cerebellar degeneration	Mukherjee et al., 2019
Niemann-Pick disease	NPC1 (AR)*	Lysosomal protein	Seizures, Jaundice, Hepatosplenomegaly, Mental retardation	Pacheco et al., 2007
Parkinson's disease	LRRK2, PARK2, PARK6 (AD)	Mitochondrial proteins	Bradykinesia, Rigidity, Tremor, Dementia	Ryan et al., 2015
Pompe disease	GAA (AR)*	Lysosomal enzyme	Muscle weakness, Cardiomyopathy, Hypotonia	Raben et al., 2012
Spastic paraplegia 15	ZFYVE26 (AR)*	Autophagosome formation	Spasticity, Hyperactive reflexes, Mental retardation	Vantaggiato et al., 2013; Denton et al., 2018
Spastic paraplegia 49	TECPR2 (AR)*	LC3/GABARAP binding protein	Developmental delay, Spasticity, Dysmorphism, Microcephaly, Hypotonia, Short stature	Oz-Levi et al., 2012
Spinocerebellar ataxia 25	ATG5 (AR)	Autophagosome formation	Developmental delay, Cerebellar degeneration, Mental retardation	Sun et al., 2015
Spinocerebellar ataxia 4	VPS13D (AR)	Lysosomal enzyme	Hyperactive reflexes, Muscle atrophy, Cerebeller degeneration	Anding et al., 2018
Vici syndrome	EPG5 (AR)*	Autolysosome formation	Cataracts, Cardiomyopathy, Developmental delay, Hypotonia, Immune deficiency, Corpus callosum agenesis	Cullup et al., 2013
Wolfram syndrome	WFS1 (AR)	Calcium homeostasis	Diabetes mellitus type 1, Optic atrophy, Hearing loss, Diabetes insipidus	Cagalinec et al., 2016
Xeroderma pigmentosum group A	XPA (AR)	DNA damage repair	Sun sensitivity, Cerebellar degeneration, Cancer, Neuropathy	Fang et al., 2014
Zellweger syndrome	PEX13 (AR)	Peroxisome biogenesis	Developmental delay, Dysmorphism, Hepatosplenomegaly, Seizures	Lee et al., 2017

For genes that are marked with an asterisk the function in mitophagy remains largely unknown, however, defects in autophagy and mitochondrial dysfunction have been reported. Abbreviations: AD, autosomal dominant; AR, autosomal-recessive; XLR, X-linked recessive; XLD, X-linked dominant.

disorders ranging from lysosomal diseases to bonafide mitophagy deficiencies (**Figures 2C,D**). In the following we will examine a few key examples of these disorders.

Defects in the Autophagic Machinery

To date, only a few monogenic diseases caused by single mutations in the autophagy core machinery have been reported. One of them, spinocerebellar ataxia-25 (SCAR25), is caused by a mutation in the *autophagy-related 5* gene (*ATG5)*, encoding a protein that is part of the ATG12-ATG5-ATG16L1 complex, which facilitates LC3/GABARAP conjugation (Mizushima, 2020). So far, two siblings have been identified with SCAR25, presenting with clinical symptoms such as truncal ataxia and intellectual disability (Kim et al., 2016). In line with the neurological phenotypes, a neuron-specific knockout of *ATG5* in mice causes neuronal degeneration, by contrast, a complete *ATG5* knockout is neonatal lethal (Kuma et al., 2004; Hara et al., 2006). Ataxia is a common feature of many mitochondrial disorders (Scheibye-Knudsen et al., 2013), however, mitochondrial viability in SCAR25 has not been investigated so far. Thus, the contribution of mitochondrial defects to the reported clinical features in SCAR25 remains speculative, since ATG5-independent mitophagy pathways have been reported (Honda et al., 2014; Hirota et al., 2015).

Mutations in members of the human WD-repeat protein interacting with phosphoinositides (WIPI) family are known to cause neurological deficits. The WIPI protein family consists of four members, WIPI1–WIPI4, that contribute to the early steps of autophagosome formation (Proikas-Cezanne et al., 2004). The family member WIPI2 localizes in a phosphatidylinositol 3-phosphate-dependent manner to the autophagosomal membrane, where it facilitates ATG16L1 recruitment and LC3 lipidation (Dooley et al., 2014; Bakula et al., 2017). Recently, patients with mutations in the *WIPI2* gene have been described with multisystemic clinical features, primarily, neurological and skeletal deficiencies that are characterized by severe mental retardation and short stature (Jelani et al., 2019). Notably, WIPI2 overexpression prevents age-related autophagy

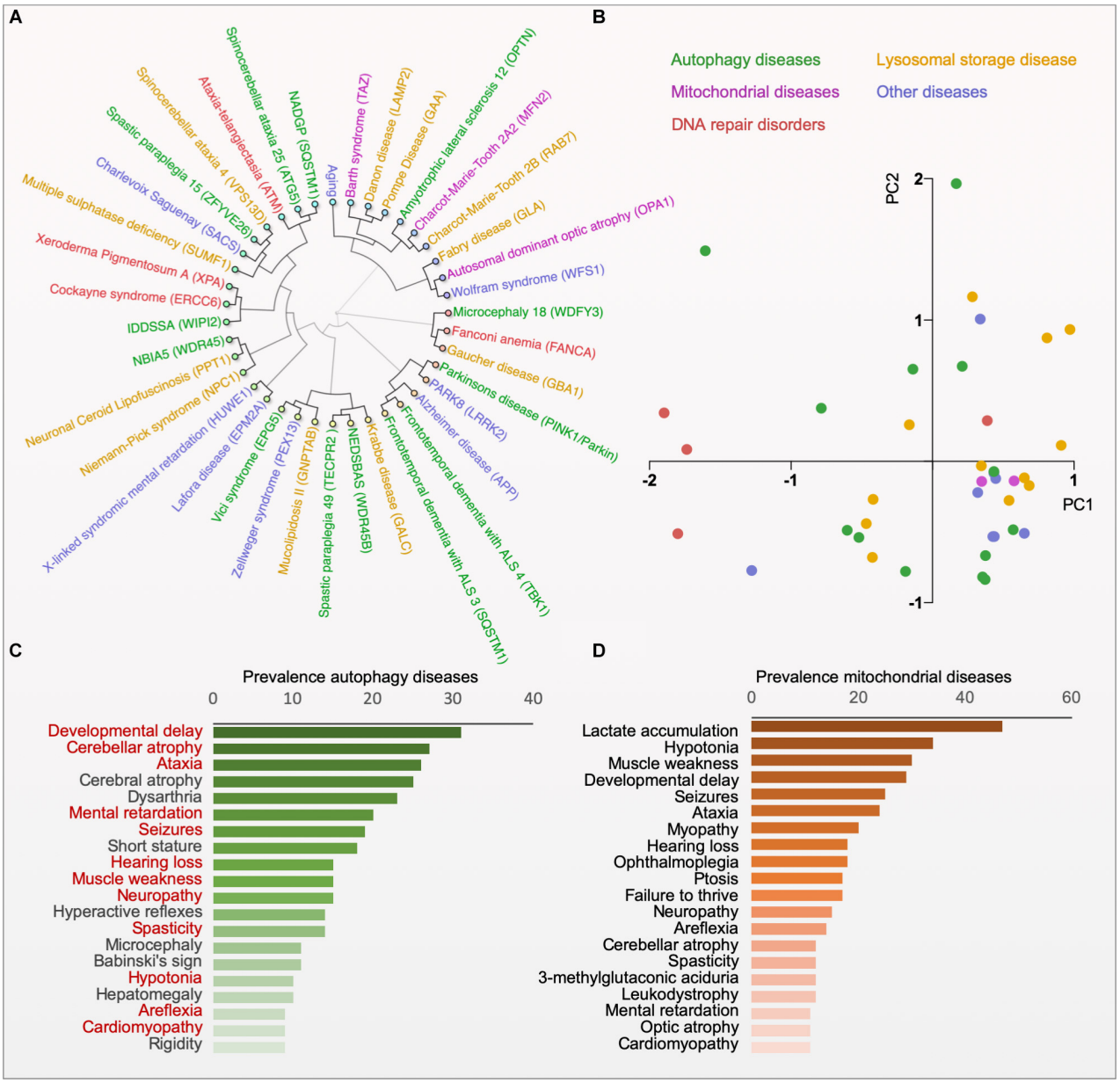

FIGURE 2 | Phenotype clustering of autophagy diseases. **(A)** Hierarchical clustering of diseases based on the published prevalence of clinical features in the diseases (for data and references see www.mitodb.com). **(B)** Principal component analysis of diseases based on the prevalence of clinical features. **(C)** The average prevalence of top-20 clinical features in all autophagy-related disorders (Red, shared with the top-20 features in mitochondrial disorders). **(D)** The average prevalence of clinical features in mitochondrial diseases.

decline in dorsal root ganglion neurons (Stavoe et al., 2019). Patients with mutations in the genes *WIPI3* (*WDR45B*) or *WIPI4* (*WDR45*) show severe and progressive neurodegenerative phenotypes (Haack et al., 2012; Hayflick et al., 2013; Saitsu et al., 2013; Suleiman et al., 2018). Notably, *WIPI4* mutations result in degeneration of the substantia nigra, a target area of the brain affected in Parkinson's disease (Mann et al., 1992). In line with these observations, *WIPI3* or *WIPI4* knockout mice show neurological defects, possibly caused by defective neuronal autophagy (Zhao et al., 2015; Ji et al., 2019). WIPI3 and −4 knockout mice display mitochondrial dysmorphology, which was also evident in WIPI4 mutant human fibroblast cells (Zhao et al., 2015; Seibler et al., 2018; Ji et al., 2019). The patient phenotypes caused by mutations in the *WIPI* genes highlight the importance of the WIPI protein members for neuronal function, however, the contribution of WIPI-mediated clearance of mitochondria in neurodegeneration remains unclear.

Deficiency in the late stage of autophagy is observed in the autosomal recessive neurological disorder, Vici syndrome. The disease is caused by mutations in the *ectopic P-granules*

autophagy protein 5 gene (*EPG5*), encoding for a Rab7 effector protein that is required for the fusion of late autophagosomes with lysosomes (Cullup et al., 2013; Wang et al., 2016). The disease is characterized by multisystemic defects that show some overlap with mitochondrial diseases, such as agenesis of corpus callosum, cardiomyopathy, immunodeficiency, cataracts and hypopigmentation (Cullup et al., 2013). Mitochondria with abnormal shape and distribution were observed in muscle tissue biopsies from patients with Vici syndrome or *EPG5* knockout mice (Cullup et al., 2013; Zhao et al., 2013). The importance of EPG5 in mitochondrial homeostasis was further highlighted by a study showing deficient mitochondrial clearance during spermatogenesis in an *EPG5*-deficient medaka fish line (Herpin et al., 2015).

Cargo recognition and degradation in selective autophagy is mediated by autophagy receptor proteins, such as optineurin and p62. Both proteins are associated with the progressive neurological disorder amyotrophic lateral sclerosis (ALS), which is primarily caused by loss of motor neurons (Maruyama et al., 2010; Fecto et al., 2011). Around 10% of ALS cases are caused by inherited single gene mutations and frequently show comorbidity with frontotemporal dementia (FTD). Interestingly, optineurin and p62 are phosphorylated by tank-binding kinase 1 (TBK1), a serine/threonine kinase that has also been implicated in ALS-FTD disease development (Cirulli et al., 2015; Freischmidt et al., 2015; Pottier et al., 2015). Thus, there is a striking correlation with mutations in multiple mitophagy players leading to ALS.

Defects in Mitochondrial Quality Control

Proteins involved in the regulation of mitochondrial quality control are essential modulators of mitophagy, consequently, understanding their molecular mechanisms may give important insights into the consequences of impaired mitophagy. In recent years, mitochondrial dysfunction has been extensively discussed as an important contributor to neurodegeneration in familial Parkinson's disease, as well as in idiopathic forms (Bose and Beal, 2016). Early onset recessive familial Parkinson's disease can be caused by mutations in the genes *Park2* (*Parkin*), *Park6* (*Pink1*), or *Park7* (*DJ-1*). All three proteins localize to mitochondria and loss of each of them leads to increased sensitivity toward oxidative stress along with mitochondrial and energetic dysfunction (Dodson and Guo, 2007). Pink1 and Parkin are directly involved in the mitophagy pathway, whereas, the precise function of DJ-1 remains under discussion. Interestingly, overexpression of Pink1 and Parkin rescues the observed phenotype caused by DJ-1 deficiency, suggesting partial redundancies in the mitophagic apparatus (Irrcher et al., 2010).

Mitochondrial fission and fusion are critical events for controlled degradation of damaged mitochondria. Optic atrophy 1 (OPA1) is an inner mitochondrial membrane protein that regulates the fusion of mitochondria, together with MFN1 and MFN2. Mutation in the *OPA1* gene has been observed to cause autosomal dominant optic atrophy (ADOA) often accompanied by myopathy and progressive ataxia (Yu-Wai-Man et al., 2010). Myopathy and neurodegeneration is also observed in patients with Charcot–Marie–Tooth syndrome caused by loss of the *MFN2* gene (Calvo et al., 2009), underscoring the importance of mitochondrial function in muscle and brain tissues. For both diseases impaired mitophagy has been reported, suggesting that dysfunctional mitophagy may contribute to the described disease pathology (White et al., 2009; Rizzo et al., 2016; Liao et al., 2017).

Defects in Lysosomal Function

Another group of diseases that may be partial driven by deficient mitophagy, are lysosomal storage disorders, a heterogenous group of more than 60 rare monogenic diseases that are caused by defects in lysosomal function (Platt et al., 2018). Some of the most well described are Gaucher disease and Niemann–Pick type C. Gaucher disease is caused by mutations in the *glucocerebrosidase* (*GBA*) gene, encoding a lysosomal enzyme required to hydrolyze the glycolipid glucosylceramide. Patients with Gaucher disease display features in multiple organs caused by lysosomal accumulation of glucosylceramide with a subset of patients display progressive neurodegeneration. Notably, the *GBA* gene represents a major risk locus for inherited Parkinson's disease supporting the idea that mitophagy is important in this disease (Goker-Alpan et al., 2004; Lwin et al., 2004). Reduced mitochondrial respiration, increased ROS production and increased alpha-synuclein accumulation can be observed in various GBA deficiency models, cellular changes that are also described to be central drivers of neuronal loss in Parkinson's disease (Osellame et al., 2013; Chen et al., 2019). Nieman Pick type C is caused by mutations in the *NPC1* gene and is characterized by developmental delay, progressive neurodegeneration, dysphagia and vertical gaze palsy, a combination of phenotypes that can also be observed in mitochondrial disorders. In patient-derived fibroblast cells and NPC1-deficient neuronal cells impaired autophagy and an accumulation of mitochondrial fragments have been observed upon lysosomal cholesterol accumulation (Pacheco et al., 2007; Elrick et al., 2012; Ordonez et al., 2012).

Secondary Defects in Mitophagy

In addition to diseases with primary defects in mitophagy, several diseases have been described with secondary mitophagic dysfunction. In the context of monogenic diseases displaying premature aging, loss of mitophagy was first described in Cockayne syndrome, a disease characterized by progressive neurodegeneration reminiscent of mitochondrial disorders (Scheibye-Knudsen et al., 2012). The pathogenesis likely involves dysregulation of uncoupling proteins (U) due to decreased activity of the PGC-1alpha transcription factor. UCPs regulate mitochondrial membrane potential and consequently a reduction in UCPs lead to increased mitochondrial membrane potential and loss of PINK1 mediated mitophagy. Accordingly, overexpression of UCP2 can rescue mitochondrial and mitophagic defects in Cockayne syndrome. Notably, the same pathogenesis is found in related DNA repair disorders xeroderma pigmentosum group A and ataxia-telangiectasia (Fang et al., 2014, 2016).

Another disease that is characterized by mitochondrial deficiency is Zellweger syndrome, which belongs to a subgroup of peroxisome biogenesis disorders (Salpietro et al., 2015). Zellweger syndrome is caused by mutations in one of 14 human

PEX genes, encoding for peroxin proteins that are required for the maintenance of peroxisomes (Waterham and Ebberink, 2012). Zellweger syndrome patients show dysmorphic features and suffer from severe neurological symptoms. Recently, PEX13 was shown to be required for mitophagy, but interestingly, dispensable for starvation-induced autophagy (Lee et al., 2017). Similarly, PEX5, an interaction partner of PEX13, has been shown to modulate autophagy via regulation of the mTOR signaling pathway (Eun et al., 2018), in line with this, mitochondrial defects can be observed in *PEX5* knockout models (Baumgart et al., 2001). However, it is still unclear, to what extent the clinical features of Zellweger syndrome are driven by mitophagic defects.

IS MITOPHAGY A THERAPEUTIC TARGET?

An increasing number of human diseases have been associated with impaired mitophagy, thus, interventions that modulate mitophagy may provide the possibility of counteracting disease development or progression (**Figure 3**). In recent years, multiple small molecules as well as lifestyle interventions have been shown to modulate autophagy, thereby causing health- and lifespan benefits in different organisms (Galluzzi et al., 2017). Due to the dependency on core autophagy regulators, mitophagy is modulated by most of the classic autophagy inducers such as the mTOR inhibitor rapamycin, the AMP-activated protein kinase (AMPK) activator AICAR as well as caloric restriction and exercise. In particular, the effectiveness of rapamycin and rapalogs has been intensively studied in the context of lifespan regulation and human disease development and rapamycin remain the most well documented compound for life- and healthspan extension in laboratory animals (Saxton and Sabatini, 2017). Further connections between longevity and mitophagy comes from work on the metabolite NAD^+ and the NAD^+-dependent acetylase Sirtuin 1 (SIRT1). Here, it has been shown that stimulation of SIRT1 through NAD^+ augmentation or small molecules leads to activation of the energy responsive kinase AMPK that in turns regulates a central autophagy regulator, Unc-51-like kinase 1 (ULK1) (Egan et al., 2011; Price et al., 2012). Further, SIRT1 and AMPK also regulate the transcription factor PGC-1alpha, a key regulator of mitochondrial function that was initially found to control UCP levels and thereby mitochondrial membrane potential (Puigserver et al., 1998; Cantó et al., 2009). Indeed, SIRT1 activation leads to UCP-2 upregulation, stimulation of mitophagy and rescue of aging features in models of premature aging (Fang et al., 2014; Scheibye-Knudsen et al., 2014). Notably, direct stimulation of AMPK through the AMP-mimetic compound AICAR regulates mitochondrial dynamics via the induction of mitochondrial fission, further highlighting the broad effect of AMPK on mitochondrial function (Toyama et al., 2016).

Due to their great diversity, natural compounds are a tremendous source for novel mitophagy modulators. Urolithin A, a gut metabolite of ellagic acid, extends health- and lifespan in *C. elegans* as well as improving muscle function in rodent models via the induction of mitophagy (Ryu et al., 2016). The effectiveness of urolithin A was further highlighted in animal models of Alzheimer's disease, where the disease pathology was ameliorated in the group of urolithin A-treated mice (Fang et al., 2019). In a human clinical trial study, the safety of urolithin A was evaluated, and signatures of improved mitochondrial function were demonstrated (Andreux et al., 2019). Similar to Urolithin A, the potency of antibacterial compound actinonin was demonstrated in Alzheimer's disease models (Fang et al., 2019). Actinonin inhibits mitochondrial translation, thereby inducing mitophagy via the activation of the PINK1/Parkin-regulated signaling pathway (Richter et al., 2013; Sun et al., 2015; Burman et al., 2017). Another natural compound that has been suggested as a potential intervention for aging and diseases is the polyamine spermidine (Eisenberg et al., 2009; Madeo et al., 2018; Schwarz et al., 2018). The administration of spermidine leads to an induction of mitophagy in cardiomyocytes, along with cardio protection in mice (Eisenberg et al., 2016). The induction of autophagy via spermidine has been associated, among others, with the inhibition of the acetyltransferase EP300 and the ATM-driven activation of the PINK1/Parkin-regulated mitophagy pathway (Pietrocola et al., 2015; Qi et al., 2016).

Transcriptional regulation of mitophagy has also been shown as a viable pathway for increased mitochondrial health. An example is the synthetic compound PMI that stimulates mitophagy via the activation of the transcription factor Nrf2, which controls the expression of mitophagy genes including p62 (East et al., 2014; Bertrand et al., 2015). PMI treatment facilitates LC3 recruitment and mitochondrial ubiquitination in a PINK1/Parkin-independent manner, notably without disrupting the mitochondrial membrane potential (East et al., 2014).

Besides targeting mitophagy core proteins, intervention strategies targeting mitochondrial proteins may present a useful approach for disorders that are characterized by abnormal mitochondrial dynamic. Mdivi-1, has been identified in a yeast screen for mitochondrial fission inhibitors and several studies indicate its therapeutic potential for the treatment of neurological disorders (Cassidy-Stone et al., 2008; Cui et al., 2010; Solesio et al., 2012). However, the specificity of Mdivi-1 toward its putative target Drp1 has recently been questioned and needs to be further clarified (Bordt et al., 2017). USP30, a deubiquitinase that targets mitochondrial proteins, may present another promising target to facilitate mitophagy, since improved mitochondrial function was obtained upon USP30 depletion in different Parkinson's disease models (Bingol et al., 2014). Notably, MF-094 has been recently identified as a selective inhibitor of USP30 that may thereby facilitate mitophagy through increased ubiquitination of outer membrane proteins (Kluge et al., 2018). Thus, a number of mitophagy modulators have been identified, yet the main goal will be the precise and specific targeting of damaged mitochondria. One possible way is to apply chimeric molecules such as the recently generated autophagy-targeting chimeric molecule (AUTAC4) that selectively targets the mitochondrial membrane for ubiquitination and subsequent degradation (Takahashi et al., 2019). These approaches may be particularly efficacious in conditions of mitophaging where the mitophagy apparatus is likely intact but mitophagy occurs at suboptimal levels.

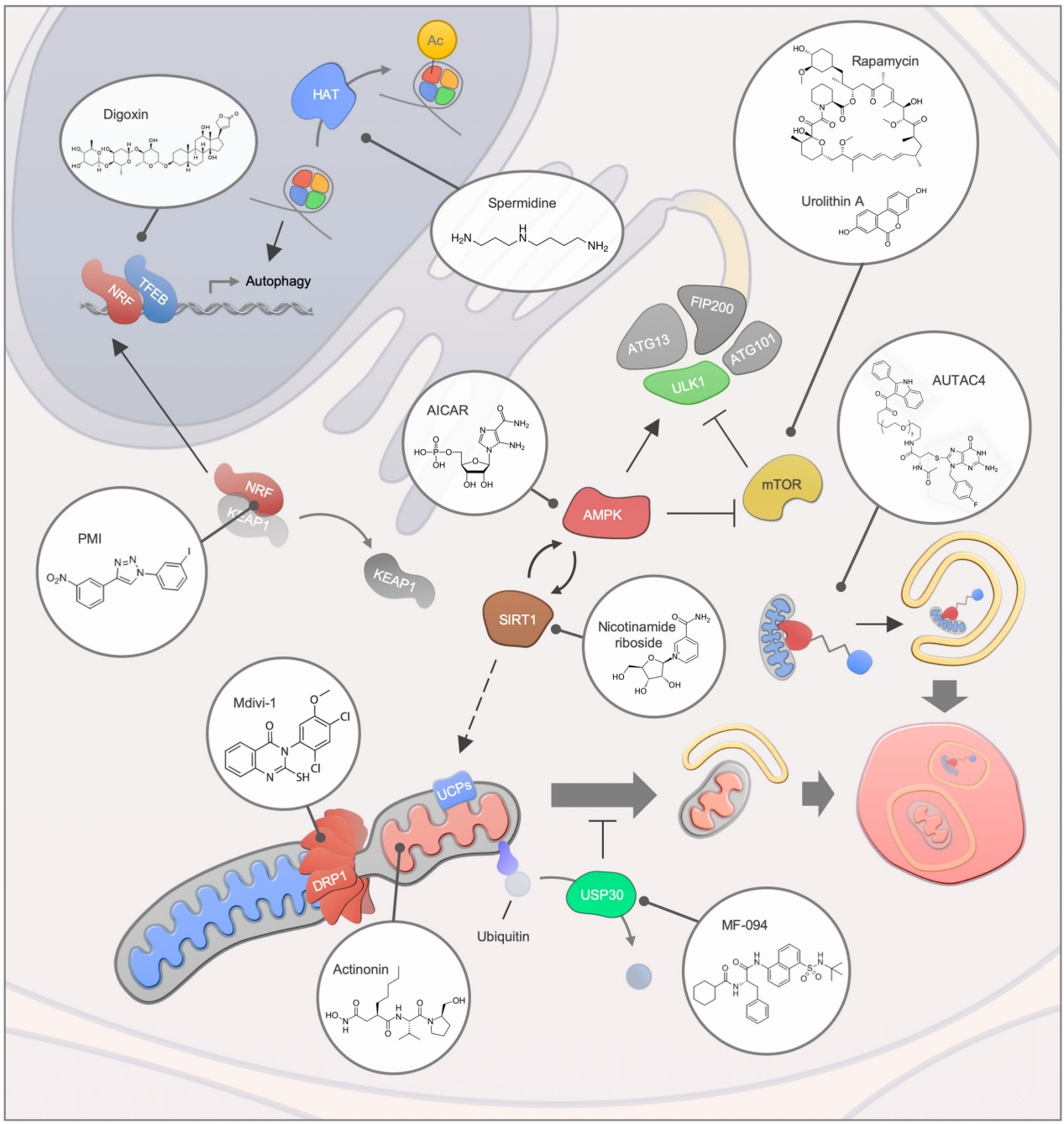

FIGURE 3 | Mitophagy interventions. An overview of different mitophagy modulating compounds and their targets. Abbreviations: Ac, Acetylation; HAT, Histone acetyltransferase.

In diseases characterized by dysfunctional lysosomes, stimulation of mitophagy may be detrimental due to an accumulation of undigested cargo material. In this regard, the inhibition of mitophagy is considered as a therapeutic strategy. In a mouse model of Pompe disease autophagy inhibition next to an enzyme replacement therapy has been proposed as a potential intervention (Raben et al., 2010). In line with this, knockdown of the mTOR pathway inhibitor TSC2 in muscle of Pompe disease mice reduced accumulation of autophagy markers and a decline in muscle atrophy was osberved (Lim et al., 2017). However, strategies to facilitate the fusion of autophagosomes and lysosomes in lysosomal storage disorders are also proposed for the treatment of several lysosomal storage disorders (Spampanato et al., 2013; Bartolomeo et al., 2017). TFEB, which controls the expression of autophagy as well as lysosomal genes and longevity (Napolitano and Ballabio, 2016),

may provide a promising target since its agonists, such as the clinically approved cardiac drug digoxin or the natural compound ikarugamycin, improve metabolic function in mice and extend lifespan in *C. elegans* (Wang et al., 2017). The therapeutic potential of TFEB in Parkinson's disease was further highlighted by a recent study that showed restored TFEB and improved neurological function upon rapamycin treatment in Q311X mutant parkin mice independently of the parkin E3 ligase (Siddiqui et al., 2015).

In summary, great progress has been made in recent years, however, the clinical safety of mitophagy modulating drugs needs to be further clarified. More refined tools that allow the distinction between mitophagy and general macroautophagy may be beneficial and could accelerate future discoveries. Altogether, this will enable us to step closer toward clinical validation of mitophagy modulators.

CONCLUDING REMARKS

Mitophagy is emerging as a central process preserving organismal and, especially, neurological health. Since most trials targeting age-associated neurodegeneration in the last decades have been disappointing, new pharmaceutical avenues are direly needed. Here, mitophagy stimulators could play a key role. Indeed, several clinical trials are underway testing the efficacy of mitophagy modulating compounds and the outcome of these studies will undoubtedly prove critical for the future translatability of the field. Nonetheless, the regulatory mechanism of mitophagy and its contribution to age-associated diseases still remains elusive and potential issues with artificially augmenting mitophagy have not been considered. However, given the central role of mitophaging in multiple age-related pathologies it appears highly likely that these new promising approaches may present possible interventions in age-associated diseases. The future is bright!

AUTHOR CONTRIBUTIONS

DB and MS-K wrote the manuscript and made the figures.

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Mitochondrial Quality Control Governed by Ubiquitin

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Mitochondria are essential organelles important for energy production, proliferation, and cell death. Biogenesis, homeostasis, and degradation of this organelle are tightly controlled to match cellular needs and counteract chronic stress conditions. Despite providing their own DNA, the vast majority of mitochondrial proteins are encoded in the nucleus, synthesized by cytosolic ribosomes, and subsequently imported into different mitochondrial compartments. The integrity of the mitochondrial proteome is permanently challenged by defects in folding, transport, and turnover of mitochondrial proteins. Therefore, damaged proteins are constantly sequestered from the outer mitochondrial membrane and targeted for proteasomal degradation in the cytosol via mitochondrial-associated degradation (MAD). Recent studies identified specialized quality control mechanisms important to decrease mislocalized proteins, which affect the mitochondrial import machinery. Interestingly, central factors of these ubiquitin-dependent pathways are shared with the ER-associated degradation (ERAD) machinery, indicating close collaboration between both tubular organelles. Here, we summarize recently described cellular stress response mechanisms, which are triggered by defects in mitochondrial protein import and quality control. Moreover, we discuss how ubiquitin-dependent degradation is integrated with cytosolic stress responses, particularly focused on the crosstalk between MAD and ERAD.

Keywords: *C. elegans*, mitochondria, proteostasis, mitochondria-associated degradation (MAD), ubiquitin, Cdc48, p97, Msp1

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INTRODUCTION

Mitochondrial integrity relies on a sophisticated network of quality control machineries, which have been evolved to counteract challenges associated with the endosymbiotic integration of this organelle into eukaryotic cells (Youle, 2019). Along with a precise coordination between nuclear and mitochondrial gene expression (Couvillion et al., 2016), transcriptional stress response programs emerged as central mitochondrial surveillance mechanisms (Andréasson et al., 2019). Mitochondrial functionality is further supported by ubiquitin-dependent degradation of proteins accumulating under stress conditions. The outer and inner mitochondrial membrane (OMM and IMM) separate the lumen into the intermembrane space (IMS) and the matrix. Mitochondria are equipped with an elaborate set of proteases acting on the different sub-compartments, to maintain the mitochondrial proteome from the inside (Koppen and Langer, 2007; Quirós et al., 2015; Glynn, 2017). Otherwise, the integrity of the mitochondrial proteome is largely supported by the UPS

localized in the cytosol (Franz et al., 2015; Bragoszewski et al., 2017; Braun and Westermann, 2017; D'Amico et al., 2017; Escobar-Henriques et al., 2020).

Besides the important role in ATP production and synthesis of amino acids, nucleotides, and iron-sulfur clusters (Lill and Mühlenhoff, 2008), mitochondria are also required for calcium buffering and apoptosis regulation (Wang and Youle, 2009; Contreras et al., 2010). Notably, mitochondria are tightly interconnected with other cellular organelles, especially the endoplasmic reticulum (ER) (Helle et al., 2013; van Vliet et al., 2014; Phillips and Voeltz, 2016). The contact between ER and mitochondria has been associated with aging and age-related diseases (Moltedo et al., 2019). In fact, the physical interaction between these tubular organelles supports the transfer of lipids, calcium ions and other metabolites, localizes to DNA nucleoids and regulates mitochondrial dynamics (Helle et al., 2013; Raturi and Simmen, 2013; van Vliet et al., 2014; Phillips and Voeltz, 2016; Moltedo et al., 2019). Recent studies suggest an intricate cooperation between ER and mitochondria in proteostasis (Dederer et al., 2019; Matsumoto et al., 2019), which constitute a newly developing research field promising for the development of therapeutic interventions as proposed for cardiac pathologies (Arrieta et al., 2020).

In this review we focus on quality control pathways that maintain mitochondrial functionality, involving cross-communication with the ER and other cellular compartments. We discuss recent discoveries on the role of the ubiquitin/proteasome-system (UPS) in mitochondrial quality control, including pathways that are constitutively active or triggered by metabolic stress to ensure mitochondrial integrity.

PROTEIN DEGRADATION MECHANISMS

Protein quality control is required at all steps originating from protein synthesis and involves a series of mechanisms dedicated to the surveillance of protein translation, transport, and turnover (Kaushik and Cuervo, 2015). Central players of the proteostasis network are molecular chaperones which mediate folding, targeting, and degradation of proteins (Klaips et al., 2018). The two major degradation pathways for proteins in the cytosol are the UPS and the autophagy-lysosomal pathway (Pohl and Dikic, 2019). In both proteolytic systems post-translational attachment of the small polypeptide ubiquitin serves as a targeting signal for protein turnover. The modification with ubiquitin (ubiquitylation) is mediated by a three-step enzymatic cascade (Kerscher et al., 2006). First, the ubiquitin-activating enzyme (E1) forms a high-energy thioester bond between its catalytic cysteine and the C-terminal glycine residue of ubiquitin, which is then transferred to a cysteine of an ubiquitin-conjugating enzyme (E2). The E2 cooperates with specific ubiquitin ligases (E3) to mediate the covalent attachment of ubiquitin mainly to a lysine residue in the selected substrate. Repeated cycles of this reaction either results in multiple mono-ubiquitylation of different lysine residues of a given substrate or formation of ubiquitin chains by targeting one of the seven lysines of ubiquitin (K6, K11, K27, K29, K33, K48, or K63) (Haakonsen and Rape, 2019). These different ubiquitin-dependent modifications termed "the ubiquitin code" serve as signals for different downstream events mediated by specialized binding proteins. Moreover, ubiquitylation can be reversed by different deubiquitylating enzymes (DUBs), which completely remove ubiquitin from substrates or trim ubiquitin chains to alter their composition (Clague et al., 2019). A prominent role of ubiquitylation is to target proteins for lysosomal or proteasomal turnover, which is often mediated by attachment of K48-linked ubiquitin chains (Dikic, 2017).

The turnover of soluble ubiquitylated proteins is mainly conducted by the 26S proteasome (Bard et al., 2018). It constitutes a multicatalytic complex of a barrel-shaped core subunit known as 20S proteasome and the 19S regulatory particle, attached to one or both ends of the 20S core. The regulatory particle is composed of a hexameric complex of AAA-ATPases that coordinates unfolding and translocation of the substrate into the core subunit. The 19S subunit also contains scaffold proteins involved in substrate recognition and deubiquitylation as well as gate opening and binding with other external factors. The core subunit is composed of four stacked heptameric rings and contains the proteolytic activity required for the cleavage of unfolded polypeptides.

In contrast to soluble substrates, proteins organized in multimeric complexes or membrane bound, require an additional extraction step prior to proteasomal degradation. This function is mainly executed by Cdc48 (p97 or VCP in vertebrates), which belongs to the family of AAA-ATPases associated with diverse cellular activities (AAA+), commonly using ATP to perform mechanochemical reactions (Sauer and Baker, 2011; Glynn, 2017). In collaboration with substrate-specific cofactors, Cdc48 binds ubiquitylated proteins and targets them to the 26S proteasome (Franz et al., 2014; Barthelme and Sauer, 2016).

Larger structures such as aggregated proteins or organelles are targeted for degradation inside lysosomes (vacuole in yeast and plants), which contain promiscuous proteolytic enzymes for degradation of engulfed cargoes. In this process termed macroautophagy (hereafter autophagy), substrates are recognized by autophagy receptors bound to autophagosomal membranes, which triggers substrate engulfment by the autophagosomal membrane and subsequent lysosomal fusion (Khaminets et al., 2016). Similar to soluble misfolded proteins, protein aggregates are targeted for autophagic degradation by ubiquitylation, which is a common feature of the two proteolytic pathways (Lu et al., 2017). Moreover, a specialized form of autophagy, called mitophagy, allows the selective turnover of entire mitochondria. This process can either be mediated by autophagy receptors residing in the OMM or by ubiquitylation of OMM proteins (Palikaras et al., 2018; Pickles et al., 2018).

UBIQUITIN-DEPENDENT SURVEILLANCE OF THE MITOCHONDRIAL PROTEOME

The degradation of ER-resident or mitochondrial proteins is regulated by two mechanistically similar ubiquitin-dependent

pathways termed ER-associated degradation (ERAD) and mitochondria-associated degradation (MAD) (Heo and Rutter, 2011; Guerriero and Brodsky, 2012; Ruggiano et al., 2014; Braun and Westermann, 2017; Mehrtash and Hochstrasser, 2018). For both tubular organelles, proteins residing in the outer membrane are directly accessible for ubiquitylation. By contrast, proteins localized inside these organelles have to be transported across the membrane to be exposed to the ubiquitin-conjugation machinery. Cdc48 triggers ERAD by retro-translocation of substrate proteins out of the ER lumen and proteasomal degradation at the cytosolic side of the ER membrane. Therefore, substrates proteins are ubiquitylated by membrane-bound ubiquitin ligase complexes. These ERAD ligases interact with accessory factors such as the UBX-domain protein Ubx2 to recruit Cdc48 for coordinating substrate extraction and turnover (Neuber et al., 2005; Schuberth and Buchberger, 2005). Increasing evidence suggests that a comparable system employs the UPS in controlling quality control of mitochondrial proteins.

UPS-Dependent Turnover of Mitochondrial Proteins

The mitochondrial mass is efficiently regulated by the balanced coordination between biogenesis and degradation. In addition to the degradation of damaged mitochondrial proteins the UPS also contributes to remodeling of the mitochondrial proteome in response to metabolic changes (**Figure 1**) (Bragoszewski et al., 2017). Mitochondria form a highly dynamic network shaped by continuous fusion and fission events with adaptive morphology according to cellular needs. These fission and fusion events are mediated by large dynamin-like GTPases that provide the mechanical force to either fuse or separate membranes. In mammals, fission is induced by DRP1 (Dnm1 in yeast), whereas fusion is driven by mitofusins (Fzo1 in yeast) in the outer membrane and by OPA1 (Mgm1 in yeast) in the inner membrane (Escobar-Henriques and Anton, 2013). Fusion is known to enhance the exchange of important molecules and can temporarily compensate for defects in mitochondrial sub-populations. On the other hand, fission promotes mitochondrial motility and allows separation of damaged organelles for mitophagy (Lackner, 2014; Roy et al., 2015; Wai and Langer, 2016). Residing in the OMM, these GTPases present cytosolic domains which are targeted for ubiquitin-dependent degradation. Thus, the UPS provides a crucial role in the regulation of mitochondrial morphology and function (Bragoszewski et al., 2017).

Notably, the first identified MAD substrate was in fact the yeast mitofusin Fzo1 (Neutzner and Youle, 2005), which is embedded into the OMM by two transmembrane domains, exposing most of its amino acid residues to the cytosol. Ubiquitylation of Fzo1 mainly depends on the F-box protein Mdm30, which is part of the multi-subunit SCF (Skp, Cullin, Fbox) ubiquitin ligase complex (Fritz et al., 2003; Escobar-Henriques et al., 2006; Cohen et al., 2008) (**Table 1**). The ubiquitin-modification of Fzo1 provides a regulatory role in the fusion of mitochondrial outer membranes, which is not necessarily linked to degradation (Anton et al., 2011; Cohen et al., 2011). In fact, proteasome-dependent turnover of Fzo1 largely depends on the nature of ubiquitylation, since the DUB Ubp2 is able to remove or trim down ubiquitin chains that are attached to Fzo1, which regulates its degradation (Anton et al., 2013) (**Table 1**).

Depending on the physiological conditions, Cdc48 also appears to be required for Fzo1 regulation (Heo et al., 2010; Esaki and Ogura, 2012). Under oxidative stress conditions, Cdc48 together with its co-factors Vms1 and Npl4 facilitates Fzo1 degradation (Heo et al., 2010). Under non-stressed conditions Fzo1 and additional other OMM proteins are targeted by another Cdc48 complex involving Npl4, Ufd1, and Doa1, also called Ufd3 (Wu et al., 2016) (**Figure 1** and **Table 1**). This might however reflect protein quality control triggered by experimental tagging of the membrane-bound proteins, since non-tagged endogenous Fzo1 is rather stabilized by Cdc48, which exerts a regulatory role during OMM fusion (Simões et al., 2018; Anton et al., 2019). Turnover of mitofusins by MAD is widely conserved among species including Marf in flies (Ziviani et al., 2010; Wang et al., 2016) and Mfn1/2 in mammals (Tanaka et al., 2010; Chan et al., 2011; Xu et al., 2011). A common role of MAD in this case is the inhibition of mitochondrial fusion by mitofusin degradation, resulting in mitochondrial fragmentation. In mammals, the E3 ligases Parkin and MARCH5 mediate p97 dependent extraction and proteasomal turnover of mitofusins, inducing mitochondrial fission and mitophagy (Karbowski et al., 2007; Ziviani et al., 2010; Chan et al., 2011; Xu et al., 2011; Wang et al., 2016). Moreover, MAD was observed to play a role in regulation of apoptosis, since it targets the anti-apoptotic BCL2 protein MCL1 for degradation in mammals (Inuzuka et al., 2011; Xu et al., 2011).

Besides Cdc48, Msp1 (ATAD1 in humans) supports extraction and degradation of mitochondrial proteins (**Figure 1** and **Table 1**). Despite being an AAA-ATPase similar to Cdc48, Msp1 contains a membrane spanning domain at the N-terminus and is mainly localized at the OMM, but is also attached to peroxisomes (Nakai et al., 1993; Chen et al., 2014; Okreglak and Walter, 2014). Msp1/ATAD1 plays a key role in mitochondrial proteostasis since it mediates the degradation of mislocalized tail-anchored proteins (TA-proteins) from the OMM. A baseline degradation of TA-proteins by the UPS occurs to maintain a dynamic stationary level and ensure insertion into the outer membrane of the correct organelle (Chen et al., 2014; Okreglak and Walter, 2014). Moreover, TA-proteins destined to the ER can mislocalize to the OMM, when their import system, termed the GET pathway in yeast and TRC in mammals, is impaired. These mislocalized TA-proteins are recognized by Msp1 at the OMM and targeted for proteasomal degradation. For instance, the TA-protein Pex15 is partly inserted in the OMM, even in the presence of the fully functional GET pathway, suggesting that TA-proteins are constitutively inserted into the OMM, where they have to be removed by Msp1 (Chen et al., 2014; Okreglak and Walter, 2014). Recently, a similar role of Msp1 was described at peroxisomes (Weir et al., 2017). Of note, the proposed ATP-dependent activity of Msp1 in the extraction of TA-proteins from mitochondrial membranes was confirmed in an *in vitro* system of reconstituted proteoliposomes (Wohlever et al., 2017).

Surprisingly, mistargeted TA-proteins extracted by Msp1 are ubiquitylated by Doa10, an E3 ligase residing in the ER membrane and degraded in a Cdc48-dependent manner

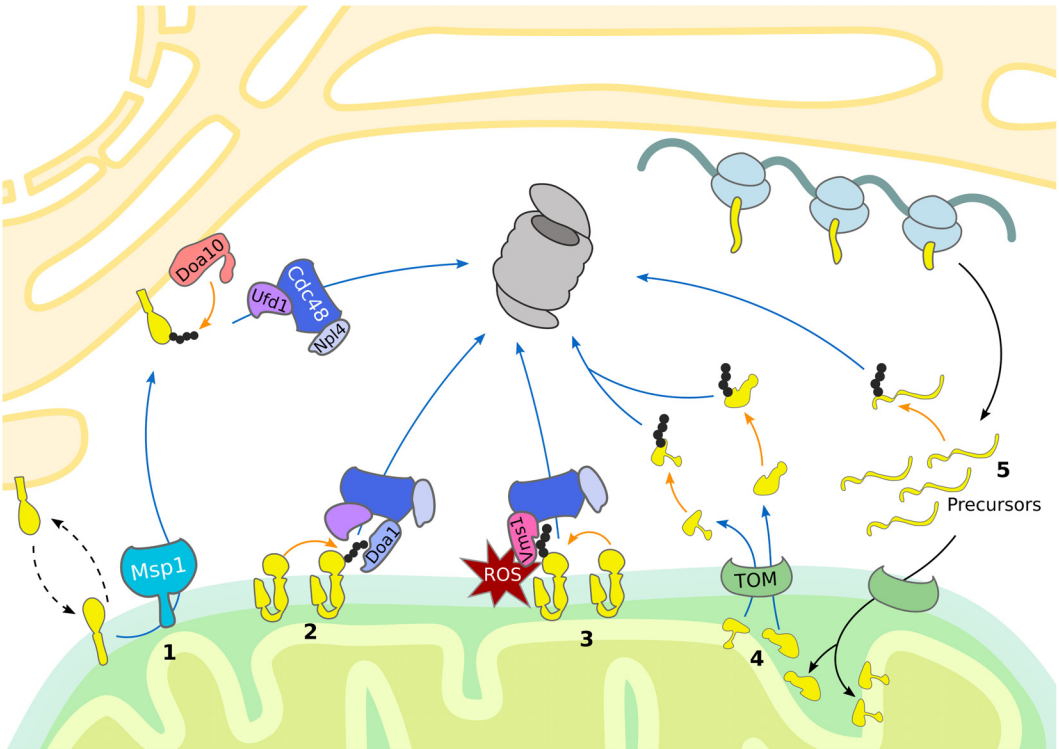

FIGURE 1 | UPS-dependent turnover of mitochondrial proteins. The cytosolic UPS mediates mitochondrial protein turnover by ubiquitylation (orange arrows) and targeting (blue arrows) of substrates for degradation by the 26S proteasome. (1) Mislocalized tail-anchored proteins are extracted from the OMM by Msp1, ubiquitylated by the ER-associated E3 ubiquitin ligase Doa10 and then translocated to the proteasome by the $Cdc48^{Ufd1/Npl4}$ complex. (2, 3) Degradation of OMM proteins occurs via Cdc48-dependent translocation to the 26S proteasome. (2) Upon oxidative stress, Vms1 translocates to the OMM where it recruits Cdc48 and its co-factor Npl4. (3) Under normal conditions OMM proteins are ubiquitylated and translocated to the 26S proteasome by Cdc48 together with the co-factors Ufd1, Npl4, and Doa1/Ufd3. (4) Proteins residing in the IMS and IMM are retro-translocated via the TOM complex into the cytosol for ubiquitin-dependent proteasomal degradation. (5) Prior import, mistargeted or damaged mitochondrial precursor proteins are degraded by the UPS.

(**Figure 1** and **Table 1**). This initial observation suggests a role of ERAD in the turnover of OMM proteins (Dederer et al., 2019; Matsumoto et al., 2019). Indeed, Msp1 co-localizes with its substrates at ER-mitochondria contact sites, which however, seem to be dispensable for Doa10/Cdc48 dependent turnover of Msp1 substrates (Matsumoto et al., 2019). Therefore, it was proposed that Msp1 extracted TA-proteins are inserted into the ER membrane for Doa10-mediated ubiquitylation. Subsequently, Cdc48 is recruited together with the cofactors Ufd1 and Npl4 to translocate mistargeted TA-proteins to the 26S proteasome (Matsumoto et al., 2019). Intriguingly, Msp1 appears to extract only monomeric proteins and not multi-complexes, suggesting that the recognition of mistargeted TA-protein is based on the weak interaction with the membrane of a single transmembrane domain (Dederer et al., 2019). Interestingly, Msp1 emerged as an MAD substrate itself, whose degradation depends on the Doa1–$Cdc48^{-Ufd1-Npl4}$ complex (Wu et al., 2016). This might indicate a role of the UPS in the regulation of mitochondrial protein half-life by controlling Msp1 level and extraction of TA-proteins.

Intriguingly, besides membrane bound proteins also inner mitochondrial proteins have been reported to be ubiquitylated. In analogy to ERAD, retro-translocation of mitochondrial proteins across IMM and/or OMM has been suggested to enable ubiquitylation at the cytosolic surface of the organelle (**Figure 1**). Indeed, such activity has been demonstrated for IMS proteins, which upon unfolding, translocate into the cytosol (Bragoszewski et al., 2015). The export of these proteins seems to depend on the translocase of the outer membrane (TOM) complex also required for import, however, further studies are required to investigate the exact mechanism of mitochondrial retro-translocation. Notably, this degradation of IMS proteins appears to be utilized to rewire the mitochondrial proteome to changing metabolic conditions upon shift from respiration to fermentation (Bragoszewski et al., 2015). A major challenge in exploring this novel mechanism is to carefully distinguish ubiquitin-dependent degradation of retro-translocated mitochondrial protein versus cytosolic precursor proteins that accumulate when mitochondrial import is impaired (**Figure 1**).

Mitochondrial Import Control

Nuclear-encoded mitochondrial proteins are synthetized as precursors in the cytosol and subsequently imported and sorted to different mitochondrial compartments. After passing through the TOM complex, precursor proteins are redistributed to their final intra-mitochondrial destination. At least five distinct but interconnected import pathways have been identified, each one

TABLE 1 | Regulators of ubiquitin-dependent mitochondrial quality control.

Function	Yeast	Mammals Other species	Function	Pathways	Reference
Translocation	Cdc48	VCP/p97 *cdc-48* (*C. elegans*) TERT94/ VCP (*D. melanogaster*)	AAA-ATPase	ERAD, MAD, RQC, mitoTAD	Heo et al., 2010; Tanaka et al., 2010; Xu et al., 2011; Brandman et al., 2012; Defenouillère et al., 2013; Kim et al., 2013; Wang et al., 2016; Wu et al., 2016; Izawa et al., 2017; Rendón et al., 2018; Verma et al., 2018; Mårtensson et al., 2019; Matsumoto et al., 2019
	Vms1	VMS1/ANKZF1 *vms-1* (*C. elegans*)	Cdc48 recruitment	MAD, RQC	Heo et al., 2010; Izawa et al., 2017; Rendón et al., 2018; Verma et al., 2018
	Ubx2		Cdc48 recruitment	ERAD, mitoTAD	Mårtensson et al., 2019; Matsumoto et al., 2019
	Doa1/ Ufd3		Cdc48 co-factor	MAD	Wu et al., 2016
	Ufd1	UFD1L	Cdc48 co-factor	MAD, ERAD, mitoTAD, RQC	Brandman et al., 2012; Wu et al., 2016; Izawa et al., 2017; Verma et al., 2018; Mårtensson et al., 2019; Matsumoto et al., 2019
	Npl4	NPL4	Cdc48 co-factor	MAD, ERAD, mitoTAD, RQC	Heo et al., 2010; Brandman et al., 2012; Wu et al., 2016; Izawa et al., 2017; Verma et al., 2018; Mårtensson et al., 2019; Matsumoto et al., 2019
	Msp1	ATAD1	AAA-ATPase	MAD, mitoCPR	Chen et al., 2014; Okreglak and Walter, 2014; Weidberg and Amon, 2018; Dederer et al., 2019; Matsumoto et al., 2019
	Cis1		Msp1 recruitment	mitoCPR	Weidberg and Amon, 2018
		Ubiquilins			Itakura et al., 2016; Whiteley et al., 2017
Ubiquitylation	Mdm30		E3 ligase	MAD	Fritz et al., 2003; Escobar-Henriques et al., 2006; Cohen et al., 2008
	Rsp5		E3 ligase	MAD	Wu et al., 2016
		Parkin	E3 ligase	MAD	Tanaka et al., 2010; Chan et al., 2011; Kim et al., 2013; Wang et al., 2016
	Ltn1	Listerin	E3 ligase	RQC	Brandman et al., 2012; Defenouillère et al., 2013; Shao et al., 2013
		MARCH5/MITOL	E3 ligase	MAD	Yonashiro et al., 2006; Karbowski et al., 2007; Park et al., 2010
	Doa10		E3 ligase	ERAD	Dederer et al., 2019; Matsumoto et al., 2019
	Cue1		Doa10 co-factor	ERAD	Dederer et al., 2019; Matsumoto et al., 2019
	Ubc6		E2	ERAD	Dederer et al., 2019; Matsumoto et al., 2019
	Ubc7		E2	ERAD	Dederer et al., 2019; Matsumoto et al., 2019
De-ubiquitylation	Ubp2		DUB	MAD	Anton et al., 2013
Transcriptional regulation	Pdr3		Transcription factor	mitoCPR	Weidberg and Amon, 2018
	Rpn4		Transcription factor	Response to clogging	Boos et al., 2019
		ATF5 *atfs-1 (C. elegans)*	Transcription factor	UPR^{mt}	Nargund et al., 2012; Fiorese et al., 2016
		ERα	Transcription factor	UPR^{mt}	Papa and Germain, 2011
	Msn2/4		Transcription factor	IPTP	Suhm et al., 2018

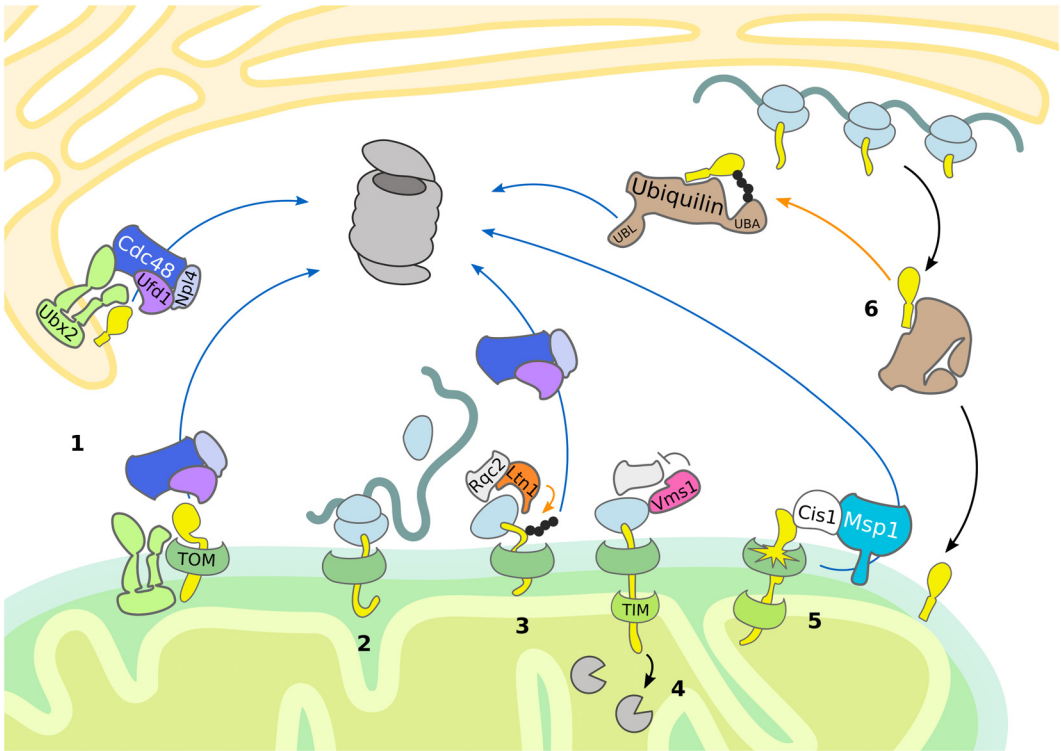

FIGURE 2 | Mitochondrial import control. The cytosolic UPS supports quality control and mitochondrial import by removing damaged proteins. Substrate ubiquitylation (orange arrows) is followed by translocation (blue arrows) and proteasomal degradation. (1) Ubx2 localizes both in the ER membrane and in proximity of the TOM complex at the OMM, where it recruits Cdc48 and its cofactors Ufd1 and Npl4 to degrade ER and mitochondrial proteins, respectively. (2, 3, 4) In case of co-translational import of mitochondrial proteins, induction of RQC is central to the handling of stalled ribosomes. (3) Ltn1-dependent ubiquitylation of the nascent polypeptide chain recruits Cdc48 with its cofactors Ufd1 and Npl4 for proteasomal targeting. (4) Vms1 counteracts Rqc2-dependent CAT-tail formation and safeguards tRNA release of the nascent polypetide, which is subsequently degraded in the matrix by mitochondrial proteases. (5) Upon mitochondrial import defects, Cis1-recruited Msp1 moderates the release of mitochondrial proteins stalled in the TOM complex. (6) In mammals, ubiquilins bind transmembrane domains of mitochondrial proteins and either support mitochondrial translocation or proteasomal targeting.

directed by a specific targeting signal (Neupert and Herrmann, 2007; Chacinska et al., 2009; Wiedemann and Pfanner, 2017; Pfanner et al., 2019). In order to avoid the import of aberrant proteins into mitochondria and to ensure mitochondrial proteostasis, specialized surveillance mechanisms monitor the nuclear-encoded proteins before and during mitochondrial import (**Figures 1**, **2**).

Interestingly, it was shown that mitochondrial precursor proteins are constantly degraded in a ubiquitin-dependent manner. For example, both the wild-type and mutant IMS protein endonuclease G (endoG) are degraded by the UPS before their import (Radke et al., 2008). However, in contrast to the mutant form, wild-type endoG can be alternatively degraded by the IMS protease Omi, supporting the idea that the proteasome plays a role in degradation of defective mitochondrial proteins prior to import. In line with this conclusion, the UPS was reported to assist the import of intermembrane proteins by constantly degrading precursor proteins before they enter mitochondria (Bragoszewski et al., 2013; Kowalski et al., 2018) (**Figure 1**).

In mammalian cells, quality control of membrane proteins recruited to mitochondria is provided by ubiquilins (Itakura et al., 2016; Whiteley et al., 2017), which are substrate receptors supporting proteasomal turnover. They typically possess a ubiquitin-binding UBA domain for recognition of ubiquitylated substrates and a ubiquitin-like UBL domain for proteasome targeting. Ubiquilins bind to mitochondrial proteins containing transmembrane domains, thereby preventing their aggregation (Itakura et al., 2016). As long as the substrate remains unmodified, the UBA domain of ubiquilin is bound to its own UBL domain, promoting mitochondrial import. Upon substrate ubiquitylation, the UBL domain is released, which results in targeting the mitochondrial substrate proteins for proteasomal degradation. Thus, ubiquilins exert an important triage function regulating mitochondrial import (**Figure 2**).

Mitochondrial targeting and protein import are governed at the mitochondrial import channel by a newly proposed MAD pathway. A subpopulation of Ubx2 binds to the TOM complex, which recruits Cdc48 to initiate degradation of partially imported proteins similarly to ERAD (Mårtensson et al., 2019) (**Figure 2** and **Table 1**). This mechanism is termed mitochondrial protein translocation-associated degradation (mitoTAD). Interestingly, Ubx2 binding with Cdc48 depends on the co-factor Ufd1, whereas the other Cdc48 partners implicated in MAD, Vms1 and

Doa1/Ufd3, are not involved in mitoTAD. However, combined deletion of Ubx2 with either Vms1 or Msp1 caused strong mitochondrial defects and an increase of ubiquitylated proteins bound to the TOM complex, suggesting redundant roles of the described mitochondrial import control pathways (Mårtensson et al., 2019). Indeed, Msp1 has been reported to function in degrading substrates from the TOM channel as well (Weidberg and Amon, 2018). Mitochondrial import stress induces the expression of Cis1, which mediates the recruitment of Msp1 to the import pore (Weidberg and Amon, 2018; Boos et al., 2019). Subsequently, Msp1 triggers proteasomal turnover of proteins clogging the TOM complex, thereby maintaining mitochondrial protein import and proteostasis (**Figure 2**) (Weidberg and Amon, 2018).

Ribosome-Associated Quality Control of Mitochondrial Proteins

The first contact of mitochondrial proteins with the cytosolic proteostasis network occurs already during translation at cytosolic ribosomes. It was recently shown that stalling of ribosomes, which indicates aberrant mRNA, defective ribosome assembly, or an accumulation of nascent protein, triggers ribosome-associated quality control (RQC) (Brandman and Hegde, 2016; Joazeiro, 2019). Stalled ribosomes are sensed by the yeast RQC complex subunit Rqc2 (NEMF in mammals), recruiting the E3 ligase Ltn1 (listerin in mammals) for polyubiquitylation of the nascent peptide. The polyubiquitin chain serves as signal to attract Cdc48 and its cofactors Ufd1 and Npl4 (UFD1 and NPLOC4 in mammals), which together shuttle the polypeptide to the proteasome. In case the accessible nascent polypeptide does not contain any lysine residues, Rqc2 can induce a peptide extension by addition of a C-terminal alanine and threonine (CAT) tail. Through this elongation, more residues of the nascent chain get exposed outside of the ribosome exit tunnel, until a lysine becomes available for Ltn1 dependent ubiquitylation. In addition, CAT-tails have been reported to induce aggregation of proteins, which might act as a protective mechanism in case of RQC failure.

Since the targeting sequence of many mitochondrial proteins is at the N-terminus, import can occur either post-translationally or co-translationally (Neupert and Herrmann, 2007; Wiedemann and Pfanner, 2017). If import and translation occur simultaneously, part of the nascent polypeptide chain is sequestered inside the mitochondrial import machinery before completion of translation and release from the ribosome. Thus, in contrast to cytosolic proteins, ubiquitylation of mitochondrial proteins emerging at the ribosome might fail due to the close proximity between the ribosome and the mitochondrial translocation machinery and reduced accessibility of the co-translationally imported nascent polypeptide chain. Consequently, Rqc2 dependent CAT tailing can induce protein aggregation inside the mitochondrial matrix and subsequent degeneration of the mitochondrial respiratory capacity (Izawa et al., 2017). The formation of protein aggregates is counteracted by Vms1 (ANKZF1 in mammals) activity, which mediates the release of nascent polypeptides from the tRNA and thereby terminates CAT tail formation (Izawa et al., 2017; Rendón et al., 2018; Verma et al., 2018) (**Figure 2** and **Table 1**).

MITOCHONDRIAL STRESS RESPONSE MECHANISMS

Based on the intricate network of ubiquitin-dependent quality control mechanisms, stressed or defective mitochondria often affect UPS activity, which largely relies on the abundance and activity of the 26S proteasome (Marshall and Vierstra, 2019). Central to this regulatory relationship are reactive oxygen species (ROS), that are generated inside mitochondria via oxidative phosphorylation (Lefaki et al., 2017). Changes in ROS level have been linked to reversible disassembly of the 26S proteasome into 20S and 19S subunits. These changes in proteasomal composition and activity might reflect a protective strategy by favoring ATP/ubiquitin-independent degradation of oxidized proteins via 20S proteasomes (Wang et al., 2010; Grune et al., 2011; Livnat-Levanon et al., 2014). In *C. elegans*, mitochondrial impairment was linked to defective turnover of cytosolic UPS model substrates despite no increase in ROS, suggesting the existence of a distinct response mechanisms regulating UPS activity (Segref et al., 2014). Mitochondrial translation accuracy has been as well-linked to cytosolic proteostasis (Suhm et al., 2018). Particularly, mitochondrial ribosome mutations in yeast either improving or reducing translation accuracy showed an increased or decreased turnover of a cytosolic proteasome substrate, respectively. However, in both studies, proteasome activity was not altered in comparison to wild-type controls, suggesting that the regulation occurs upstream of proteasomal degradation (Segref et al., 2014; Suhm et al., 2018).

Interestingly, recent studies have demonstrated that specialized stress response pathways induced by mitochondrial impairment affect the UPS by regulating gene expression in the nucleus and protein translation in the cytosol (Papa and Germain, 2011; Nargund et al., 2012; Wrobel et al., 2015; Weidberg and Amon, 2018; Boos et al., 2019). Along with the well-known heat shock response (HSR) in the cytosol (Richter et al., 2010) and the unfolded protein response (UPR) in the ER (Walter and Ron, 2011), a mitochondrial UPR (UPR^{mt}) has been thoroughly investigated (Münch, 2018; Shpilka and Haynes, 2018). These three stress response mechanisms are characterized by one or more signal transduction pathways that activate transcription of protective genes, encoding molecular chaperones, proteases, and UPS components. Consequently, induction of HSR, UPR, and UPR^{mt} supports proteostasis by inducing UPS function.

Even though first detected in mammalian cells (Martinus et al., 1996; Zhao et al., 2002), the UPR^{mt} mechanism has been characterized in *C. elegans* (Yoneda, 2004; Haynes et al., 2010; Nargund et al., 2012). Central to this mitochondrial stress response is the transcription factor ATFS-1, which translocates into the nucleus when mitochondrial import is blocked (Nargund et al., 2012). Although the downstream transcriptional regulation induced in case of mitochondrial stress is similar to *C. elegans*, the regulatory signaling of the mammalian UPR^{mt} emerged

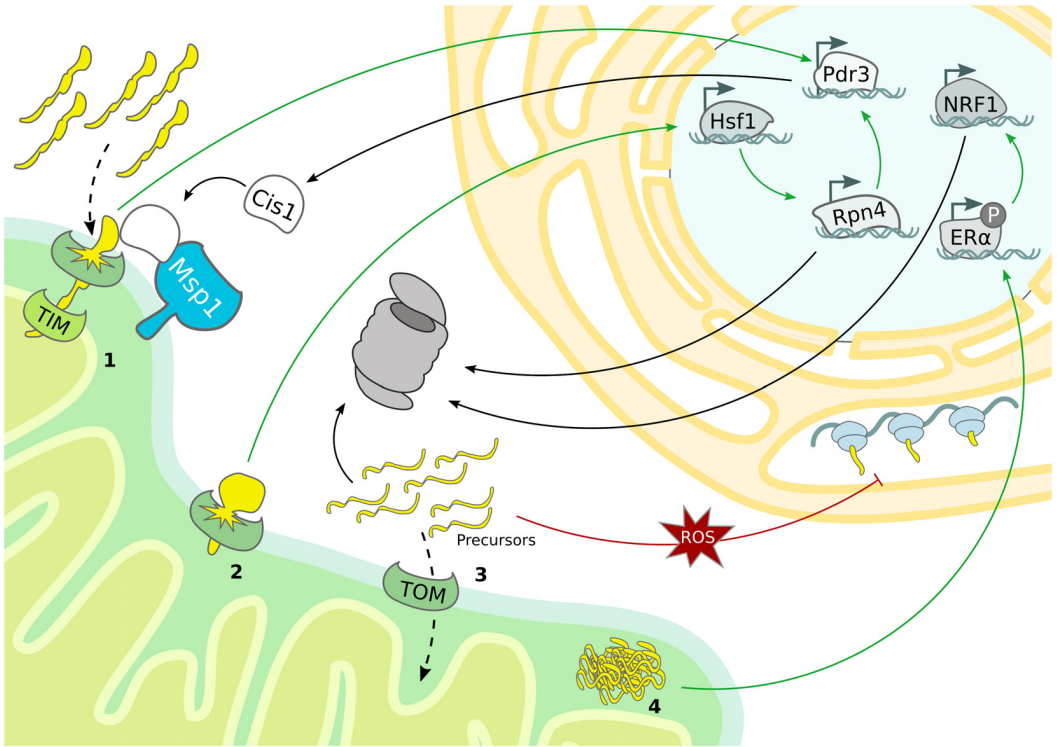

FIGURE 3 | Mitochondrial stress response mechanisms. Mitochondrial stress pathways regulate nuclear gene transcription and cytosolic protein translation to sustain proteostasis. (1) Overexpression of bipartite signal-containing proteins activates the mitoCPR, which triggers Pdr3-dependent expression of MDR genes, from which Cis1 promotes the recruitment of Msp1 to the TOM complex. (2) TOM clogging activates Hsf1-dependent expression of Rpn4, which drives expression of proteasomal subunits and mitoCPR-induced Pdr3. (3) The accumulation of mitochondrial precursor proteins caused by import defects boosts proteasomal activity and depletes general protein translation in the cytosol. (4) In mammals, protein aggregation in the IMS induces proteasome activity by ligand-independent activation of ERα and subsequent upregulation of NRF1.

to be more complex and a general consensus model is still missing (Münch, 2018). Notably, a specialized mammalian UPR^{mt} was reported to enhance proteasomal activity in response to protein aggregation in the IMS (Papa and Germain, 2011). The proposed molecular mechanism is based on the ligand-independent activation of the estrogen receptor alpha (ERα), which upregulates NRF1, a transcription factor involved in the expression of proteasomal subunits (**Figure 3** and **Table 1**) (Papa and Germain, 2011). Accordingly, the *C. elegans* NRF homolog SKN-1 has also been identified as a downstream target of the UPR^{mt} (Nargund et al., 2012). In yeast, increased proteasome activity was detected in consequence of mitochondrial import defects, indicating an UPR activated by the mistargeting of proteins (UPR^{am}) (Wrobel et al., 2015). Thus, defective mitochondrial import generates an accumulation of misfolded proteins in the cytosol, which aggravates proteasomal assembly and proteolytic activity (**Figure 3**).

The use of deep transcriptome sequencing, combined with proteomics allowed to monitor transcriptional and translational changes over time after 'clogging' of the TOM complex in yeast. Under these conditions the heat shock responsive transcription factor Hsf1 is activated and triggers the expression of molecular chaperones. Interestingly, one Hsf1-dependent target gene encodes the transcription factor Rpn4, which specifically upregulates proteasomal subunits, constituting a second layer of response that is activated upon prolonged stress (Boos et al., 2019). In addition to Hsf1 activation, the expression of nuclear encoded respiratory chain subunits is downregulated, which might serve to reduce the mitochondrial import load (**Figure 3**). Another transcriptional stress response activated by import defects is the mitochondrial compromised protein import response (mitoCPR) (Weidberg and Amon, 2018). Here, import inhibition caused by overexpression of bipartite signal-containing proteins, which are normally inserted into the IMS, provokes expression of multi drug resistance (MDR) response genes by the transcription factor Pdr3. One of the most upregulated genes is Cis1, which is recruited to the OMM and, by interacting with Tom70 and Msp1, supports the Msp1 and proteasome-dependent degradation of non-imported mitochondrial precursor proteins. Interestingly, the correct functionality of this pathway is fundamental for cell survival upon defective mitochondrial import but not under normal growth conditions (**Figure 3** and **Table 1**) (Weidberg and Amon, 2018).

Although a general transcriptional program has not been identified yet, an intricate cooperation of the different quality control pathways becomes evident. For example, Rpn4 is not only initiated upon TOM "clogging," but also in import-defective

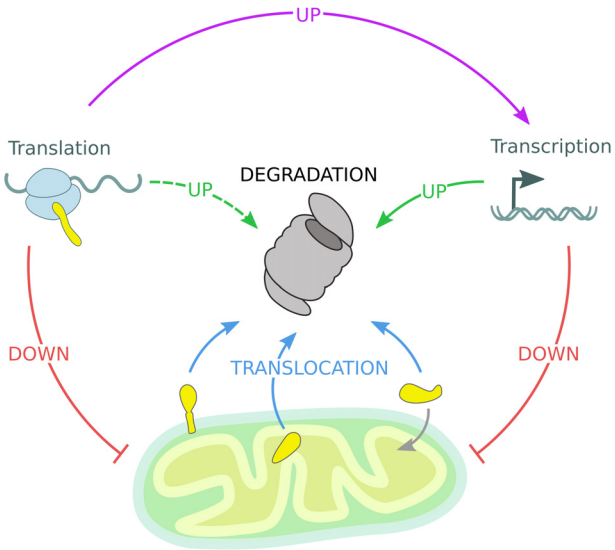

FIGURE 4 | Mitochondrial quality control governed by the UPS. The mitochondrial proteome is regulated by constant degradation of mitochondrial proteins, which are sequestered from the organelle and translocated (blue arrows) to the 26S proteasome for degradation. In addition, mitochondrial precursor proteins are degraded by the 26S proteasome if not efficiently imported into mitochondria. Upon mitochondrial stress, transcription and translation of mitochondrial genes are diminished, reducing the substrate load of both the mitochondrial import machinery and the 26S proteasome (red arrows). Moreover, transcription of protective genes is induced to enhance UPS activity (solid green arrow). Stress-induced inhibition of global protein translation triggers the expression of specialized transcription factors responsible for the activation of inducible stress response programs mentioned before (violet arrow), while other gene products might support UPS function (dashed green arrow).

yeast mutants related to UPR^{am} (Wrobel et al., 2015; Boos et al., 2019). However, in contrast to clogging the TOM channel (Boos et al., 2019), import-defects do not induce the expression of proteasome subunits but of proteasome assembly chaperones (Wrobel et al., 2015). These discrepancies suggest that Rpn4 might be activated in case of mitochondrial defects, but probably distinct transcriptional programs characterize specific types of import-related stress. Moreover, the Pdr3-dependent transcriptional induction of Cis1 upon accumulation of bipartite signal-containing proteins also requires Rpn4 (**Figure 3**) (Boos et al., 2019).

An additional cellular strategy to avoid UPS overload under prolonged mitochondrial stress conditions is based on suppression of protein translation both inside and outside of the organelle (Münch, 2018; Samluk et al., 2018; Shpilka and Haynes, 2018). The predominant mechanism of cytosolic translation inhibition is mediated by phosphorylation of the eukaryotic transition initiation factor 2 alpha (eiF2α), which prevents formation of the translation initiation complex (Sonenberg and Hinnebusch, 2009). However, oxidative stress generated upon dysfunctional mitochondrial import was recently reported to reduce cytosolic translation independently of eiF2α phosphorylation. For instance, the translation machinery can be directly modulated by the redox status of proteins participating in translation (**Figure 3**) (Topf et al., 2018). Besides reducing global protein translation, mitochondrial import defects have also been proposed to induce translation of particular mRNAs (Wang and Chen, 2015; Topf et al., 2016). Specialized translation of stress-related transcription factors has already been described as a downstream event of eiF2α phosphorylation, which therefore trigger numerous stress responses, including the UPR^{mt} (Fiorese et al., 2016; Pakos-Zebrucka et al., 2016; Quirós et al., 2017).

Even though a common mechanism has not been identified yet, it is obvious that diverse, overlapping mitochondrial signaling pathways are activated to support proteostasis and cellular survival. These pathways influence gene expression to adapt mitochondrial and cytosolic protein degradation pathways under mitochondrial stress conditions.

CONCLUSION

Each cellular sub-compartment is equipped with specialized quality control machineries, which are intricately connected and cross-communicate. The reported studies support the idea that ubiquitin-dependent mitochondrial quality control pathways efficiently adapt in response to environmental and metabolic changes. However, in case of acute stress conditions, specialized response programs are induced to adjust the UPS capacity and thereby restore organellar proteostasis (Pakos-Zebrucka et al., 2016; Braun and Westermann, 2017; D'Amico et al., 2017; Pickles et al., 2018; Andréasson et al., 2019; Zheng et al., 2019; Escobar-Henriques et al., 2020). Besides understanding the mechanistic details of individual pathways, the regulation of cell-type specific and organismal composition of mitochondrial quality control need to be further addressed. Mechanistically, ubiquitin-dependent mitochondrial proteostasis follows a series of common regulatory events: stress sensing, substrate targeting and modification, protein translocation, and proteasomal degradation. Mitochondrial quality control is further regulated by nuclear gene transcription and cytosolic protein translation events (**Figure 4**). Overall, these proteostasis strategies are highly adaptive and can efficiently and dynamically modulate the stability of the mitochondrial proteome according to cellular needs.

FUTURE PERSPECTIVES

Regulation of mitochondrial quality control by the UPS emerged to be conserved in all eukaryotes, involving Cdc48/p97 and the 26S proteasome. However, mechanistic details on substrate selection and ubiquitin ligases remain largely unclear. In contrast to proteasomal degradation of OMM proteins, little is known about the turnover of intra-mitochondrial proteins. Especially how mitochondrial substrates are retro-translocated from the different inner mitochondrial compartments into the cytosol is of central importance for understanding the regulation of MAD. Conversely, a novel functional role of the mitochondrial translocation machinery has been identified, which seems to

import cytosolic aggregation-prone proteins into mitochondria for efficient degradation (Ruan et al., 2017). A more detailed view on the protein shuttling between mitochondria and cytosol will extend our current view on the cellular mechanisms dedicated to proteostasis maintenance and the reciprocal role of mitochondrial and cytosolic proteolytic systems.

Besides the high degree of mechanistic similarities between the MAD and ERAD pathways, mitochondrial quality control is further defined by functional mitochondria-ER interactions. For example, substrates extracted from the OMM by Msp1 are targeted to the ER for subsequent proteasomal degradation (Dederer et al., 2019; Matsumoto et al., 2019). Moreover, mitochondrial precursor proteins have been identified to associate with the ER membrane before being imported. The recently proposed ER-surface mediated targeting (ER-SURF) model describes the association of mitochondrial precursor proteins with the ER membrane and their rerouting to mitochondria by the ER-localized chaperone Djp1 (Hansen et al., 2018). Thus, the interaction between mitochondria and ER might play a conceptual role in protein quality control of mitochondrial proteins.

Mitochondrial impairments have been associated to several pathologies not only limited to metabolic diseases or myopathies, but including cancer (Vyas et al., 2016; Denisenko et al., 2019), pulmonary hypertension (Chen et al., 2019) and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Kim and Mook-Jung, 2019; Tapias, 2019). Thus, further understanding of mitochondrial surveillance mechanisms might help to establish therapeutic interventions for treatment of mitochondrial pathologies. The multiple layers of mitochondrial regulation that can lead to disease progression if defective makes mitochondrial quality control a challenging but exciting research field, which is more and more integrated in the context of specialized cellular pathways, from basic research to clinical studies.

AUTHOR CONTRIBUTIONS

All authors conceived, wrote the manuscript, and critically revised the manuscript. SR designed the figures.

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A Healthy Heart and a Healthy Brain: Looking at Mitophagy

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Mitochondrial dysfunction is a hallmark of aging and is a major contributor to neurodegenerative diseases and various cardiovascular disorders. Mitophagy, a specialized autophagic pathway to remove damaged mitochondria, provides a critical mechanism to maintain mitochondrial quality. This function has been implicated in a tissue's ability to appropriately respond to metabolic and to bioenergetic stress, as well as to recover from mitochondrial damage. A global decline in mitophagic flux has been postulated to be linked to pathological alterations that occur in the heart and the brain as well as a general age-dependent decline in organ function. Cellular observation suggests multiple mechanistically distinct pathways converge upon and activate mitophagy. Over the past decade, additional molecular components within mitophagy have been discovered, including several disease-associated genes that are functionally implicated in mitophagy. However, the pathophysiological role of mitophagy, and how it is regulated within normal physiology or various disease states, is less well established. Here, we will review the evidence that a decline in mitophagy contributes to impaired mitochondrial homeostasis and may be particularly detrimental to postmitotic neurons and cardiomyocytes. We will discuss mitophagy's pathological significance in both neurodegenerative diseases and cardiovascular disorders. Additionally, signaling pathways regulating mitophagy are reviewed, with emphasis placed on how these pathways might contribute to disease progression. Understanding mitophagy's role in the mechanisms of disease pathogenesis should allow for the development of more efficient strategies to battle pathological conditions associated with mitochondrial dysfunction.

Keywords: mitophagy, neurodegenerative diseases, cardiovascular disorders, mitochondrial, autophagy

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INTRODUCTION

Autophagy is the regulated process that targets unnecessary or dysfunctional cellular components for lysosomal-mediated removal (Mizushima et al., 1998; Klionsky and Emr, 2000; Kim and Lee, 2014; Yan and Finkel, 2017). This process is a crucial recycling mechanism to maintain cellular homeostasis under normal physiological states as well as in disease conditions as it facilitates the orderly degradation of damaged organelles and other cellular components, (Levine, 2005; He et al., 2012; Kim and Lee, 2014; Fernández et al., 2018). The removal of mitochondria through autophagy, a process called mitophagy, is an important element of mitochondrial quality control

(Youle and Narendra, 2011; Palikaras et al., 2018). This system mediates the selective engulfment of defective or damaged mitochondria by autophagosomes and their subsequent catabolism by lysosomes, preserving overall mitochondrial homeostasis (Youle and Narendra, 2011; Palikaras et al., 2018). Significant evidence suggests that mitophagy may be required for adaptation to various stresses, and that dysregulation of mitophagy may contribute to a host of diseases, most notably neurodegenerative conditions such as Parkinson's disease (PD) (Narendra et al., 2008; Geisler et al., 2010; Lazarou et al., 2015). The products of two PD-associated genes, the phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and the cytosolic E3 ligase Parkin, can sense mitochondrial damage and mediate ubiquitin-dependent mitophagy (Narendra et al., 2008; Youle and Narendra, 2011; Pickrell and Youle, 2015; Yamano et al., 2016; Palikaras et al., 2018). Loss-of-function mutations in the regulatory kinase PINK1 and the ubiquitin ligase Parkin have each been identified as a cause for familial, early-onset PD, suggesting that impaired mitophagy may contribute to the loss of dopaminergic neurons that occurs during the PD disease progression (Kitada et al., 1998; Valente et al., 2004; Lazarou et al., 2015; Palikaras et al., 2017; Harper et al., 2018). Similarly, the preservation of mitochondrial function through mitophagy is also important for mitochondria-rich and bioenergetically demanding organs such as the heart (Murphy et al., 2016; Bravo-San Pedro et al., 2017). Cardiac mitophagy may be required for the myocardium to recover from mitochondrial damage that occurs, for instance, during cardiac hypertrophy or ischemic injury (Murphy et al., 2016; Bravo-San Pedro et al., 2017). Evidence suggests a decline in mitophagy may contribute to the myriad of pathological events that occur under metabolic stress or in the elderly heart (Eisenberg et al., 2016; Leon and Gustafsson, 2016; Lesnefsky et al., 2016; Murphy et al., 2016; Shirakabe et al., 2016a,b).

Considerable interest has been focused on elucidating the molecular mechanisms of mitophagy, particularly on PINK1/Parkin's ability to catalyze the reaction to ubiquitinate a range of outer mitochondrial membrane (OMM) proteins (Narendra et al., 2008, 2010; Matsuda et al., 2010; Lazarou et al., 2015; Palikaras et al., 2017; Harper et al., 2018). PINK1 is a mitochondrial-targeted kinase whose stability is regulated, at least in part, by mitochondrial membrane potential (MMP) (Narendra et al., 2008, 2010; Matsuda et al., 2010; Lazarou et al., 2015). When MMP is dissipated following mitochondrial damage, PINK1 accumulates on the OMM, where it can phosphorylate ubiquitin attached to OMM and recruit Parkin to impaired mitochondria, thereby triggering its latent E3 ubiquitin ligase activity to ubiquitinate OMM proteins (Narendra et al., 2008, 2010; Matsuda et al., 2010; Lazarou et al., 2015). This post-translational tagging of OMM proteins with ubiquitin can serve as a signal to recruit receptors such as optineurin and NDP52, which act as a signal facilitating autophagosomal engulfment of individual damaged mitochondria (Itakura et al., 2012; Lazarou et al., 2015) (**Figure 1**). PINK1/Parkin-independent mechanisms can also initiate mitophagy (Novak et al., 2010; Hanna et al., 2012). The proapoptotic Bcl2 family proteins Nix and Bnip3 on the OMM participate in autophagic engulfment of mitochondria through direct interaction with LC3 on autophagosomes (Novak et al., 2010; Hanna et al., 2012) (**Figure 1**). Newly formed erythrocytes, also known as reticulocytes, eliminate their membrane-bound organelles, including mitochondria, during the course of development (Koury et al., 2005; Schweers et al., 2007). Nix regulated mitophagy may contribute to the rapid and coordinated clearance of mitochondria, also called the programmed mitochondrial degradation, in the process of erythropoiesis, whereas loss of Nix in mice impairs mitochondrial degradation during erythroid maturation (Schweers et al., 2007; Sandoval et al., 2008). The OMM protein FUNDC1 (FUN14 domain containing 1) can also bind LC3 to recruit autophagosome and promote mitophagy (Liu et al., 2012; Chen et al., 2016) (**Figure 1**). FUNDC1-mediated mitophagy may depend on its phosphorylation status regulated by the Unc-51 Like Autophagy Activating Kinase 1 (ULK1), casein kinase 2 (CK2), and PGAM5 phosphatase (Chen et al., 2014, 2016; Palikaras et al., 2018). Recent progress has demonstrated a crucial role for the inner mitochondrial membrane (IMM) proteins in mitophagy regulation. Notably, IMM protein prohibitin 2 (PHB2), a component of the mitochondrial prohibitin complex, may serve as a mitochondrial receptor for mitophagy upon mitochondrial depolarization (Wei et al., 2017).

While there has been considerable mechanistic insight into the regulatory pathways of mitophagy, investigation into the role of mitophagy in healthy and disease conditions has only just begun. Mouse tissue analysis has revealed a variation in basal mitophagy levels (Sun et al., 2015; McWilliams et al., 2018b), which might be required for continuous mitochondrial housekeeping. Stressed-induced mitophagy has been implicated in a tissue's ability to recover from mitochondrial damage, as well as appropriately responding to metabolic and bioenergetic stressors (Sun et al., 2016; Bravo-San Pedro et al., 2017; Palikaras et al., 2018). In energetically demanding tissues such as the heart and the brain, the mitophagic removal might require exquisite regulatory mechanisms, which merit further investigation. Additionally, recent studies have demonstrated an essential role for programmed mitophagy that directs the developmental metabolic transitioning of cardiac mitochondria (Gong et al., 2015). In summation, the preservation of mitochondrial function is crucial for all tissues, but it is undoubtedly critical for bioenergetically demanding organs such as the brain and the heart. Here, we will review how mitophagy represents a major pathway to help sustain mitochondrial quality and how alterations in mitophagy can contribute significantly to both neurodegenerative and cardiovascular diseases (**Figure 1**). The ability to genetically and pharmacologically modulate mitophagic flux may provide considerable insight into mitochondria related disease mechanisms and allow for the development of novel therapeutic approaches.

MITOPHAGY IN NEURODEGENERATIVE DISEASES

There is a growing appreciation regarding the critical role of altered mitochondrial function in the pathogenesis of

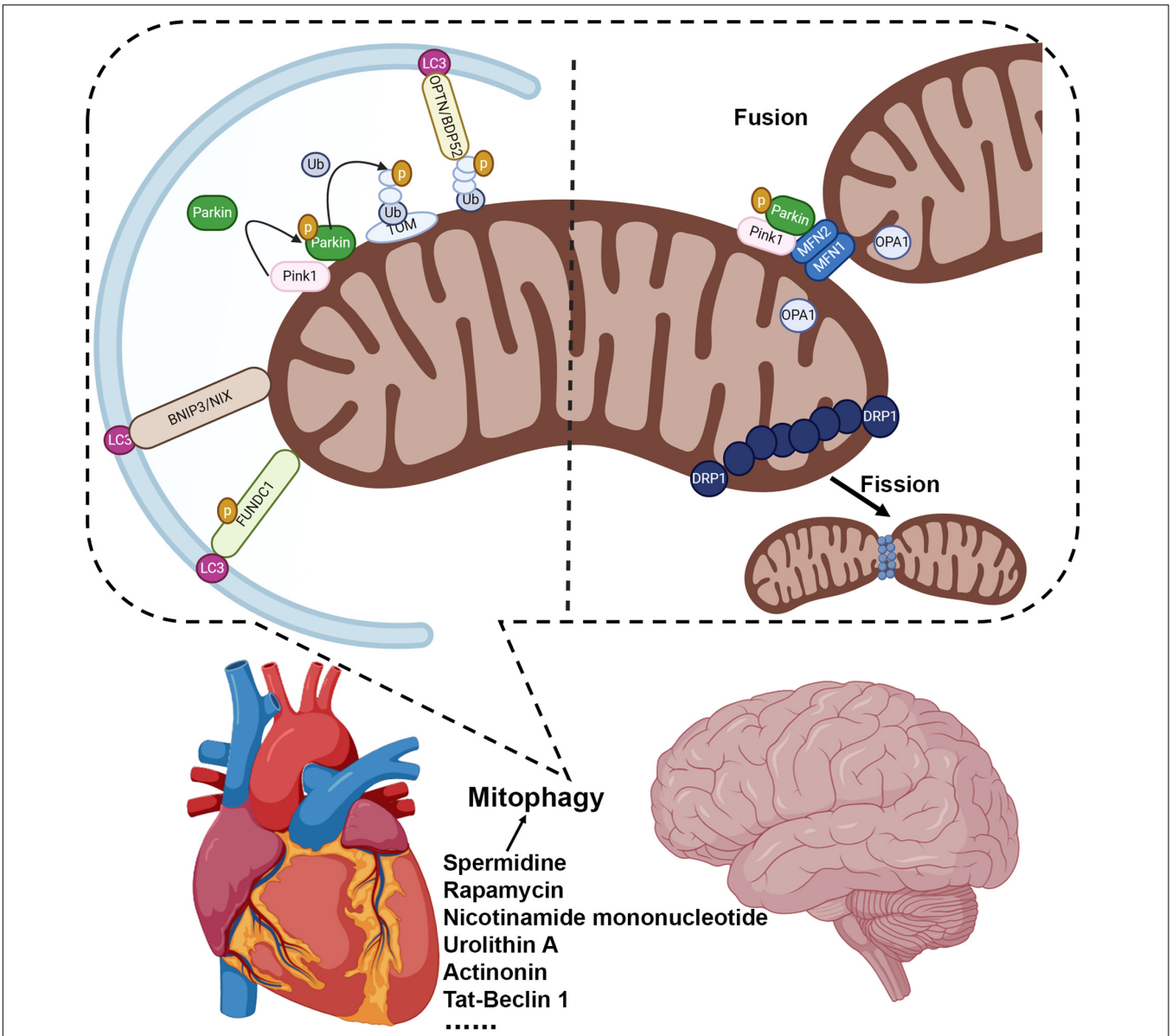

FIGURE 1 | Mitophagy is a critical mechanism to maintain mitochondrial quality in the brain and heart. Mitophagy can be regulated by multiple mechanistically distinct pathways: the PINK1/Parkin dependent mitophagy, which is mediated by mitophagy receptors such as optineurin and NDP52; BNIP3 and NIX regulated mitophagy to facilitate autophagic engulfment of mitochondria; and FUNDC1 mediated mitophagy. Manipulating autophagy/mitophagy activity may preserve mitochondrial function and serve as a novel therapeutic strategy, such as spermidine (Eisenberg et al., 2016), rapamycin (Sciarretta et al., 2012; Yan et al., 2013; Dai et al., 2014; Ranek et al., 2019), nicotinamide mononucleotide (Fang et al., 2019), urolithin A (Ryu et al., 2016; Fang et al., 2019), actinonin (Fang et al., 2019), and Tat-Beclin 1 (Shoji-Kawata et al., 2013; Shirakabe et al., 2016b).

neurodegenerative diseases (Valente et al., 2004; Youle and Narendra, 2011; Subramaniam and Chesselet, 2013; Hsieh et al., 2016; Palikaras et al., 2018). Selective identification and removal of damaged or dysfunctional mitochondria through mitophagy is thought to be an effective mechanism in maintaining neuronal mitochondrial homeostasis (Hwang et al., 2015; Hsieh et al., 2016; McWilliams et al., 2018b). Evidence suggests a decline in mitophagy might contribute to many neurodegenerative diseases (Burchell et al., 2013; Bingol et al., 2014; Fivenson et al., 2017; Fang et al., 2019).

Attention to mitophagy has been augmented by its link to genes related to inherited forms of early-onset PD (Kitada et al., 1998; Valente et al., 2004; Lazarou et al., 2015; Bingol and Sheng, 2016; Sliter et al., 2018). PD is one of the most common neurodegenerative disorders characterized by a series of motor impairments including tremors, rigidity, bradykinesia (slowness of movement), and postural instability (poor balance), which are caused by the progressive loss of dopaminergic neurons in the substantia nigra of the brain (Ascherio and Schwarzschild, 2016). Although medical and surgical treatments

may provide symptomatic relief, there is no cure for PD (Deep-Brain Stimulation for Parkinson's Disease Study Group, 2001; Narendra et al., 2009; Gao et al., 2011; Brod et al., 2012; Richard et al., 2012; Burchell et al., 2013; Hauser et al., 2013; Olanow et al., 2014; Gan-Or et al., 2015; Ascherio and Schwarzschild, 2016). Identification of genes mutated in monogenic forms of PD has provided valuable insight into the etiology of the disease (Narendra et al., 2009; Burchell et al., 2013; Gan-Or et al., 2015; Ascherio and Schwarzschild, 2016). Specifically, mutations or variants of PINK1 and Parkin have been found in the inherited forms of early-onset PD's patient (Kitada et al., 1998; Valente et al., 2004; Lazarou et al., 2015; Bingol and Sheng, 2016; Sliter et al., 2018). Biochemical and genetic studies reveal the products of these two genes, PINK1 and Parkin, normally function within the same genetic pathway to govern mitochondrial quality control (Lazarou et al., 2015; Bingol and Sheng, 2016; Sliter et al., 2018). PINK1 can detect and accumulate on the damaged mitochondria, which results in activation of Parkin's E3 ubiquitin ligase activity and recruitment of Parkin to the dysfunctional mitochondrion (Narendra et al., 2008; Matsuda et al., 2010; Hasson et al., 2013; Lazarou et al., 2015). Parkin then ubiquitinates OMM proteins to promote mitophagy (Hasson et al., 2013; Lazarou et al., 2015; Sliter et al., 2018). The realization that PINK1 and parkin can work together in the same pathway to coordinate mitophagy strengthens the notion that mitophagy may play an important role in PD (Hasson et al., 2013; Lazarou et al., 2015; Sliter et al., 2018). Therefore, the biochemical mechanisms of PINK1/Parkin mediated mitophagy, along with their roles in various models of PD, have been studied extensively over the past 10 years. While the generation of Parkin and PINK1 mutant flies has elucidated the functions of these genes in regulating mitochondrial integrity (Clark et al., 2006; Park et al., 2006; Palikaras et al., 2018), PINK1 and Parkin knockout mouse models poorly recapitulate dopamine neurodegeneration and the pathophysiology of human PD (Goldberg et al., 2003; Itier et al., 2003; Kitada et al., 2007; Billia et al., 2011; Hasson et al., 2013; Lazarou et al., 2015; Sliter et al., 2018). A significant portion of basal mitophagy occurs within the PD-relevant dopamine neurons of the substantia nigra pars compacta and in microglia, indicating a critical role of mitophagy in these cells under physiological conditions (McWilliams et al., 2018b). However, PINK1 or Parkin does not appear to influence basal mitophagy (McWilliams et al., 2018a,b). This data suggests that the precise role of PINK1/Parkin under physiological conditions remains to be defined. The pathophysiological basis of the cross-regulation between various mitochondrial quality control pathways will be of prime importance in understanding how mitophagy occurs and how it relates to PD progression.

Insight into the mechanisms underlying mitophagy and the importance of mitochondrial quality control have extended to other common neurodegenerative diseases associated with mitochondrial dysfunction such as Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) (Trushina et al., 2004; Ye et al., 2015; Palikaras et al., 2017; Chakravorty et al., 2019; Evans and Holzbaur, 2019). Impaired mitophagy is closely related to AD, another important age-related neurodegenerative disease (Fivenson et al., 2017; Fang et al., 2019). AD is an irreversible, progressive brain disorder, characterized by cognitive defects and a progressive decline in memory (Fivenson et al., 2017; Fang et al., 2019). Like PD, the symptoms of AD are related to the loss of important neurons in certain areas of the brain, including the hippocampus, entorhinal cortex, temporal lobe, parietal lobe, and frontal lobe (Fivenson et al., 2017; Fang et al., 2019). Although the etiology of AD remains unclear, the accumulation of intracellular hyperphosphorylated tau (p-tau) and extracellular amyloid β-peptide (Aβ) have been identified in the onset and progression of the disease (Braak and Braak, 1995; Fivenson et al., 2017; Fang et al., 2019; Thal et al., 2019). Aβ and p-tau pathologies can contribute to mitochondrial defects, and in AD, neurons may display mitochondrial dysfunction and a bioenergetic deficit (Mattson et al., 2008; Kapogiannis and Mattson, 2011; Fang et al., 2019). Impairment of mitochondrial function has been identified in the brain tissues of AD mouse models, as well as in human samples of both sporadic and familial types of AD (Lustbader et al., 2004; Fang et al., 2019). Mitochondria dysfunction can accelerate Aβ deposit at the cellular level and contribute to hyperphosphorylation of tau in neurons (Esposito et al., 2006; Mattson et al., 2008; Kapogiannis and Mattson, 2011; Fang et al., 2019). Accumulating evidence demonstrates that a decline in mitophagy may contribute to impaired mitochondrial homeostasis in AD (Fivenson et al., 2017; Chakravorty et al., 2019; Fang et al., 2019). Under AD-linked pathophysiological conditions, Parkin-mediated mitophagy is altered in AD mutant neurons and in AD patient brains (Ye et al., 2015). Inadequate mitophagy capacity may contribute to the aberrant accumulation of dysfunctional mitochondria in AD-affected neurons (Ye et al., 2015; Fivenson et al., 2017; Chakravorty et al., 2019; Fang et al., 2019). Interestingly, using postmortem human AD brain samples, induced pluripotent stem cell-derived human AD neurons, and a set of AD animal models, a recent study demonstrates defective mitophagy in AD (Fang et al., 2019). Of note, pharmacological restoration of mitophagy ameliorates memory loss in both *Caenorhabditis elegans* and mouse models of AD (Fang et al., 2019). Thus, targeting the mitochondrial quality control system may provide a novel therapeutic strategy for AD.

Mitochondrial dysfunction also has been implicated in the pathology of HD, another progressive neurodegenerative disease caused by a genetic mutation in the huntingtin gene (Shirendeb et al., 2011; Hwang et al., 2015). In HD, the pathological expansion of CAG trinucleotide repeat encoding a polyglutamine (polyQ) tract in the amino-terminal region of the Huntingtin protein results in an abnormal polyglutamine stretch and in accumulation of polyQ-expanded HTT (Mattson et al., 2008; Song et al., 2011; Martin et al., 2015). Due to the loss of GABAergic neurons in the basal ganglia HD is characterized by motor dysfunction, psychiatric disturbance, and a decline in cognition (Ross and Tabrizi, 2011). The mutant huntingtin protein may affect a wide range of cellular pathways and functions, including transcriptional regulation, axonal trafficking of vesicles, mitochondrial function, ubiquitin-mediated proteolysis, and the autophagic systems (Luthi-Carter and Cha, 2003; Gauthier et al., 2004; Trushina et al., 2004;

Wong and Holzbaur, 2014; Martin et al., 2015). Damaged mitochondria and impaired mitophagy are closely related to neuronal death and disease progression in HD (Mattson et al., 2008; Song et al., 2011; Martin et al., 2015). Mutant HTT can bind the mitochondrial fission GTPase dynamin−related protein−1, and perturb mitochondrial dynamics in HD, leading to the accumulation of damaged mitochondria and increased reactive oxygen species (ROS) (Shirendeb et al., 2011; Song et al., 2011). Research has indicated that mutant HTT mediated transcriptional dysregulation of autophagy-related genes, cargo recognition failure of autophagosomes, and impaired trafficking of lysosomes contribute to inefficient autophagy in HD (Martinez-Vicente et al., 2010). Additionally, recent studies demonstrate that mutant HTT can interact with the Ser/Thr-kinase ULK1 and p62/SQTM12 (Lim et al., 2015; Wold et al., 2016), thereby potentially regulating mitophagy. Furthermore, expression of mutant HTT with expanded polyglutamine repeats may alter GAPDH-mediated mitophagy, thus contributing to the pathology of HD (Hwang et al., 2015). Using mt-Keima mice to measure *in vivo* mitophagic flux, markedly reduced levels of mitophagy have been observed in mutant HTT-expressing mice (Sun et al., 2015). Therefore, the presence of the expanded polyQ tract can affect the neuronal mitophagy, and consequently promote mitochondrial dysfunction, contributing to disease progression in HD.

Defects in mitophagy appear to have significance with regards to familial ALS, also known as motor neuron disease or Lou Gehrig's disease (Evans and Holzbaur, 2019). ALS is characterized by a progressive degeneration of motor neurons in the spinal cord and brain (Evans and Holzbaur, 2019). Several of the ALS-associated genes have been functionally implicated in autophagy or the mitophagy process (Evans and Holzbaur, 2019). These include VCP, encoding valosin-containing protein, implicated in autophagy (Buchan et al., 2013) and the maintenance of lysosomal function (Papadopoulos et al., 2017), as well as the noncoding sequence of the C9ORF72 gene, mutations of which account for approximately 40% of familial ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Floeter et al., 2017). C9ORF72 may regulate ULK1 and may function in the regulation of lysosomal fusion or function (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Floeter et al., 2017). Mutations in the serine/threonine kinase Tank-binding kinase 1 (TBK1) and the autophagy receptor optineurin (OPTN) are associated with ALS (Moore and Holzbaur, 2016). ALS-linked mutations in OPTN and TBK1 can interfere with mitophagy suggesting, an inefficient turnover of damaged mitochondria may represent a key pathophysiological mechanism contributing to neurodegenerative disease. The TBK1-OPTN axis can target damaged mitochondria and promote autophagosome formation around mitochondria triggering mitophagy (Lazarou et al., 2015; Palikaras et al., 2017; Harper et al., 2018). Inhibition of TBK1 or expression of ALS-linked TBK1 mutant can block efficient autophagosome formation (Moore and Holzbaur, 2016). In neurons, an ALS-associated mutation in OPTN is sufficient to disrupt mitochondrial function under basal conditions, due to the slow kinetics of mitophagy (Evans and Holzbaur, 2020). Mutant Cu/Zn superoxide dismutase (SOD1) also causes alterations of mitochondrial function in ALS (Palomo et al., 2018). Interestingly, a recent study demonstrates that Parkin genetic ablation slows down motor neuron demise, delays ALS disease progression, and thus extends the lifespan of the SOD1−G93A mutant mice (Palomo et al., 2018). Therefore, understanding the molecular basis for autophagy and mitophagy in familial ALS should provide considerable insight into the disease-causing mechanisms involved in ALS pathogenesis, and may lead to the development of more effective therapeutic approaches for ALS.

Mitochondrial defects have been linked to neuronal dysfunction and the pathogenesis of neurodegenerative diseases. In neurons, mitophagy serves as a major pathway required for the preservation of mitochondrial function. Mitophagy deficit's role in neurodegenerative diseases has only been recently recognized despite the significant advancements in understanding the molecular and the cellular mechanisms that govern mitophagy. Given the fact that neurons have an exceptionally high demand for ATP, the quality control of mitochondria is essential for neuronal functions. The same can be said for cardiomyocytes, in which mitochondria occupy approximately one-third of the cell volume (Schaper et al., 1985; Murphy et al., 2016; Bertero and Maack, 2018). Therefore, defective mitophagy may impair mitochondrial homeostasis and can be particularly detrimental for these terminally differentiated cells such as the postmitotic neurons and cardiomyocytes (Youle and Narendra, 2011; Bravo-San Pedro et al., 2017; Palikaras et al., 2018). Interestingly, researchers recently have noticed a link between various cardiovascular abnormalities and neurodegenerative diseases. For instance, Aβ aggregations have been reported to be present in the hearts of patients with idiopathic dilated cardiomyopathy (Gianni et al., 2010). And recent studies have demonstrated compromised myocardial function and intramyocardial deposits of Aβ in AD patients (Troncone et al., 2016). Likewise, clinical studies on HD patients at various stages of disease progression have revealed a high incidence of cardiovascular events (Melik et al., 2012; Stephen et al., 2015; Kobal et al., 2017). Although neurons and cardiomyocytes vary a lot in their structure and function, these findings depict a possible biological framework linking neurodegenerative diseases to cardiovascular risks. However, evidence of a possible role that mitochondria play calls for further analysis of this connection.

MITOPHAGY IN CARDIOVASCULAR DISEASE

The heart demands a substantial amount of energy to fuel myocardial contraction and is subsequently rich in mitochondria (Murphy et al., 2016; Bertero and Maack, 2018). The cardiac mitochondria account for ∼30% of myocellular volume and possess the capacity to use multiple metabolic substrates to generate ATP under a wide range of physiological and pathological conditions (Schaper et al., 1985; Murphy et al., 2016; Bertero and Maack, 2018). Also, mitochondria are essential organelles in the regulation of metabolic substrate utilization, cell death, calcium storage, and ROS levels (Wallace et al., 2010;

Mishra and Chan, 2016; Sun et al., 2016). Mitophagy plays a critical role in preserving mitochondrial quality in normal cardiovascular physiology and in pathological circumstances. During the early perinatal period, changes in oxygen and nutrient availability catalyze a switch in cardiac substrate preference from glucose to fatty acids (Gong et al., 2015; Dorn, 2016). Recent studies have demonstrated an essential role for mitophagy in the normal perinatal transformation of myocardial metabolism (Gong et al., 2015). During cardiac stress and injury, mitophagy increases to help clear damaged mitochondria, as well as prevent oxidative damage and cell death (Hoshino et al., 2013; Kubli et al., 2013). In response to pressure overload-induced mitochondrial dysfunction, mitophagy can be activated dynamically and play a protective role against heart failure (Shirakabe et al., 2016b). Over the past decade, significant progress has been made in understanding of the physiological and pathological roles of cardiac mitophagy (Billia et al., 2011; Murphy et al., 2016; Tong and Sadoshima, 2016; Bravo-San Pedro et al., 2017; Wang et al., 2018).

The functional significance of cardiac mitophagy has been revealed by analyzing loss-of-function mouse models of autophagy achieved by cardiac-specific ATG5 or ATG7 ablation (Nakai et al., 2007; Taneike et al., 2010). In most cases, a functional macroautophagy machinery is needed for selective removal of damaged mitochondria, while ATG5 and ATG7 are essential genes for optimal autophagic responses (Youle and Narendra, 2011; Palikaras et al., 2018). Mice with temporally controlled cardiomyocyte-specific deletion of ATG5 exhibit left ventricular dilatation, with an accumulation of damaged mitochondria in the heart (Nakai et al., 2007; Taneike et al., 2010). ATG7-dependent activation of cardiac mitophagy recently has been reported to protect the myocardium from the metabolic stress of a high-fat diet (Tong et al., 2019). Therefore, interventions designed to stimulate autophagy in the cardiovascular system may prevent the accumulation of damaged mitochondria under cardiac stress and exhibit potential cardioprotective effects. Eisenberg et al. reported that the polyamine spermidine, delivered as a dietary supplement, can enhance cardiac autophagy/mitophagy, ameliorate age-related cardiac hypertrophy and preserve diastolic function in older mice (Eisenberg et al., 2016). Interestingly, a functional autophagy system in cardiomyocytes is required for the cardioprotective effects of spermidine (Eisenberg et al., 2016). Furthermore, genetic studies have demonstrated the key role of the Ser/Thr-kinase ULK1 in mediating mitophagy and early autophagosome formation (Wu et al., 2014). ULK1-regulated mitophagy is activated in response to energetic stress and may play an essential role in preserving mitochondrial function following myocardial ischemia (Saito et al., 2019).

The impact of PINK1 and Parkin has been extended in the cardiovascular system. In Drosophila models, genetic deletion of either PINK1 or Parkin can lead to mitochondrial dysfunction and poor cardiac contractibility (Guo, 2012; Bhandari et al., 2014). Genetic deletion of PINK1 in mice results in cardiac hypertrophy and progressive cardiac dysfunction (Billia et al., 2011). These mice also exhibit increased infarct size in response to ischemia-reperfusion injury (Siddall et al., 2013). Furthermore, PINK1-mediated mitochondrial quality control could be important during acute cardiac mitochondria stress following exhaustive exercise (Sliter et al., 2018). Mice with Parkin deletion demonstrate normal baseline cardiac phenotypes (Kubli et al., 2013; Piquereau et al., 2013), but exhibit an increased sensitivity to stress conditions from myocardial infarction (MI) or cardiac aging, along with a decline in cardiac mitophagy and accumulation of dysfunctional mitochondria (Hoshino et al., 2013; Kubli et al., 2013). It is worth mentioning those cardiac abnormalities, such as cardiomyopathy, arrhythmia, and sudden cardiac death, are still under investigation, though rare may occur in PD patients (Scorza et al., 2018). Therefore, under specific cardiac pathophysiological circumstances, PINK1 and Parkin may serve as a regulatory mechanism of mitophagy in the heart. Additional mitochondrial quality control pathways regulated by NIX and BNIP3 (Dorn, 2010), FUNDC1 (Zhang et al., 2017; Lampert et al., 2019) or general autophagy may compensate for the loss of PINK1/Parkin-mediated mitophagy. The functional significance of PINK1 and Parkin may require further investigation.

Recent progress also has linked mitophagy to the processes of mitochondrial dynamism, fission and fusion (Youle and Narendra, 2011; Palikaras et al., 2018; Pickles et al., 2018). Cell biological observations suggest that a group of fission and fusion proteins regulate mitochondrial morphology depending on the metabolic status (Mishra and Chan, 2016). Mitofusins, MFN1, and MFN2, as well as optic atrophy protein 1 (OPA1) promote the mitochondrial fusion (Mishra and Chan, 2016), whereas the dynamin-related protein 1 (DRP1) mediates mitochondrial fission (Friedman and Nunnari, 2014; Mishra and Chan, 2014, 2016) (**Figure 1**). MFN2 can be phosphorylated by the PINK1 kinase on the OMM, which facilitates Parkin translocation to promote mitophagy (Chen and Dorn, 2013). MFN2 deletion in mouse hearts disrupts mitophagic flux independent of its activity in fission/fusion regulation (Song et al., 2014). Mitochondrial fission, on the other hand, allows for mitochondrial fragmentation and has been suggested to regulate mitophagy (Rambold et al., 2011; Mishra and Chan, 2014, 2016). Several studies have demonstrated that genetic manipulation of DRP1 in mouse hearts can alter myocardial mitochondrial function and mitophagy (Kageyama et al., 2014; Song et al., 2015; Shirakabe et al., 2016b). Cardiomyocyte-specific homozygous deletion of DRP1 suppresses mitophagy, and leads to dilated cardiomyopathy in mouse models, while the heterozygous DRP1 knockout mice are more susceptible to ischemia/reperfusion injury (Ikeda et al., 2015). Furthermore, in the murine heart, DRP1 may mediate mitophagy in response to mitochondrial dysfunction under pressure overload, while haploinsufficiency of DRP1 exacerbates the progression of heart failure (Shirakabe et al., 2016b). The particular role for fission/fusion mediated mitophagy in healthy and in diseased hearts, as well as how PINK1 and Parkin participate in the regulatory mechanisms associated with mitochondrial fission/fusion is still under investigation (Kageyama et al., 2014; Song et al., 2015).

Cardiovascular disease imposes a huge burden worldwide, in terms of mortality, morbidity, and healthcare costs

(Dokainish et al., 2017; Benjamin et al., 2019). Despite significant progress in understanding the pathophysiology of the disease, the prevalence of cardiovascular disease and its mortality rates remain high (Dokainish et al., 2017; Benjamin et al., 2019). Although preclinical studies suggest the potential benefit of mitochondria-targeted therapies in cardiovascular disease, it remains to be established whether the preservation of mitochondrial function through modulating mitophagy will result in improved clinical outcomes in patients (Murphy et al., 2016; Bravo-San Pedro et al., 2017). A better understanding of the regulatory mechanisms of mitophagy in the heart and its pathophysiologic role in cardiovascular disease are needed to develop effective mitophagy-targeted therapeutic agents and translate this innovative treatment strategy.

PERSPECTIVES

Mitophagy serves as a critical mechanism to eliminate damaged mitochondria and is regulated by multiple mechanistically distinct pathways (Youle and Narendra, 2011; Palikaras et al., 2018). Cellular level studies have provided valuable insight into the signaling pathways regulating mitophagy, as well as mapping out how and when mitophagy occurs in a wide range of physiological and pathological conditions to counter cellular stressors such as ROS or damaged mitochondria (Youle and Narendra, 2011; Bravo-San Pedro et al., 2017; Palikaras et al., 2018). A better understanding of mitochondrial turnover mechanisms, with an improved focus on how these pathways might contribute to disease pathogenesis, should allow for the development of more efficient strategies to battle numerous pathological conditions associated with mitochondrial dysfunction.

Mitophagy is an important element of overall mitochondrial quality control. Defective mitophagy is thought to contribute to normal aging as well as various neurodegenerative and cardiovascular diseases (Youle and Narendra, 2011; Sun et al., 2016). In fact, aging by itself is a major risk factor for the pathophysiology of cardiovascular and neurodegenerative diseases (Eisenberg et al., 2016; Fivenson et al., 2017; Bonora et al., 2018). Increasing evidence suggests that mitophagy failure accelerates aging (Eisenberg et al., 2016; Sun et al., 2016; Fivenson et al., 2017; Bonora et al., 2018). Interestingly, a marked age-dependent decline in mitophagy has been observed in the hippocampus of the mouse brain (Sun et al., 2015), an area where new memory and learning are encoded. Similar effects have been noted in mouse models of HD (Sun et al., 2015). This strengthens the hypothesis that mitophagy might regulate neuronal homeostasis and that a decline in mitophagy might predispose to age-dependent neurodegeneration. Age-related mitochondrial function deterioration is underlined as a key feature of other diseases, such as obesity, diabetes, and cancer (Eisenberg et al., 2016; Sun et al., 2016; Fivenson et al., 2017; Bonora et al., 2018). Therefore, maintaining a healthy mitochondrial network via functional mitophagy may serve as an attractive therapeutic strategy in the treatment of a wide range of age-related diseases, and potentially regulate longevity.

The emergence of nutritional and pharmacological interventions to modulate autophagy/mitophagy and to serve as a potential therapeutic model is quite encouraging. Accumulation of ubiquitinated OMM proteins has been proposed to act as a signal for selective mitophagy (Youle and Narendra, 2011; Palikaras et al., 2018). As described above, ubiquitination of mitochondrial proteins is positively regulated, in part, by the E3 ubiquitin ligase, Parkin (Narendra et al., 2008, 2010; Matsuda et al., 2010; Youle and Narendra, 2011). In contrast, removal of ubiquitin is achieved by the action of resident mitochondrial deubiquitinases, most notably USP30, thereby acting to antagonize mitophagy (Bingol et al., 2014; Cunningham et al., 2015; Bingol and Sheng, 2016; Gersch et al., 2017). Inhibition of USP30 enzyme activity may provide an unambiguous avenue to pursue the role of mitophagy as a therapeutic target. In addition, the natural polyamine spermidine can preserve cardiac diastolic function in older mice by inducing mitophagy (Eisenberg et al., 2016). Inhibitors of mTOR can induce autophagy, and protect against various cardiac pathologies, and prolong lifespan in diverse species (Sciarretta et al., 2012; Yan et al., 2013; Dai et al., 2014; Ranek et al., 2019). Recently, three promising candidates that may stimulate and reinvigorate mitophagy process have been demonstrated to reduce the accumulation of amyloid-beta and phosphorylated tau in Alzheimer's mouse brains (Fang et al., 2019). These compounds, including nicotinamide mononucleotide, urolithin A, and actinonin, can improve symptoms of AD and dementia symptoms in preclinical models (Fang et al., 2019). In addition, Tat-Beclin 1 peptide, derived from a region of the autophagy protein, beclin 1, can promote autophagy/mitophagy and improve mitochondrial function in heart failure animal models (Shirakabe et al., 2016b). Therefore, identifying more efficient and specific agents that can modulate the clearance of defective mitochondria are likely to have significant therapeutic benefits.

Recent advances have greatly expanded our knowledge of how mitophagy functions in health and disease. The magnitude and kinetics of mitophagy in various disease conditions, however, remains to be elucidated. Although mitophagy has many potential benefits, under certain conditions, uncontrolled or overactive mitophagy may disrupt organelles' integrity and may prove detrimental to cell health (Liu et al., 2013; Saito and Sadoshima, 2015). Therefore, it is essential to elucidate how mitophagy collaborates with other cellular processes, such as autophagy, mitochondrial fission/fusion, and mitochondrial biogenesis to restore unhealthy mitochondria and maintain overall mitochondrial homeostasis. Furthermore, it is critical to determine when and to what extent manipulating mitophagy activity may regulate and keep mitochondria in health to prevent disease progression.

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HL, RZ, and NS designed the outline of the review and wrote the draft of the review. JK, AM, and SO provided scientific comments and wrote part of the review.

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REVIEW
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The Long and the Short of PTEN in the Regulation of Mitophagy

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Mitophagy is a key mitochondrial quality control mechanism for effective and selective elimination of damaged mitochondria through the autophagy-lysosome machinery. Defective mitophagy is associated with pathogenesis of important human diseases including neurodegenerative diseases, heart failure, innate immunity, and cancer. In the past two decades, the mechanistic studies of mitophagy have made many breakthroughs with the discoveries of phosphatase and tensin homolog (PTEN)-induced kinase protein 1 (PINK1)-parkin-mediated ubiquitin (Ub)-driven pathway and BCL2/adenovirus E1B 19 kDa protein-interacting proteins 3 (BNIP3)/NIX or FUN14 domain containing 1 (FUNDC1) mitochondrial receptor-mediated pathways. Recently, several isoforms of dual phosphatase PTEN, such as PTEN-long (PTEN-L), have been identified, and some of them are implicated in the mitophagy process via their protein phosphatase activity. In this review, we aim to discuss the regulatory roles of PTEN isoforms in mitophagy. These discoveries may provide new opportunities for development of novel therapeutic strategies for mitophagy-related diseases such as neurodegenerative disorders via targeting PTEN isoforms and mitophagy.

Keywords: mitophagy, PINK1, Parkin, BNIP3, PTEN, PTEN-L

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INTRODUCTION

Autophagy is an evolutionarily conserved process to degrade or recycle intracellular materials through lysosomes or vacuoles (Mizushima, 2018). In mammalian cells, there exist three different types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Among them, macroautophagy (referred to as autophagy hereafter) is the most well-studied form, which is orchestrated by a group of proteins encoded by autophagy-related-genes (*ATGs*) and characterized by the formation of double-membraned autophagosomes (Zachari and Ganley, 2017; Dikic and Elazar, 2018; Mizushima, 2018). The formation of autophagosomes can be briefly divided into three main steps: (1) The initiation step is regulated by unc51-like activating kinase 1 (ULK1) complex comprised of ULK1, ATG13, FIP200, and ATG101 to form the phagophore; (2) the vesicle nucleation step is regulated by Beclin1-ATG14 and Vps34/class III phosphatidylinositol 3-kinases (PI3K) complex to generate phosphatidylinositol 3-phosphate (PI3P); and (3) the vesicle elongation step is mediated by two ubiquitination conjugation systems, ATG12-ATG5-ATG16L1 and LC3-PE (phosphatidylethanolamine) systems, as well as ATG9-containing vesicles to form the autophagosomes (Mizushima et al., 2011; Hurley and Young, 2017; Lahiri et al., 2019). Autophagy can be either a general non-selective process to randomly

uptake cargos for degradation (bulk autophagy) or a selective process to remove or degrade specific organelles, aggregated proteins, DNA, and/or invading pathogens (selective autophagy). Up to date, several types of selective autophagy have been recognized, including mitophagy, ribophagy, xenophagy, reticulophagy, lysophagy, and aggrephagy (Rogov et al., 2014; Kirkin, 2020).

Among them, mitophagy represents the most well-studied form of selective autophagy to degrade dysfunctional or superfluous mitochondria through the autophagy-lysosome machinery, which is regulated by multiple factors with distinct posttranslational modifications (Montava-Garriga and Ganley, 2020; Wang et al., 2020). The phenomenon of mitophagy was first described by Christian De Duve and Robert Wattiaux in 1966 when they observed that mitochondria were engulfed by autophagic vacuoles (De Duve and Wattiaux, 1966). The term of "mitophagy" was coined by John J. Lemasters to distinguish this selective autophagy that degrades mitochondria from the bulk autophagy (Lemasters, 2005). Mitophagy is usually initiated by an "eat me" signal, such as labeling damaged mitochondria with ubiquitin (Ub) or autophagy receptors (Harper et al., 2018; Pickles et al., 2018; Wang et al., 2020). Owing to its critical role in maintaining mitochondrial homeostasis and close implication in multiple human diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD) (Williams and Ding, 2018; Lou et al., 2019), the machinery of mitophagy has drawn substantial attention in the past two decades. The discoveries of PINK1-Parkin-mediated Ub-driven pathway and BNIP3/NIX or FUNDC1 receptor-mediated pathways represent the milestones in the mitophagy field. In this review, we will discuss some of these key factors, especially the newly identified protein phosphatase, in the regulation of mitophagy.

PINK1-PARKIN-MEDIATED UBIQUITIN-DRIVEN MITOPHAGY

One breakthrough in the understanding of the molecular mechanisms of mitophagy is the discovery of PINK1-Parkin-mediated pathway (Narendra et al., 2008, 2010; Vives-Bauza et al., 2010). PINK1 (encoded by the *PARK6* gene) is a serine/threonine kinase, which was identified in 2001 (Unoki and Nakamura, 2001) and contains a mitochondrial targeting sequence (MTS) at its N-terminus as well as an outer mitochondrial localization signal (OMS) next to the transmembrane domain (TMD) (Okatsu et al., 2015a). Two homozygous mutations, including G→A in transition in exon 4 and G→A transitions in exon 7, in *PINK1* were found in autosomal recessive early onset familial forms of PD patients (Valente et al., 2004). Parkin (encoded by the *PARK2* gene) is an E3 Ub ligase, which was identified in 1998 and was named "Parkin" due to its important roles in the pathogenesis of autosomal recessive juvenile parkinsonism (AR-JP) (Kitada et al., 1998; Lucking et al., 1998; Abbas et al., 1999). Parkin contains a Ub-like (UBL) domain, a classic RING (RING1) domain, three zinc-coordinating domains termed in between RING (IBR) domain, a RING2 domain, and a RING0 domain that is a Parkin unique domain (Hristova et al., 2009; Trempe et al., 2013; Walden and Muqit, 2017). Numerous studies have reported that PINK1 and Parkin work in the same pathway to remove dysfunctional mitochondria and to maintain mitochondrial homeostasis, with the well-established feedforward model of PINK1-Parkin mitophagy activation (Harper et al., 2018; Pickles et al., 2018; Wang et al., 2020).

When mitochondria are healthy, PINK1 is constantly maintained at a low level due to mitochondrial import, protease cleavage, and proteasome degradation (Jin et al., 2010; Deas et al., 2011; Lazarou et al., 2012; Sekine et al., 2019). Upon mitochondrial damage and depolarization, PINK1 is rapidly accumulated on the outer mitochondrial membrane (OMM) and activated through dimerization and autophosphorylation (Okatsu et al., 2012, 2013; Aerts et al., 2015; Rasool et al., 2018). Therefore, PINK1 acts as a mitochondrial damage sensor to initiate mitophagy. Once activated, PINK1 phosphorylates mitochondrial pre-existing Ub at Ser 65 (pSer65-Ub) (Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014; Shiba-Fukushima et al., 2014). pSer65-Ub serves as a key receptor to recruit Parkin from cytosol to mitochondria through direct binding (Shiba-Fukushima et al., 2014; Okatsu et al., 2015b). Binding to pSer65-Ub releases the UBL domain of Parkin from its RING1 domain (Sauve et al., 2015; Wauer et al., 2015a; Aguirre et al., 2017), which promotes the phosphorylation of the UBL domain by PINK1 at Ser 65 (pSer65-Parkin) (Kondapalli et al., 2012; Shiba-Fukushima et al., 2012; Wauer et al., 2015a; McWilliams et al., 2018). Subsequently, the phospho-UBL domain rebinds to the RING0 domain of Parkin to release the catalytic RING2 domain to achieve full activation (Gladkova et al., 2018; Sauve et al., 2018). Activated Parkin then conjugates more Ub onto OMM proteins for PINK1 phosphorylation, which mediates further rounds of Parkin translocation to mitochondria; thus, PINK1, pSer65-Ub, and Parkin form a positive feedforward amplification loop to initiate mitophagy.

Another important function of pSer65-Ub is to recruit autophagy receptors, such as NDP52 (CALCOCO2) and Optineurin (OPTN) to damaged mitochondria, a process that is TANK-binding kinase 1 (TBK1) dependent (Heo et al., 2015; Lazarou et al., 2015; Richter et al., 2016). TBK1 is a serine/threonine kinase and phosphorylates these autophagy receptors to promote their binding ability to various Ub chains (Heo et al., 2015; Richter et al., 2016). Interestingly, activation of TBK1 also requires OPTN binding to Ub chains in the presence of PINK1 and Parkin (Heo et al., 2015; Richter et al., 2016). In the prevailing model of mitophagy, after binding to the pSer65-Ub chains, OPTN and/or NDP52 recruit phagophore onto mitochondria by directly binding to LC3 through their LC3-interacting regions (LIR motifs) (Gatica et al., 2018; Palikaras et al., 2018). However, emerging studies suggest that LC3/GABARAP family proteins are dispensable in the selective recognition of damaged mitochondria, based on the observation that, in LC3/GABARAP knockout cells, mitochondria can still be engulfed by autophagosomes (Itakura et al., 2012; Nguyen et al., 2016; Padman et al., 2019). One very recent study has highlighted the role of NDP52 to recruit ULK1 complex to damaged mitochondria (Vargas et al., 2019). NDP52 directly interacts with

FIP200 in a TBK1-dependent manner to recruit ULK1 complex, leading to autophagosome biogenesis on damaged mitochondria and initiation of autophagy machinery.

Interestingly, besides PINK1-mediated pSer65-Ub, several other PINK1-independent phosphorylation sites of Ub have been identified, including pThr7-Ub, pSer20-Ub, and pSer57-Ub (Wauer et al., 2015b). Among them, pSer57-Ub has been reported to hyperactivate Parkin (George et al., 2017). Obviously, more studies are needed to understand the functional implication of such Ub phosphorylation in mitophagy. In addition to Ub and Parkin as described above, a number of additional PINK1 substrates have been reported. For instance, PINK1 phosphorylates mitofusin 2 (MFN2) at Thr 111 and Ser 442, leading to Parkin mitochondrial recruitment through promoting the interaction between MFN2 and Parkin, suggesting that MFN2 may serve as a mitochondrial receptor for Parkin (Chen and Dorn, 2013). However, another study indicates that MFN2 antagonizes mitophagy through tethering mitochondria and endoplasmic reticulum (ER) and limiting the accessibility of other mitochondrial proteins to PINK1 and Parkin (McLelland et al., 2018). It is known that some OMM proteins such as MFN2 undergo ubiquitination and proteasomal degradation at the beginning of the mitophagy (Tanaka et al., 2010; Ding et al., 2012; McLelland et al., 2018). Therefore, it is possible that such a process may facilitate mitophagy by removing the barrier among PINK1, Parkin, and other mitochondrial proteins. PINK1 can also phosphorylate Miro (also called RhoT) at Ser156, which recruits Parkin onto mitochondria and results in ubiquitination and proteasomal degradation of Miro, and thus blocking mitochondrial motility (Wang et al., 2011; Shlevkov et al., 2016). Interestingly, a recent report found that Miro, through direct protein–protein interaction, recruits Parkin at healthy mitochondria independent of PINK1, and such pre-existing Parkin is essential for Parkin further recruitment and activation upon mitochondrial damage in a PINK1-dependent manner (Safiulina et al., 2019). In addition, in a phosphoproteomic screening study for PINK1 substrates, Lai and colleagues reported that the phosphorylation of Rab GTPases such as Rab8A at the conserved Ser 111 is indirectly regulated by PINK1, and this phosphorylation can block the phosphorylation of Rab8A at Thr72 by leucine-rich repeat kinase 2 (LRRK2), suggesting the interplay of PINK1 with other PD-related genes (Lai et al., 2015; Vieweg et al., 2019). Thus, identification of more PINK1 substrates will not only provide new insights into the molecular mechanisms of PINK1-Parkin-mediated mitophagy but also provide deeper understanding of the molecular mechanisms of important neurodegenerative disorders such as PD.

BNIP3/NIX (BNIP3L)-MEDIATED MITOPHAGY

BNIP3, a member of prodeath BCL2 family proteins, was first found as an E1B 19-kDa interacting proteins (Boyd et al., 1994). NIX (also named BNIP3L) is a homolog of BNIP3 with ∼55% identical similar amino acid sequence (Matsushima et al., 1998). Both proteins contain an atypical BCL2-homology 3 (BH3) domain and C-terminal TMD, which is essential for their proapoptotic activity and mitochondrial localization (Yasuda et al., 1998; Imazu et al., 1999). Moreover, BNIP3 and NIX both contain an identical LIR motif, which makes them to interact with LC3s/GABARAP subfamilies and recruit autophagosomes to sequester damaged mitochondria, especially under hypoxia conditions (Novak et al., 2010; Hanna et al., 2012; Birgisdottir et al., 2013). Under hypoxia, the expression of BNIP3 and NIX are increased through the transcriptional regulation of hypoxia-inducible factor 1α (HIF-1α) or FOXO3 (Sowter et al., 2001; Mammucari et al., 2007; Zhang et al., 2008). Mutation of the LIR motif abolishes the interaction of BNIP3/NIX with LC3 and thereby attenuates mitochondrial clearance (Novak et al., 2010; Hanna et al., 2012; Zhu et al., 2013), while phosphorylation of the LIR motif enhances the interaction with LC3 and promotes mitophagy (Zhu et al., 2013; Rogov et al., 2017). However, the kinase(s) and phosphatase(s) regulating this phosphorylation of LIR remain to be identified.

It should be noted that NIX, but not BNIP3, plays an important role in the development of reticulocytes through the regulation of mitophagy. Mitochondria were not cleared in reticulocytes when NIX is deficient (Diwan et al., 2007; Schweers et al., 2007; Zhang and Ney, 2008; Zhang J. et al., 2012). Interestingly, treatment with mitochondrial uncoupling agents could restore the removal of mitochondria in the absence of NIX, suggesting that the regulatory effect of NIX on mitophagy was probably due to its role in regulating mitochondrial depolarization (Sandoval et al., 2008; Zhang and Ney, 2008). However, there is still no direct evidence to show that NIX could cause mitochondrial depolarization, and further studies are thus needed.

Intriguingly, several studies have revealed the crosstalk between BNIP3/NIX receptor-mediated pathway and PINK1-Parkin-mediated pathway. For instance, both BNIP3 and NIX can promote Parkin mitochondrial recruitment (Ding et al., 2010; Lee et al., 2011), while NIX can also be ubiquitinated by Parkin to promote autophagy receptor recruitment to damaged mitochondria (Gao et al., 2015). In addition, BNIP3 is able to inhibit PINK1 proteolytic degradation and stabilize PINK1 on OMM to facilitate Parkin mitochondrial recruitment and mitophagy (Zhang et al., 2016). These findings suggest that these pathways cooperate with each other to ensure efficient mitophagy.

FUNDC1-MEDIATED MITOPHAGY

FUNDC1 is another important hypoxia-induced mitophagy receptor (Liu et al., 2012). As a mitochondrial outer membrane protein, FUNDC1 contains three TMDs and an LIR motif in its N-terminus exposed to the cytosol that interacts with LC3 to recruit autophagosome (Liu et al., 2012; Wu et al., 2016). Mutation or deletion of LIR motif of FUNDC1 significantly reduces or blocks mitophagy (Liu et al., 2012). Similar to the cases of other mitophagy key factors, the activity of FUNDC1 is also regulated by phosphorylation and dephosphorylation. Under normal conditions, FUNDC1 is phosphorylated by Src

and CK2 at the sites of Tyr18 and Ser13, which blocks the interaction of FUNDC1 with LC3 (Liu et al., 2012; Chen et al., 2014). Another study showed that FUNDC1 can be phosphorylated by ULK1 at Ser17 to promote mitophagy (Wu et al., 2014). However, upon induction of hypoxia, Src and CK2 are inhibited, then phosphoglycerate mutase family member 5 (PGAM5), one unique mitochondrial phosphatase, dephosphorylates FUNDC1 at Ser13, which in turn promotes the interaction between FUNDC1 and LC3 to facilitate mitophagy (Chen et al., 2014). Interestingly, the same group reported that FUNDC1 is accumulated at the ER-mitochondrial contact site in response to hypoxia, which is essential for the mitochondrial recruitment of DRP1 to facilitate mitochondrial fission prior to mitophagy (Wu et al., 2016).

CANONICAL PTEN (PTEN-SHORT) AS A NEGATIVE REGULATOR OF MITOPHAGY

PTEN is a powerful tumor suppressor with both lipid phosphatase and protein phosphatase activity, which was identified in 1997 (Li and Sun, 1997; Li et al., 1997; Steck et al., 1997). PTEN contains 403 amino acids with a N-terminal phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P2]-binding domain (PBD), a catalytic phosphatase domain, a C2 domain, a C-tail domain, and a PDZ-binding motif (**Figure 1A**; Lee et al., 1999). Loss of PTEN leads to cancer, neurological disorders, metabolic diseases, and tissue homeostasis defects (Backman et al., 2001; Kwon et al., 2006; Chen et al., 2018; Lee et al., 2018). PTEN is also vital for embryonic development, as its homozygous deletion causes lethality in mice (Di Cristofano et al., 1998; Stumpf and den Hertog, 2016). All these findings reveal that PTEN's function is not only important for tumor suppression but also vital for other biological processes.

The probably most important function of PTEN is to block the activation of pro-oncogenic class I PI3K–AKT–mTOR signaling pathway through its lipid phosphatase activity (Cantley and Neel, 1999). PI3K phosphorylates PI(4,5)P2 to generate phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P3], which recruits AKT at the cell membrane, and then AKT is phosphorylated via PDK1 and mTORC2 to indirectly activate mTORC1 (King et al., 2015). PTEN opposes this pathway through dephosphorylating PI(3,4,5)P3 to PI(4,5)P2 via its lipid phosphatase activity, leading to reduced AKT phosphorylation and inactivation (Worby and Dixon, 2014). Thus, the phosphorylation level of AKT has been widely used as an indicator for PTEN activity.

Due to the inhibitory effects of PTEN on the PI3K–AKT–mTOR signaling pathway, several studies have shown that PTEN can positively regulate autophagy (Arico et al., 2001; Ueno et al., 2008; Cai et al., 2018). Intriguingly, two independent groups reported that inhibition of AKT signaling impaired PINK1 accumulation, Parkin recruitment, and subsequent efficient mitophagy in response to mitochondrial depolarization (McCoy et al., 2014; Soutar et al., 2018). However, the role of PTEN in the regulation of mitophagy is still largely unclear. Harper and colleagues reported that RAB7A could be directly phosphorylated by TBK1 at Ser 72 (pSer72-RAB7A) to facilitate the efficient recruitment of ATG9A vesicles to damaged mitochondria and promote PINK1-Parkin-mediated mitophagy, and non-phosphorylated RAB7A failed to support this process (Heo et al., 2018). Importantly, PTEN has been found to dephosphorylate pSer72-RAB7A via its protein phosphatase activity (Shinde and Maddika, 2016; Hanafusa et al., 2019), thus suggesting a potential role of PTEN in regulating mitophagy. A more direct study showed that deletion of PTEN increased MFN2 expression and rescued mitophagic flux via the AMP-activated protein kinase (AMPK)–cAMP response element-binding protein (CREB) pathways (Li et al., 2019). Interestingly, both PTEN and MFN2 have a distribution at ER-mitochondrial contact site (de Brito and Scorrano, 2008; Bononi et al., 2013; Naon et al., 2016). As discussed above, MFN2 can be phosphorylated by PINK1 and serves as a mitochondrial receptor for Parkin (Chen and Dorn, 2013). Moreover, phosphorylated MFN2 dissociates mitochondria from ER to initiate mitophagy (McLelland et al., 2018). Thus, it will be interesting to explore whether PTEN can dephosphorylate MFN2 at the ER-mitochondrial contact site to suppress mitophagy. In addition, overexpression of PTEN inhibits mitophagy via blockage of Toll-like receptor 4 (TLR4)–c-JUN N-terminal kinase (JNK)–BNIP3 pathway (Li M. et al., 2018).

Moreover, several *in vivo* studies have highlighted that PTEN deletion in dopamine neurons provides neuroprotective effects in both genetic and neurotoxin-induced PD mouse models (Diaz-Ruiz et al., 2009; Domanskyi et al., 2011; Zhang Y. et al., 2012). Another study showed that the protein level of PTEN is significantly increased in neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-hydrochloride (MPTP)-treated mice and 1-methyl-4-phenylpyridinium (MPP^+)-treated SH-SY5Y cells, leading to enhanced neurotoxicity and apoptosis (Zhao et al., 2020). In addition, inhibition of PTEN is able to attenuate amyloid-β (Aβ)-induced synaptic toxicity and rescue cognitive function in AD models (Knafo et al., 2016). Consistently, a PTEN inhibitor, bisperoxovanadium-pic [bpV(pic)], provides neuroprotective effects in Aβ-induced neurotoxicity in a human neuroblastoma cell model (Liu et al., 2017). Apparently, more studies are needed to explore whether the above processes are due to the regulative effects of PTEN on mitophagy.

NOVEL PTEN-L (PTEN-LONG) AS A BRAKE OF MITOPHAGY

PTEN-L is the first characterized isoform of canonical PTEN, which was identified in 2013 (Hopkins et al., 2013). PTEN-L and PTEN shares the same mRNA, but PTEN-L translates from a non-AUG start codon (CUG start codon), adding an alternatively translated region (ATR) at the N-terminus of PTEN (Hopkins et al., 2013). PTEN-L can be secreted from one cell and taken up by other neighboring cells to inhibit PI3K–AKT signaling pathway both *in vitro* and *in vivo* (Hopkins et al., 2013). Intriguingly, Liang et al. reported that PTEN-L (also termed as PTENα) is a mitochondrial protein to regulate mitochondrial energy metabolism (Liang et al., 2014). They

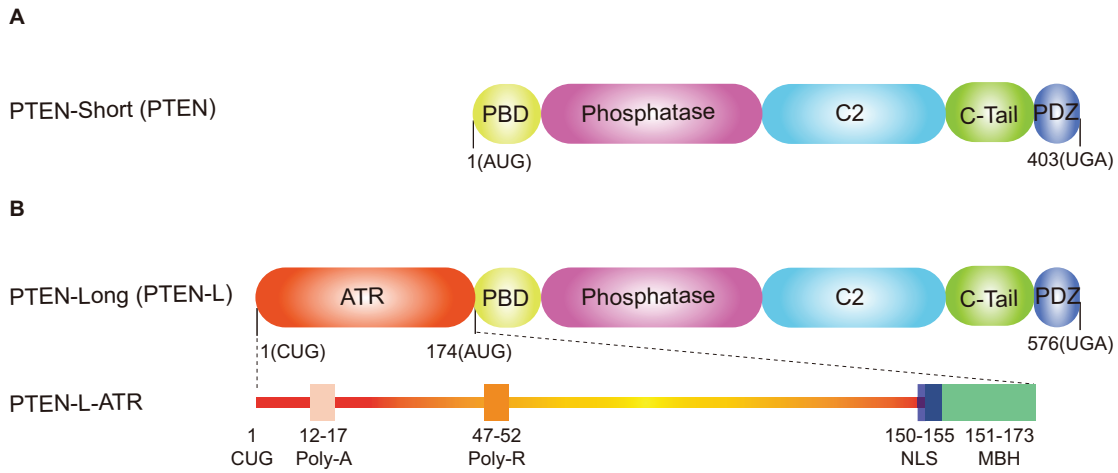

FIGURE 1 | Domain structure of phosphatase and tensin homolog (PTEN) isoforms. **(A)** PTEN-short (canonical PTEN), translated from an AUG start codon, contains five functional domains: a N-terminal PtdIns (4,5) P2 (PIP2)-binding domain (PBD), a dual phosphatase domain, a C2 domain, a C-tail domain, and PDZ-binding motif. **(B)** PTEN-long (PTEN-L) is translated from a CUG start codon upstream from the classic AUG start codon. In addition to the same five functional domains with the canonical PTEN, PTEN-L contains an alternatively translated region (ATR) adding 173 amino acids at the N-terminus. The extended ATR is composed of a secreted polyalanine signal sequence (Poly-A, residues 12–17), a cell permeable polyarginine motif (Poly-R, residues 47–52), a nuclear localization sequence (NLS, QKKPRH, residues 150–155) as well as a membrane-binding α-helix (MBH, residues 151–173).

found that somatic deletion of PTEN-L resulted in much smaller mitochondria with irregular shape and led to mitochondrial depolarization (Liang et al., 2014). It is known that, in addition to the same domains with canonical PTEN (PTEN-short), the extended ATR of PTEN-L contains a secreted polyalanine signal sequence (Poly-A), a cell permeable polyarginine motif (Poly-R), a nuclear localization sequence (NLS, QKKPRH) as well as a membrane-binding α-helix (MBH) (**Figure 1B**; Hopkins et al., 2013; Malaney et al., 2013; Masson et al., 2016; Shen et al., 2019). In addition, most parts of the ATR are intrinsically disordered and probably contain various postmodification sites and protein-binding motifs (Malaney et al., 2013; Masson et al., 2016), indicating that PTEN-L may modify distinct substrates compared with PTEN.

Recently, our group has revealed that PTEN-L functions as a protein phosphatase for Ub and antagonizes the PINK1-Parkin-mediated mitophagy pathway (Wang et al., 2018a,b). First, topology assay and immunogold electron microscopy revealed that a significant proportion of PTEN-L was associated with the mitochondrial outer membrane. Second, PTEN-L overexpression blocked mitophagy induced by mitochondrial damage agents including carbonyl cyanide 3-chlorophenylhydrazone (CCCP), combination of oligomycin and antimycin A (O/A), and valinomycin, whereas PTEN-L knockout accelerated mitophagic flux. Third, PTEN-L overexpression was able to strongly prevent Parkin mitochondrial recruitment, autoubiquitination, and subsequent activation of its E3 ligase activity. Finally, PTEN-L could dephosphorylate various types of pSer65-Ub chains *in vivo* and *in vitro* via its protein phosphatase activity but independent of its lipid phosphatase activity, leading to the disruption of the feedforward amplification loops formed by PINK1, Parkin, and pSer65-Ub chains. Since Ub modification is a vital posttranslational process in mitophagy, deubiquitinating enzymes (DUBs) become potential regulators to maintain the mitochondrial homeostasis, especially in the PINK1-Parkin-mediated Ub-driven mitophagy pathway. There are more than 100 putative DUB genes in humans, which can be grouped into two classes: cysteine proteases and metalloproteases. Among them, ubiquitin-specific proteases (USPs), which are encoded by 58 different genes, such as USP30, USP15, and USP8, have been widely studied in the field of mitophagy (Bingol et al., 2014; Cornelissen et al., 2014; Durcan et al., 2014; Marcassa et al., 2018; Ordureau et al., 2020). Recently, USP36 has been reported as a positive regulator of mitophagy; knockdown of USP36 impairs Parkin mitochondrial translocation, leading to blockage of mitophagy (Geisler et al., 2019). Interestingly, they also found that the protein level of PTEN-L was increased after USP36 knockdown, which was associated with reduced pSer65-Ub level and consistent with our findings (Geisler et al., 2019).

Intriguingly, Li et al. demonstrated that PTEN-L promotes mitophagy through interaction with Parkin by its MBH motif to promote Parkin self-association and mitochondrial localization (Li G. et al., 2018). Further studies are thus needed to examine the precise role of PTEN-L in this pathway and more importantly to explore whether PTEN-L is implicated in the pathology of mitophagy-related diseases, such as PD and AD.

CONCLUSION AND FUTURE DIRECTIONS

Mitochondria are one of the essential organelles in eukaryotic cells, with critical functions including energy (ATP) production, cell survival/cell death, cell signaling, and immune response. Dysfunctional mitochondria are implicated in many pathological processes and diseases such as cell death, inflammation,

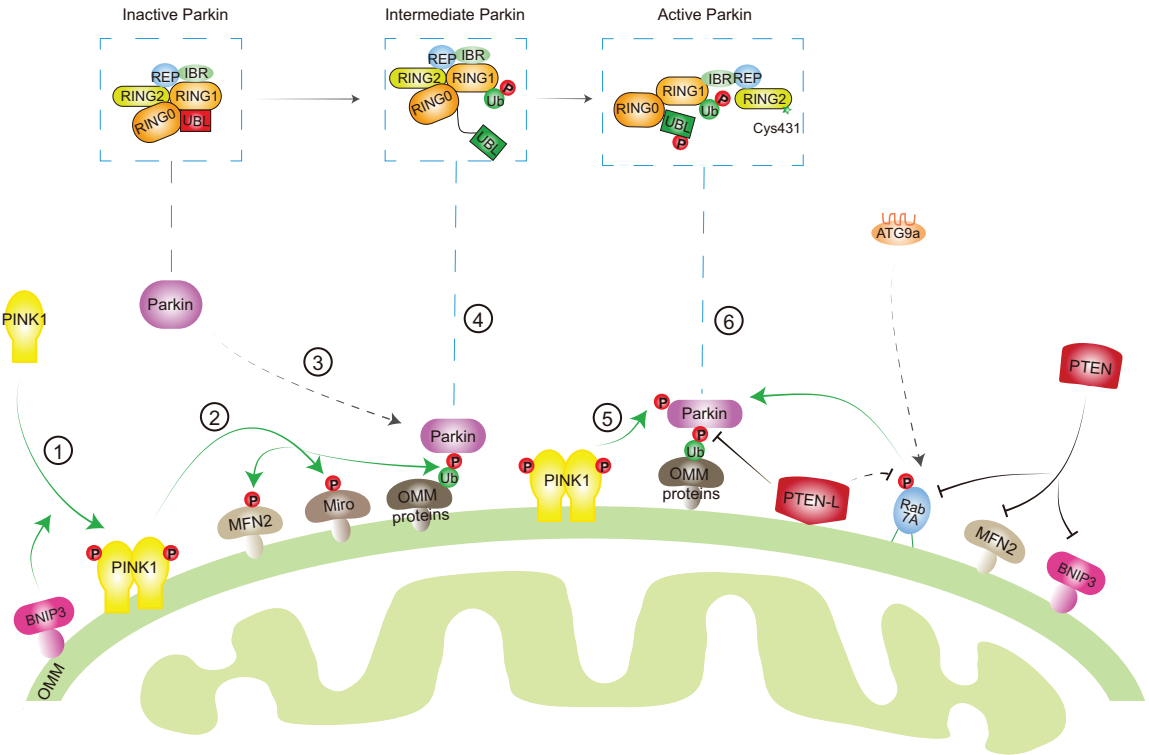

FIGURE 2 | Key effectors involved in mitophagy machinery. When mitochondria are healthy, phosphatase and tensin homolog-induced kinase protein 1 (PINK1) is imported into the mitochondria, cleaved by protease, and degraded by proteasome, while Parkin keeps in an inactive conformation in the cytosol through intradomain–domain interactions. Upon mitochondrial damage or depolarization, PINK1 is stabilized and activated at the outer mitochondrial membrane (OMM) ①, which leads to the phosphorylation of its downstream targets, such as ubiquitin (Ub) ②. Parkin has a high affinity to phosphorylated Ub (pSer65-Ub), which recruits Parkin from cytosol to mitochondria ③. Several other factors, such as mitofusin 2 (MFN2), Miro, Rab7A, as well as BCL2/adenovirus E1B 19 kDa protein-interacting proteins 3 (BNIP3) are also involved in Parkin mitochondrial recruitment. Binding to pSer65-Ub releases the Ub-like (UBL) domain of Parkin from RING1 domain, partially activating Parkin ④. Then, PINK1 phosphorylates the UBL domain at Ser65 ⑤, which drives the phospho-UBL to rebind to the RING0 domain of Parkin to expose RING2′ catalytic site (Cys431) and fully activate Parkin ⑥. On the other hand, phosphatase and tensin homolog long (PTEN-L) located at OMM dephosphorylates Ub to inhibit mitophagy, whereas PTEN in the cytosol suppresses mitophagy through targeting Rab7A, MFN2, or BNIP3.

neurodegenerative diseases, and cancer. Thus, removal of damaged mitochondria by mitophagy has been shown to be an important mitochondrial quality control mechanism to maintain the mitochondrial homeostasis. However, this process must be restricted to dysfunctional mitochondria. Excessive degradation of essential mitochondria will cause cell death (Ordureau and Harper, 2014; Shi et al., 2014; Guo et al., 2016; Sharma et al., 2019). In addition, during the mitochondria fission process, the membrane potential of healthy mitochondria is temporarily compromised (Twig et al., 2008), which possibly activates PINK1-Parkin pathway to remove healthy mitochondria. Therefore, the mitophagy machinery is orchestrated by key mitophagy effectors with reversible posttranslational modifications, such as phosphorylation and dephosphorylation, to determine a finely tuned mitophagic activity in response to diverse stresses (**Figure 2**).

We now appreciate that phosphorylation of Ub by PINK1 (pSer65-Ub) plays central roles in the regulation of Ub-dependent mitophagy pathway. pSer65-Ub levels are very low in healthy mitochondria, but dramatically increased after mitochondrial damage and also increased during aging or in PD patient brain, which highlights its roles in diseases (Fiesel et al., 2015; Hou et al., 2018). Although PINK1 is the only reported kinase to generate pSer65-Ub, pSer65-Ub could be detected in PINK knockout cells (Ordureau et al., 2014) and in PINK1-deficient yeast (Swaney et al., 2015), suggesting another kinase exists to phosphorylate Ub at Ser 65. However, the function of PINK1-independent pSer65-Ub remains largely unclear. Another question is whether pSer65-Ub can be involved in other selective autophagy, such as xenophagy, which shares several key factors with mitophagy, including TBK1, NDP52, OPTN, and SQSTM1.

Recent studies have indicated that PTEN family proteins are involved in the regulation of both PINK1-Parkin-mediated Ub-driven and BNIP3 receptor-mediated mitophagy. Some important questions need to be further addressed. First is how the cells determine the expression level of different PTEN isoforms to function under different conditions. Second is whether there is a specific recruitment of PTEN-L and PTEN to mitochondria in response to mitochondrial damage. Third and more importantly is whether PTEN isoforms can serve as molecular targets for

development of novel interventional approaches in the regulation of mitophagy to benefit mitophagy-related human diseases.

AUTHOR CONTRIBUTIONS

LW and H-MS designed the outline of the review and wrote the draft of the manuscript. GL wrote part of the review.

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REVIEW
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RNA-Binding Proteins Implicated in Mitochondrial Damage and Mitophagy

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The mitochondrial lifecycle comprises biogenesis, fusion and cristae remodeling, fission, and breakdown by the autophagosome. This cycle is essential for maintaining proper cellular function, and inhibition of any of these processes results in deterioration of bioenergetics and swift induction of apoptosis, particularly in energy-craving cells such as myocytes and neurons. Regulation of gene expression is a fundamental step in maintaining mitochondrial plasticity, mediated by (1) transcription factors that control the expression of mitochondrial mRNAs and (2) RNA-binding proteins (RBPs) that regulate mRNA splicing, stability, targeting to mitochondria, and translation. More recently, RBPs have been also shown to interact with proteins modulating the mitochondrial lifecycle. Importantly, misexpression or mutations in RBPs give rise to mitochondrial dysfunctions, and there is strong evidence to support that these mitochondrial impairments occur early in disease development, constituting leading causes of pathogenesis. This review presents key aspects of the molecular network of the disease-relevant RBPs, including transactive response DNA-binding protein 43 (TDP43), fused in sarcoma (FUS), T-cell intracellular antigen 1 (TIA1), TIA-related protein (TIAR), and pumilio (PUM) that drive mitochondrial dysfunction in the nervous system.

Keywords: mitochondria, RNA-binding proteins, TDP43, FUS, TIA1, TIAR, PUM, mitophagy

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INTRODUCTION

Adenosine triphosphate (ATP) production by mitochondria is essential for most cellular activities. In addition to ATP generation, however, mitochondria are heavily involved in calcium homeostasis, production and modulation of reactive oxygen species (ROS), and in the execution of apoptosis.

Mitochondria are highly dynamic organelles characterized by rapid movement and undergo some five fusion-fission cycles every hour to properly maintain their function (Twig et al., 2008; Pernas and Scorrano, 2016). Mitochondrial fusion is the process in which mitochondria fuse together to spread metabolites, proteins, and DNA throughout the network to maintain mitochondrial (mt) DNA replication and oxidative phosphorylation (OXPHOS) capacity (Chen et al., 2005, 2010; Silva Ramos et al., 2019). It is mediated by optic atrophy 1 (OPA1), and mitofusin-1 and 2 (MFN1/2) (Chen et al., 2003; Olichon et al., 2003). Mitochondrial fission, on the other hand, is the process in which mitochondria divide to separate dysfunctional/depolarized mitochondrial sections in a daughter mitochondrion that will be targeted by autophagy, otherwise known as mitophagy (Twig et al., 2008). It is primarily regulated by dynamin-related protein 1 (DRP1) and dynamin-2 (DYN2) with the aid of adaptor proteins mitochondrial fission 1 (FIS1), mitochondrial

fission factor (MFF), and mitochondrial dynamics proteins 49 and 51 (MiD49/51) (Smirnova et al., 1998; Yoon et al., 2003; Gandre-Babbe and van der Bliek, 2008; Otera et al., 2010; Palmer et al., 2011; Lee et al., 2016). Additionally, folds of the inner membrane of the mitochondrion (known as cristae) that are formed to increase the surface area for housing the electron transport chain (ETC) complexes and ATP synthase continuously remodel to improve mitochondrial function (Enriquez, 2016). Collectively, these mitochondrial morphology events comprise the mitochondrial life cycle.

Mitochondrial dynamics are altered according to the energy requirements of the cell, nutrient availability, stress, and aging, and depend on transcriptional and post-transcriptional mechanisms. While transcription factors mediate the expression of nuclear and mitochondrial genes, RNA-binding proteins (RBPs) regulate splicing, stability, localization, and translation events. More recently, RBPs have been shown to interact directly with proteins on mitochondrial surface, too. In this review, we present findings that implicate RBPs misregulation in mitochondrial damage. We focus on transactive response DNA-binding protein 43 (TDP43), fused in sarcoma (FUS), T-cell intracellular antigen 1 (TIA1), TIA-related protein (TIAR), and pumilio (PUM), as there is substantial experimental data that show their involvement in mitochondrial pathology. General features, such as the neurological symptoms associated with their perturbation, molecular and cellular function, target mRNAs and subcellular localization have been described in our previous review, and are thus not described here (Ravanidis et al., 2018).

TDP43

Mutations or deregulation of transactive response DNA binding protein 43 (*TDP43* or *TARDBP*) expression have been associated with a spectrum of neurodegenerative diseases including frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) (Ravanidis et al., 2018). Electron microscopy (EM) analyses of patient brain samples as well as cellular and animal models of TDP43 proteinopathy revealed prominent mitochondrial impairment, including abnormal cristae architecture and diminished cristae surface area (Wang et al., 2019). Further, increased TDP43 expression induced mitochondrial dysfunction, including decreased mitochondrial membrane potential and elevated production of ROS (Wang et al., 2019; **Figure 1**).

Alzheimer's disease (AD) pathology includes mitochondrial perturbations such as alterations in respiratory function, mitochondrial biogenesis, and mitophagy (Cai and Tammineni, 2017; Chakravorty et al., 2019). Using the APP/PS1 transgenic mouse model co-expressing the familial AD Swedish mutations ($APP^{K595N,M596L}$) and mutant human presenilin 1 (PSEN1-ΔE9) under stress conditions, Davis et al. (2018), found increased accumulation of the N-terminal (27 kDa, N27) and C-terminal (30 kDa, C30) fragments of TDP43 in mitochondria. Immunoprecipitation from cortex lysates, to reveal the interacting partners of TDP43, showed enrichment for mitochondrial proteins, including prohibitin-2 (PHB2) and voltage-dependent anion channel 1 (VDAC1). PHB2 is a scaffold protein and a mitophagy receptor located in the inner mitochondrial membrane. It is involved in targeting mitochondria for autophagic degradation by interacting with microtubule-associated protein 1A/1B-light chain 3 (LC3) conjugated to phosphatidylethanolamine (LC3-II), which is found in autophagosomal membranes (Lahiri and Klionsky, 2017; Wei et al., 2017). Accordingly, PHB2 knockdown was shown to drastically reduce mitochondrial clearance (Wei et al., 2017). In addition, PHB2 is involved in mitochondrial membranes' fusion by stabilizing indirectly the long forms of dynamin-like GTPase OPA1, which mediates mitochondrial inner membrane fusion and cristae morphogenesis. Loss of PHB2 impairs the stability of OPA1, affects mitochondrial ultrastructure, and induces the perinuclear clustering of mitochondria (Merkwirth et al., 2012). Overexpression of TDP43 was found to increase PHB2 levels, whereas TDP43 knockdown reduced PHB2 and LC3-II expression in HEK293T cells treated with carbonyl cyanide m-chlorophenylhydrazone (CCCP), an inducer of mitophagy (Davis et al., 2018). Accordingly, an increase in the E3 ubiquitin ligase parkin (PRKN)-positive punctate staining (indicative of mitophagy) in cells treated with CCCP was observed, which was enhanced with TDP43 overexpression and reduced when TDP43 levels were knocked down (Davis et al., 2018). In parallel with these findings, in NSC34 cells that exhibit motor neuron features, overexpression of full length or C-terminal fragments of TDP43 (TDP25 and TDP35) led to increased levels of LC3-II and decreased levels of autophagy receptor p62 (SQSTM1) (Hong et al., 2012). Collectively, these results suggest that TDP43 overexpression is linked to enhanced mitophagic flux.

TDP43 expression also affects mitochondrial dynamics. Using transgenic mice expressing full-length human TDP43, Xu et al. (2010) observed aggregates of mitochondria, with decreased cristae and vacuoles within the mitochondrial matrix, adjacent to the nucleus, accompanied by enhanced levels of FIS1 and pro-fission phosphorylation of DRP1 at Ser616, both key mediators of the mitochondrial fission machinery (Taguchi et al., 2007). Conversely, a marked reduction in MFN1 expression, which plays an essential role in mitochondrial fusion, was observed (Xu et al., 2010).

Corroborating evidence came from Wang et al. (2013), showing that overexpression of wild-type TDP43 in primary motor neurons reduced mitochondrial length and density in neurites. Further, transgenic mice overexpressing wild-type or mutant TDP43 displayed significantly shorter, smaller, and damaged mitochondria (Wang et al., 2013). In contrast, artificial miRNA-mediated suppression of TDP43 in primary motor neurons resulted in significantly increased mitochondrial length and density in dendrites (Wang et al., 2013). In addition, co-expression of MFN2 with mutant TDP43 completely prevented all TDP43-induced mitochondrial abnormalities (Wang et al., 2013).

Informative findings have also arisen from work in *Drosophila*. Khalil et al. (2017) found that overexpression of human wild-type TDP43 in neurons resulted in abnormally small mitochondria. The mitochondrial fragmentation was correlated with a specific

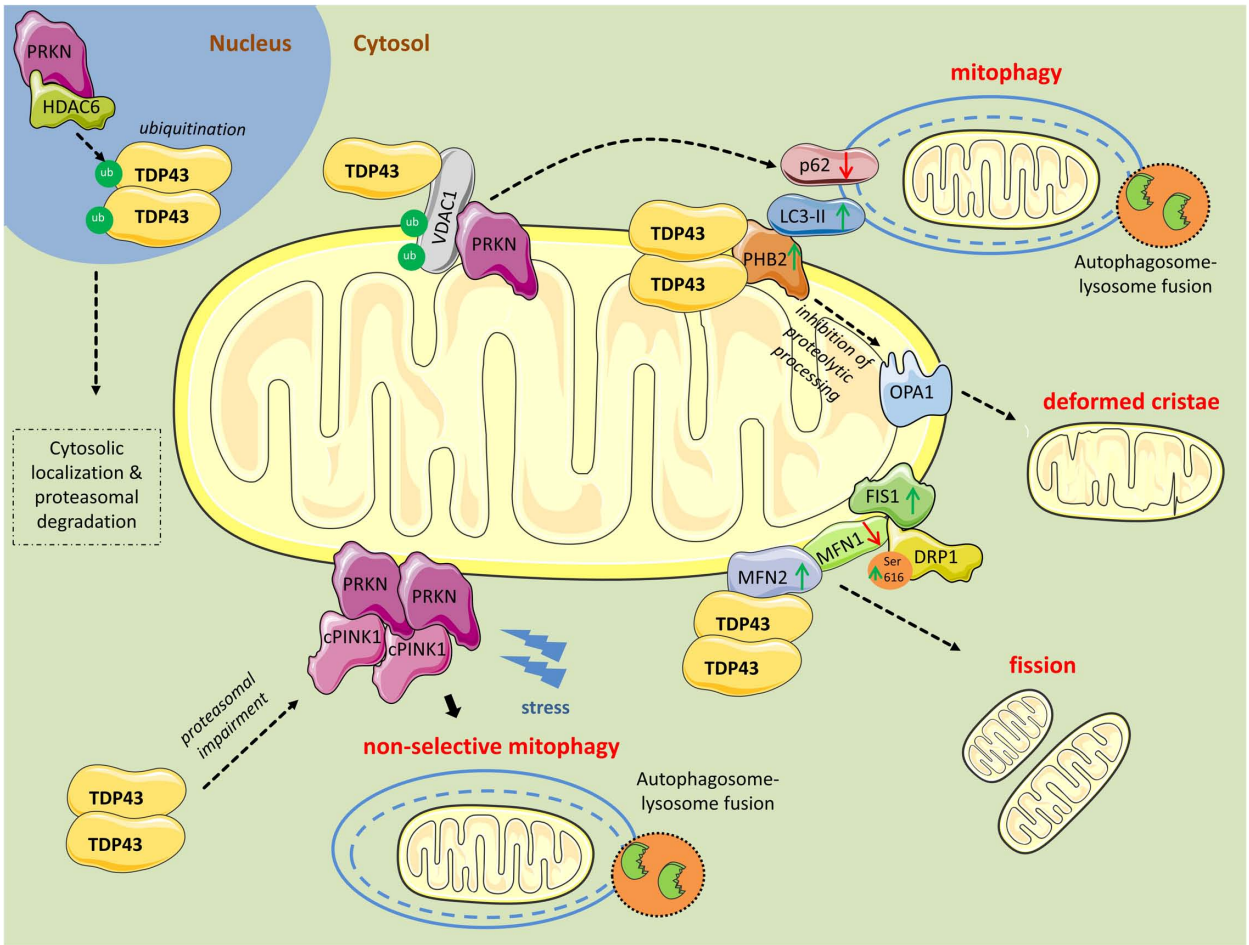

FIGURE 1 | Mitochondrial perturbations induced by TDP43. PRKN in complex with HDAC6, ubiquitinates nuclear TDP43 promoting its cytoplasmic localization and proteasomal degradation. However, as revealed from research in aging or neurodegenerative diseases, TDP43 often persists in the cytosol and forms aggregates. Excess cytosolic TDP43 interacts with VDAC1, located in the outer mitochondrial membrane, but it is still unclear if interferes with its functions. Polyubiquitination of VDAC1 by PRKN is essential for driving mitophagy. Moreover, cytosolic TDP43, translocated to the outer mitochondrial membrane, directly interacts with PHB2 and, in parallel, increases its protein levels. PHB2 is known to interact with LC3-II to induce mitophagy. PHB2 is also involved in mitochondrial membranes fusion by stabilizing indirectly the long forms of OPA1. Additionally, TDP43 directly interacts with MFN2, a mitochondrial membrane protein regulating mitochondrial fusion, and possibly stabilizes its expression. Concurrently, TDP43 leads to reduced levels of another fusion protein, MFN1, and increases levels of FIS1 and DRP1 phosphorylated at Ser616, proteins promoting mitochondrial fission. Finally, TDP43 downregulates *PRKN* mRNA and protein levels, and impairs the proteasome, leading to the accumulation of cleaved PINK1 (cPINK1) in the cytosol. During stress conditions cPINK1 aggregates recruit PRKN to the mitochondria launching mitophagy in otherwise healthy mitochondria (non-selective mitophagy).

decrease in the levels of Marf, the MFN ortholog in *Drosophila*. Importantly, overexpression of Marf or inactivation of pro-fission Drp1 ameliorated the defects (Khalil et al., 2017). Similar mitochondrial dysfunctions were observed in another *Drosophila* study, and likewise the mitochondrial fission defects were rescued by co-expression of mitochondrial pro-fusion genes Marf, Opa1, and the dominant negative mutant form of Drp1 (Altanbyek et al., 2016).

Using immunoprecipitation from cortical human brain tissue, TDP43 was found to also interact directly with pro-fusion factor MFN2 (Davis et al., 2018). Knocking down TDP43 in HEK293T cells led to a reduction in MFN2 expression levels, whereas TDP43 overexpression marginally increased MFN2 levels (Davis et al., 2018). Previously, MFN2 repression was shown to inhibit mitophagy and result in the accumulation of damaged mitochondria in muscles during aging (Sebastian et al., 2016), indicating that changes in the balance of mitochondrial fission/fusion machinery affect not only architecture dynamics but mitophagy as well.

Under steady-state conditions, PTEN-induced kinase 1 (PINK1), a mitochondrial serine/threonine kinase, is imported in the inner mitochondrial membrane where it is cleaved by the serine protease presenilin-associated rhomboid-like (PARL) (Yamano and Youle, 2013). Following cleavage, PINK1 is released into the cytosol where it is recognized by the N-end rule E3 enzymes, ubiquitin protein ligase E3 component N-Recognin 1 (UBR1), UBR2, and UBR4 for constitutive and rapid proteasome-mediated degradation (Yamano and Youle, 2013).

When mitochondria are damaged, PINK1 is not cleaved and is subsequently anchored to the outer mitochondrial membrane where it recruits and activates, via phosphorylation, the E3 ubiquitin ligase PRKN to trigger selective mitophagy (Pickrell and Youle, 2015). Both PINK1 and PRKN exhibit mutations that have been linked to autosomal recessive early-onset Parkinson's disease (PD) (Kitada et al., 1998; Hatano et al., 2004; Rohe et al., 2004; Valente et al., 2004).

Using human TDP43 knock-in flies, TDP43-infected mouse primary neurons, TDP43-transfected HEK293T cells, and $TDP43^{Q331K}$ transgenic mice, Sun et al. (2018), showed that TDP43 downregulates *PRKN* mRNA and protein levels via mechanisms requiring both the RNA-binding and the protein-protein interaction functions of TDP43. Unlike *PRKN*, TDP43 did not regulate *PINK1* at the mRNA level. Instead, overexpression of TDP43 lead to cytosolic aggregates of cleaved PINK1 due to impaired proteasomal activity, and compromised mitochondrial respiration (Sun et al., 2018). Upregulation of PRKN expression or RNAi-mediated downregulation of PINK1 levels suppressed TDP43-induced degenerative phenotype in *Drosophila,* indicating that PRKN and PINK1 are important components of TDP43-induced proteinopathy (Sun et al., 2018). Additionally, it has been reported that accumulation of cleaved PINK1 induces non-selective mitophagy and non-apoptotic cell death (Lim et al., 2015). In this article, it is shown that cleaved PINK1 cytosolic aggregates trigger PRKN translocation to healthy mitochondria, leading to non-selective mitophagy (Lim et al., 2015).

In another study, PRKN was shown to ubiquitinate nuclear TDP43, and together with HDAC6, promote cytosolic TDP43 accumulation reminiscent of ubiquitinated wild-type or mutant TDP43 found in the cytosol in several neurodegenerative diseases (Hebron et al., 2013). Moreover, *Prkn* knockout mice exhibited high levels of TDP43, underscoring an indispensable role for PRKN in mediating TDP43 clearance and cytosolic localization (Wenqiang et al., 2014).

A dual regulation of mitophagy and apoptosis by PRKN via VDAC1, a direct partner of TDP43 in mitochondria (Davis et al., 2018), has also been revealed. Previously, VDACs have been shown to mediate mitophagy via recruitment of PRKN in the mitochondria (Geisler et al., 2010; Sun et al., 2012; Li et al., 2014). More recently, PRKN was shown to mono- or poly-ubiquitinate VDAC1. Polyubiquitination was required for PRKN-mediated mitophagy, whereas mono-ubiquitination was required for mitochondrial calcium influx and apoptosis (Ham et al., 2020). The role of TDP43 in the mono- or poly-ubiquitination of VDAC1 by PRKN has yet not determined.

FUS

Mutations in the FUS or translocated in liposarcoma (*FUS/TLS*) gene give rise to familial ALS and occasionally FTLD-FUS, both displaying FUS-positive inclusions (Ravanidis et al., 2018). Interestingly, however, in the majority of FTLD-FUS cases, no *FUS* mutations have been identified, but rather an increase in wild-type FUS expression highlighting a dose-dependent role in neurodegeneration (Sabatelli et al., 2013; Deng et al., 2015). Several systems have been used to model FUS-proteinopathies, in all of which wild-type or ALS-mutant FUS overexpression led to progressive neurodegeneration reiterating findings in patients (Huang et al., 2011; Ravanidis et al., 2018).

Several studies implicate mitochondrial damage as an early event that precedes cell death in FUS proteinopathies (Deng et al., 2015, 2018; So et al., 2018; **Figure 2**). Deng et al. (2015) showed that overexpression of wild-type or ALS-associated mutant FUS in HEK293 cells reduced the mitochondrial membrane potential and increased the production of mitochondrial ROS. Increased levels of ROS drive mitochondrial translocation of the pro-fission protein DRP1 in ASTCa1 cells, leading to mitochondrial fragmentation (Wu et al., 2011). Likewise, Deng et al. (2015) observed mitochondrial fragmentation in wild-type or mutant FUS-overexpressing HT22 cells, cultured neurons, and transgenic fly motor neurons. They then performed EM to compare healthy control and FTLD-FUS brain mitochondria. While in controls most mitochondria appeared healthy with well-organized cristae as packed-stacks of membrane sheets and with only a few FUS-immunostaining signals, in FTLD patients mitochondria displayed a marked loss or disruption of cristae with frequent detection of "onion-like" deformed shapes and FUS-immuno-positive signals, in close association with the mitochondria (Deng et al., 2015).

Similarly, So et al. (2018), using transgenic hFUS mice, revealed that FUS, which is abundant at the pre-synaptic terminal of the neuromuscular junction (NMJ), caused a significant decrease in the number of mitochondria, while many of those that remained had pronounced abnormalities including disorganized cristae and large vacuoles as early as postnatal day 15. Interestingly, mitochondria in the post-synaptic muscle endplate were abundant and of normal appearance, consistent with other studies demonstrating that mitochondria at distal axon terminals undergo the earliest damage in the course of ALS disease (Magrane et al., 2012; Ruffoli et al., 2015).

Deng et al. (2015) moved on to demonstrate that heat shock protein 60 kDa (HSP60), an ATP-dependent mitochondrial chaperone, interacted with FUS and mediated FUS mitochondrial localization. siRNA-based downregulation of HSP60 levels reduced mitochondrially localized FUS without altering its overall cellular levels; in fact, levels of nuclear and cytoplasmic FUS increased as a result. Accordingly, HSP60 downregulation increased the size of mitochondria and partially rescued mitochondrial defects as well as neurodegenerative phenotypes caused by wild-type or mutant FUS overexpression in transgenic fly photoreceptors. Finally, they found that HSP60 protein levels were elevated in the brains of FTLD-FUS patients (Deng et al., 2015). These observations indicate that HSP60 plays an important role in mediating the translocation of excess FUS in mitochondria, a critical early step in mitochondrial impairment and thereafter neurodegeneration.

Additional mechanisms by which FUS induces mitochondrial damage have been brought forward. Wild-type or mutant FUS were found to interact with the mitochondrial ATP synthase β-subunit (ATP5B) (Deng et al., 2018), which is the essential catalytic subunit of mitochondrial ATP synthase

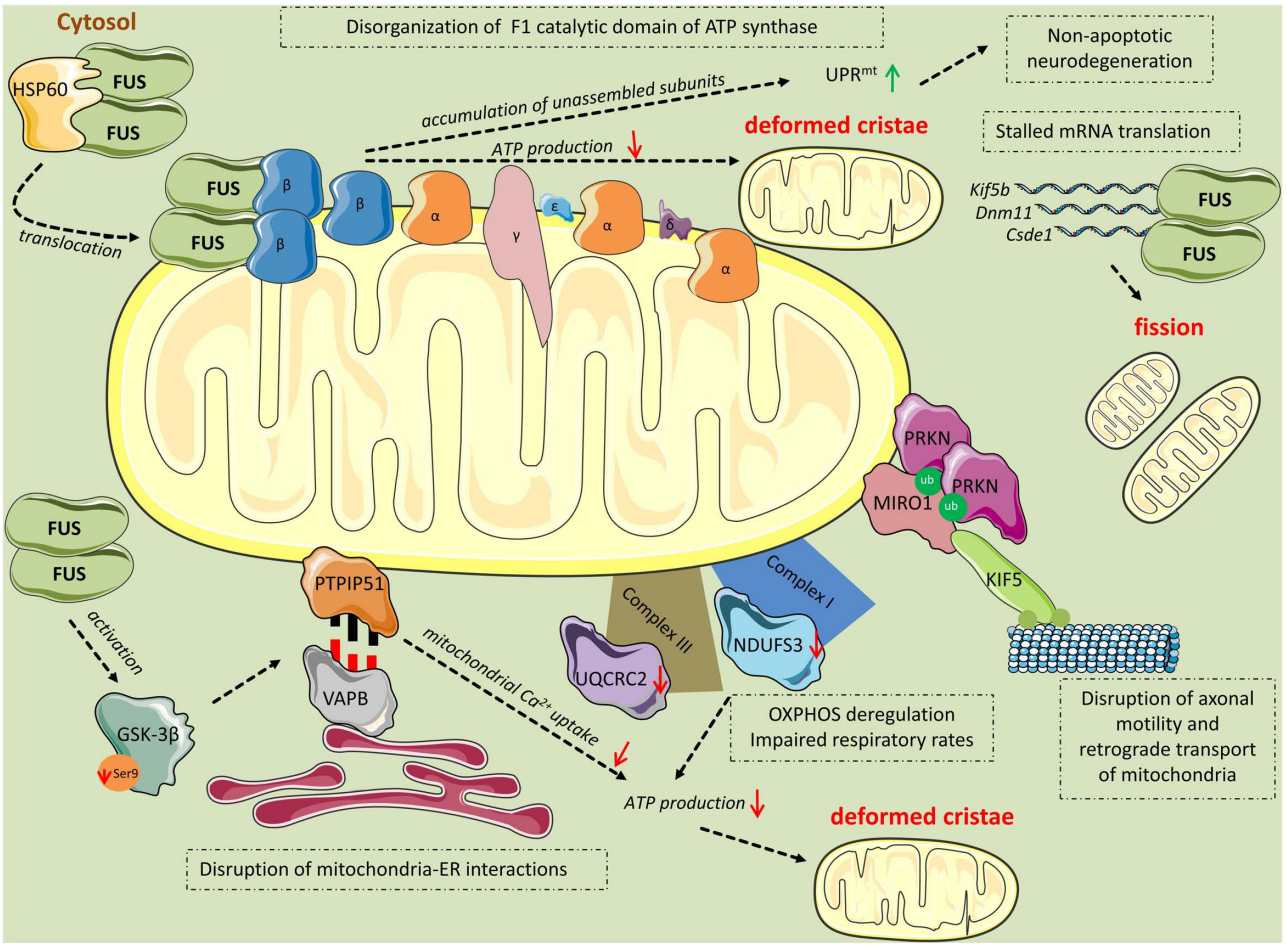

FIGURE 2 | Mitochondrial perturbations induced by FUS. HSP60 mediates FUS translocation to the outer mitochondrial membrane. Mitochondrial-localized FUS binds to the β subunit of the F1 catalytic domain of ATP synthase (Complex V). The binding leads to disassembly of the F1 domain and accumulation of unassembled ATP synthase subunits, including ATP5B, which activates the UPR^{mt} response leading to non-apoptotic cell death. Additionally, disruption of the F1 domain of the ATP synthase complex results in impaired ATP production and thereafter, deformed cristae. FUS induces mitochondrial perturbations in several other manners while being in excess in the cytoplasm. Mutant FUS binds to mature mRNAs coding for important mitochondrial proteins including *Kif5b*, *Dnm1l,* and *Csde1,* inhibiting their translation. This inhibition progressively leads to mitochondrial fission. Excess FUS drives the accumulation of PINK1 and PRKN proteins. As a consequence, RHOT1, also known as Miro1, a component of the primary motor/adaptor complex that anchors kinesin to the mitochondrial surface and a direct target of PRKN, is ubiquitinated leading to disruption in axonal motility and retrograde transport of mitochondria. Additionally, FUS has an impact on the OXPHOS process by deregulating the expression of the subunits NDUFS3 and UQCRC2 of Complexes I and III, respectively. OXPHOS deregulation leads to respiratory impairment and subsequent ATP production deterioration and deformed cristae. Finally, FUS decreases the levels of ser9 phosphorylation in GSK-3β, leading to increased GSK-3β activity. Activated GSK-3β deregulates the interaction of mitochondrial tethered membrane protein PTPIP51 and the inner protein of the ER, VAPB, disrupting mitochondria-ER associations. The ER-mitochondria disruption decreased Ca^{2+} uptake by mitochondria following release from ER stores, resulting in reduced ATP production and deformed mitochondria.

(Wang and Oster, 1998). FUS binding to ATP5B disrupted the assembly of ATP synthase super-complex, suppressing ATP synthesis (Deng et al., 2018). Previously, ATP synthase complex assembly has been closely associated with mitochondrial cristae formation (Paumard et al., 2002). ATP synthase mutants show disorganized cristae in yeast (Paumard et al., 2002; Strauss et al., 2008), which could explain the disruption or loss of cristae observed following FUS overexpression (Deng et al., 2015, 2018; So et al., 2018).

On top of that, whereas ATP synthase complex activities and formation were decreased, mitochondrial ATP5B protein levels were increased in FUS-overexpressing HEK293 cells and flies (Deng et al., 2018). This has given rise to an accumulation of unassembled ATP synthase subunits, including ATP5B, which activated the mitochondrial unfolded protein response (UPR^{mt}) (Deng et al., 2018). UPR^{mt} is an adaptive mechanism to ensure mitochondrial proteostasis and quality control. However, excessive activation of UPR^{mt} following severe or extended mitochondrial stresses can induce non-apoptotic neurodegeneration (Martinez et al., 2017). That is likely the case here, as downregulation of UPR^{mt} genes ameliorated wild-type

or mutant FUS-induced retinal degeneration in flies (Deng et al., 2018).

A different perspective was brought forward by Stoica et al. (2016). They found that wild-type or ALS-associated mutant FUS decreased the endoplasmic reticulum (ER)-mitochondria associations in NSC34 motor neuron cells and in spinal cord motor neurons from FUS transgenic mice (Stoica et al., 2016). Specifically, they showed that FUS disrupted the interaction between the integral ER protein, vesicle-associated membrane protein-associated protein B (VAPB), and the outer mitochondrial membrane protein, protein tyrosine phosphatase interacting protein 51 (PTPIP51) that serve as scaffolds to tether the two organelles (De Vos et al., 2012). This disruption was accompanied by a decrease in Ca^{2+} uptake by the mitochondria following its release from ER stores. Since mitochondrial ATP production is linked to Ca^{2+} levels (De Vos et al., 2012), uncoupling of ER-mitochondria by FUS resulted in impaired ATP production (Stoica et al., 2016). Immunoprecipitation revealed that FUS did not bind either VAPB or PTPIP51. Instead, FUS reduced the inhibitory phosphorylation of ser9 in GSK-3β, resulting in its activation (Stoica et al., 2016). Previously, the same group has shown that GSK-3β inhibition increases the VAPBPTPIP51 interaction; however, the precise mechanism is not yet determined (Stoica et al., 2014). Hence, using the GSK-3β inhibitors AR-A014418 and CT99021H, they showed that FUS-induced defects in ER-mitochondria association as well as mitochondrial Ca^{2+} levels were restored (Stoica et al., 2016). Considering that damaged ER-mitochondria associations have also been described in AD and PD (Zampese et al., 2011; Cali et al., 2012; Hedskog et al., 2013; Ottolini et al., 2013; Guardia-Laguarta et al., 2014), this indicates that perturbation of the ER–mitochondrial axis may be a general feature in neurodegeneration.

Another way by which disease-causing FUS mutations induce mitochondrial impairment and neurotoxicity was deciphered by Nakaya and Maragkakis (2018). They showed that unlike wild-type FUS that predominantly binds pre-mRNAs, the ALS-associated R495X FUS mutant binds mature mRNAs in the cytoplasm (Nakaya and Maragkakis, 2018). Although R495X binding had only a moderate effect on mRNA levels, it significantly reduced the translation of mRNAs that are associated with mitochondrial function such as *Kif5b*, *Dnm1l,* and *Csde1* (Nakaya and Maragkakis, 2018). These alterations were accompanied by a reduction in mitochondrial size, as previously reported (Deng et al., 2015, 2018; So et al., 2018). Importantly, by introducing multiple mutations in the RRM RNA-binding domain of R495X FUS, to reduce its RNA-binding ability (Daigle et al., 2013), they partially abrogated R495X-induced effects on mRNA translation, mitochondrial size, and neurotoxicity, uncovering a novel RNA-mediated pathway of FUS proteinopathy (Nakaya and Maragkakis, 2018).

Insights into the role of PRKN in FUS-mediated mitochondrial dysfunction were revealed by Cha et al. (2020). Using *Drosophila* flies, they showed that when PRKN was co-overexpressed with FUS, it was able to rescue locomotive defects (Cha et al., 2020). At the cellular level, PRKN co-overexpression did not lead to any significant mitochondrial morphological improvements compared to the flies only overexpressing FUS; in fact, PRKN overexpressed alone also exhibited fragmented mitochondria (Cha et al., 2020). Instead, they found that PRKN restored the expression of mitochondrial subunits I (NDUFS3) and III (UQCRC2), which are significantly decreased in FUS-induced ALS flies. As a result, flies overexpressing both FUS and PRKN had partially restored ATP levels (Cha et al., 2020). Interestingly, complex III is one of the five mitochondrial distinct multi-subunit complexes (I–V) whose activity is reported to be dampened in spinal cord tissues of ALS patients (Sasaki and Iwata, 2007). Taken together, these observations demonstrated a protective role of PRKN in FUS-induced mitochondrial dysfunction.

Contradictory findings concerning the role of PRKN in FUS-mediated defects have also been reported (Chen et al., 2016). Overexpression of wild-type or mutant FUS in HEK293 cells lead to the accumulation of PINK1 and PRKN proteins (Chen et al., 2016). As a consequence, the Ras homolog family member T1 (RHOT1, also known as Miro1), a component of the primary motor/adaptor complex that anchors kinesin to the mitochondrial surface and a direct target of PRKN, was ubiquitinated leading to the disruption in axonal motility and retrograde transport of mitochondria (Chen et al., 2016). Previously, Miro1 was shown to be phosphorylated by PINK1, which promoted its proteasomal degradation by PRKN (Wang et al., 2011; Liu et al., 2012). RNAi-mediated downregulation of both PINK1 and PRKN restored locomotive defects in FUS transgenic flies (Chen et al., 2016). As the PINK1/PRKN pathway also promotes mitochondrial fission (Poole et al., 2008; Yu et al., 2011), Chen et al. (2016) proposed that the upregulation of PINK1 and PRKN is partly responsible for mitochondrial fragmentation induced by wild-type and mutant FUS overexpression.

TIA1 AND TIAR

T-cell intracellular antigen 1 and TIA-related/like protein share an extended identity in the amino acid sequence, and like other RBPs, they translocate to the cytoplasm following cellular stress conditions forming stress granules (SG) (Ravanidis et al., 2018). Missense mutations in the *TIA1* gene cause both Welander distal myopathy (WDM) (Hackman et al., 2013) and ALS, characterized by delayed SG disassembly and accumulation of non-dynamic SGs that harbor TDP43 (Mackenzie et al., 2017).

Early in the analysis of TIA1 cell models, it became evident that TIA1 and TIAR affect mitochondrial dynamics (**Figure 3**). Using EM, Carrascoso et al. (2017) found that TIA1 or TIAR overexpression in HEK293 cells promoted mitochondrial clustering and fission. Closer inspection of mitochondria revealed changes in cristae organization, with many cristae having a slightly wider and more loosely organized intermembrane space than those of control cells (Carrascoso et al., 2017). Further, the mtDNA/nDNA ratio was similar between control and TIA1- or TIAR- overexpressing cells, suggesting that the changes in mitochondria were linked to reorganization dynamics rather than *de novo* mitochondrial

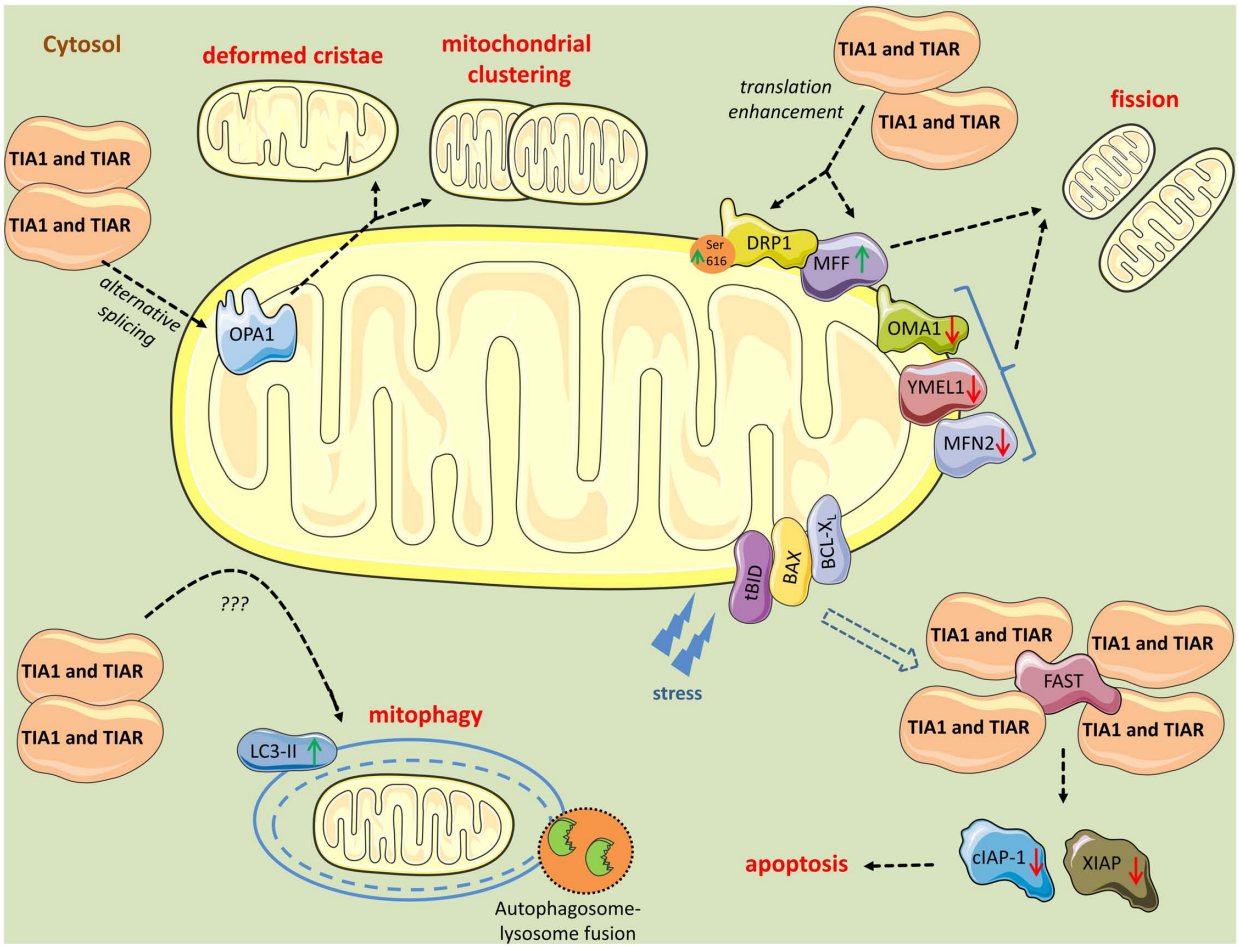

FIGURE 3 | Mitochondrial perturbations induced by TIA1 and TIAR. TIA1 and TIAR mediate exon 4b inclusion in the pre-mRNA of *OPA1* generating the OPA1 variant 5, which is associated with a smaller mitochondria, mitochondrial clustering, and remodeling around the perinuclear region. Further, cytosolic TIA1 enhances the translation of *MFF* mRNA and promotes DRP1 translocation to mitochondria leading to mitochondrial fragmentation. In parallel, TIA1 and TIAR induce modest downregulation of the pro-fusion proteases OMA1, YMEL1, and MFN2, further contributing to the pro-fission phenotype and mitophagy. TIA1 also has pro-apoptotic properties inhibited by FAST. FAST is released from its mitochondrial tether during stress, a process mediated by tBID and BAX. Following its release, FAST binds to TIA1 and prevents TIA1-mediated silencing of mRNAs encoding inhibitors of apoptosis, such *cIAP-1* and *XIAP*. When TIA1 is in excess, it binds FAST and obstruct its anti-apoptotic events. Finally, TIA1 and TIAR increase LC3-II levels, yet the mechanism is unknown, leading to increased mitophagic events.

biogenesis. Mitochondrial respiration and ATP production were impaired as a result (Carrascoso et al., 2017). When switched from glucose to galactose or fatty acids as cell culture substrates, to promote a switch from glycolysis to OXPHOS and determine the degree of mitochondrial dependency in cell growth, TIA1- or TIAR- overexpressing cells showed reduced proliferation rates (Carrascoso et al., 2017). Additionally, they displayed increased mitophagy rates and ROS production. Enhanced cleaved poly (ADP-ribose) polymerase 1 (PARP1) levels and delay in G1/S cycle phase transition, phenomena of early apoptosis, correlated with increased mitophagy (Carrascoso et al., 2017). Increased mitochondrial DNA damage were also observed in TIA1- or TIAR- overexpressing cells following H_2O_2 treatment suggestive of impaired antioxidant defense (Carrascoso et al., 2017). Collectively, these results indicate that TIA1 or TIAR provoke respiratory deficiency and compromised mitochondrial function (Carrascoso et al., 2017).

Mechanistically, TIA1 and TIAR mediated exon 4b inclusion in the pre-mRNA of *OPA1* generating the OPA1 variant 5. OPA1 is a dynamin-like GTPase that regulates cristae junction numbers and stability, and the different OPA1 protein isoforms (eight in humans) relay instructions that help determine fusion, build cristae, and tune the morphology of mitochondria (Olichon et al., 2007; Song et al., 2007; Glytsou et al., 2016). OPA1v5, specifically, promotes mitochondrial clustering and remodeling around the perinuclear region (Song et al., 2007; Carrascoso et al., 2017). Ablation of TIA1 or TIAR in mouse embryonic fibroblasts (MEFs) favored the expression of short forms of OPA1, and the appearance of elongated mitochondria indicative of fusion phenotypes (Carrascoso et al., 2017). Furthermore, knockdown of OPA1 or overexpression of OPA1v5 triggered mitochondrial

clustering mimicking TIA1 or TIAR effects (Carrascoso et al., 2017). In addition, proteases associated with fusion (OMA1, YMEL1, and MFN2) were modestly downregulated in TIA1- or TIAR-overexpressing cells, whereas the fission-associated protein MFF was slightly upregulated, further contributing to the pro-fission phenotype (Carrascoso et al., 2017).

Tak et al. (2017) independently reported similar mitochondrial phenotypes following TIA1 modulation, but provided different mechanistic insights. Likewise, they showed that TIA1 overexpression in CHANG liver cells enhanced mitochondrial fission, while downregulation enhanced mitochondrial elongation. In addition, TIA1 downregulation increased mitochondrial activity, including the rate of ATP synthesis and oxygen consumption (Tak et al., 2017). Further, they identified *MFF* 3'UTR as a direct target of TIA1 and showed that TIA1 promoted mitochondrial fragmentation by enhancing MFF translation. Accordingly, *Tia1*-null MEF cells had decreased levels of MFF and mitochondrial DRP1, thereby leading to mitochondrial elongation (Tak et al., 2017).

Studies investigating the p.E384K mutant form of TIA1 ($TIA1^{WDM}$) responsible for WDM revealed similar findings (Carrascoso et al., 2019). $TIA1^{WDM}$ overexpression in HEK293 cells resulted in mitochondrial fission and mitochondrial swelling with an abnormal distribution of cristae. This led to decreased mitochondrial membrane potential and enhanced redox status (Carrascoso et al., 2019). Additionally, there was an increase in the formation of autophagosomes and autolysosomes, as well as mitophagic and apoptotic rates (Carrascoso et al., 2019). Taken together, these results revealed that similar to wild-type TIA1, disease-associated mutant TIA1 overexpression has a negative impact on mitochondrial dynamics, leading to mitochondrial dysfunction and cell death.

Sanchez-Jimenez and Izquierdo (2013) used *Tia1* and *Tiar* knock-out MEFs to study the molecular and cellular consequences. They found that TIA1 and TIAR knockout cells had two to threefold more mitochondria, six to sevenfold higher mitochondrial membrane potential, and twofold higher ROS levels. Mitochondria had atypical morphology, with some being enlarged and others being fragmented (Sanchez-Jimenez and Izquierdo, 2013). These alterations were associated with nuclear DNA damage, revealed by 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) staining, and high rates of autophagy, possibly as a compensatory mechanism toward survival. Consequently, TIA1 and TIAR knockout MEFs displayed defects in cell proliferation and increased cell death (Sanchez-Jimenez and Izquierdo, 2013).

A different perspective by which TIA1 is promoting apoptosis was brought forward by Li et al. (2004b). They proposed that during stress, TIA1 silences (Kedersha et al., 2000; Anderson and Kedersha, 2002), among others, the translation of mRNAs encoding inhibitors of apoptosis, and that the Fas-activated serine/threonine kinase (FAST) phosphoprotein is counteracting this function (Li et al., 2004b). They showed that FAST, which is tethered to the outer mitochondrial membrane in association with BCL-X_L (Li et al., 2004a), is a constitutive pro-survival protein (Li et al., 2004b). RNAi-mediated silencing of endogenous FAST in HeLa cells resulted in apoptosis, whereas overexpression of FAST inhibited both Fas- and UV- induced apoptosis (Li et al., 2004b). Mechanistically, they found that a FAST mutant lacking its TIA1-binding domain did not inhibit apoptosis, and overexpressed TIA1 inhibited the antiapoptotic effects of FAST. They proposed that in response to stress, tBID and BAX move to the outer mitochondrial membrane, where they sequester BCL-X_L, releasing FAST from its mitochondrial tether. FAST then binds to TIA1 and prevents TIA1-mediated silencing of mRNAs, including those encoding inhibitors of apoptosis, such as *cIAP-1* and *XIAP* (Li et al., 2004b). Hence, FAST serves as a cellular sensor of mitochondrial stress, that in response to stress, modulates TIA1-regulated posttranscriptional silencing responses.

PUMILIO

Pumilio belongs to the evolutionary conserved Pumilio and FBF (PUF) family of RBPs comprised two paralogous members in vertebrates (Pum1 and 2), and one in *Drosophila* (Pum). It is an important mediator of neurological processes, including olfactory learning and motor function (Ravanidis et al., 2018). In humans, a *PUM1* mutation is associated with adult-onset ataxia, whereas haploinsufficiency due to deletions or missense variants cause developmental delay and seizures (Gennarino et al., 2018).

Several study systems ranging from yeast to mice highlighted the role of PUFs in regulating mitochondrial biogenesis and mitophagy (**Figure 4**). In yeast, Puf3p was shown to specifically associate with 135 mRNAs, 87% of which are nucleus-encoded mitochondrial mRNAs (Gerber et al., 2004). Among these mitochondrial mRNAs, 59% (80 genes) are involved in protein biosynthesis, including structural components of the ribosome; 16% (22 genes) in mitochondrial organization and biogenesis; 13% (17 genes) in aerobic respiration; 9% (12 genes) in mitochondrial translocation; 9% are tRNA ligases (12 genes); and 7% are translational regulators (nine genes) (Gerber et al., 2004). Interestingly, when Puf3p was deleted in yeast, Puf3p-associated mRNAs were not only selectively increased compared to all other mRNAs (Gerber et al., 2004), but also mislocalized away from mitochondria (Eliyahu et al., 2010), indicating that Puf3p regulates the stability and localization of mRNAs expressing mitochondrial proteins. Consequently, yeast strains overexpressing Puf3p exhibited respiratory dysfunction and abnormal mitochondrial morphology and motility (Gerber et al., 2004; Garcia-Rodriguez et al., 2007).

Studies in *Drosophila* backed yeast findings. Work by Gehrke et al. (2015), revealed that nuclear mRNAs encoding respiratory chain complexes (*nRCC*) are localized in a PINK1/Tom20-dependent manner to the mitochondrial outer membrane, where they are de-repressed and translated by PINK1/PRKN pathway through the displacement of translation repressors, including PUM and hnRNPF; in this case, PINK1 displayed an RNA-binding capacity competing with PUM for mRNA-binding, while PRKN mono-ubiquitinated the RBPs lowering their affinity for *nRCC* mRNAs (Gehrke et al., 2015). Accordingly, inhibiting PUM via RNAi was found to increase, whereas PUM overexpression decreased *nRCC* mRNAs abundance (Gehrke et al., 2015). In addition,

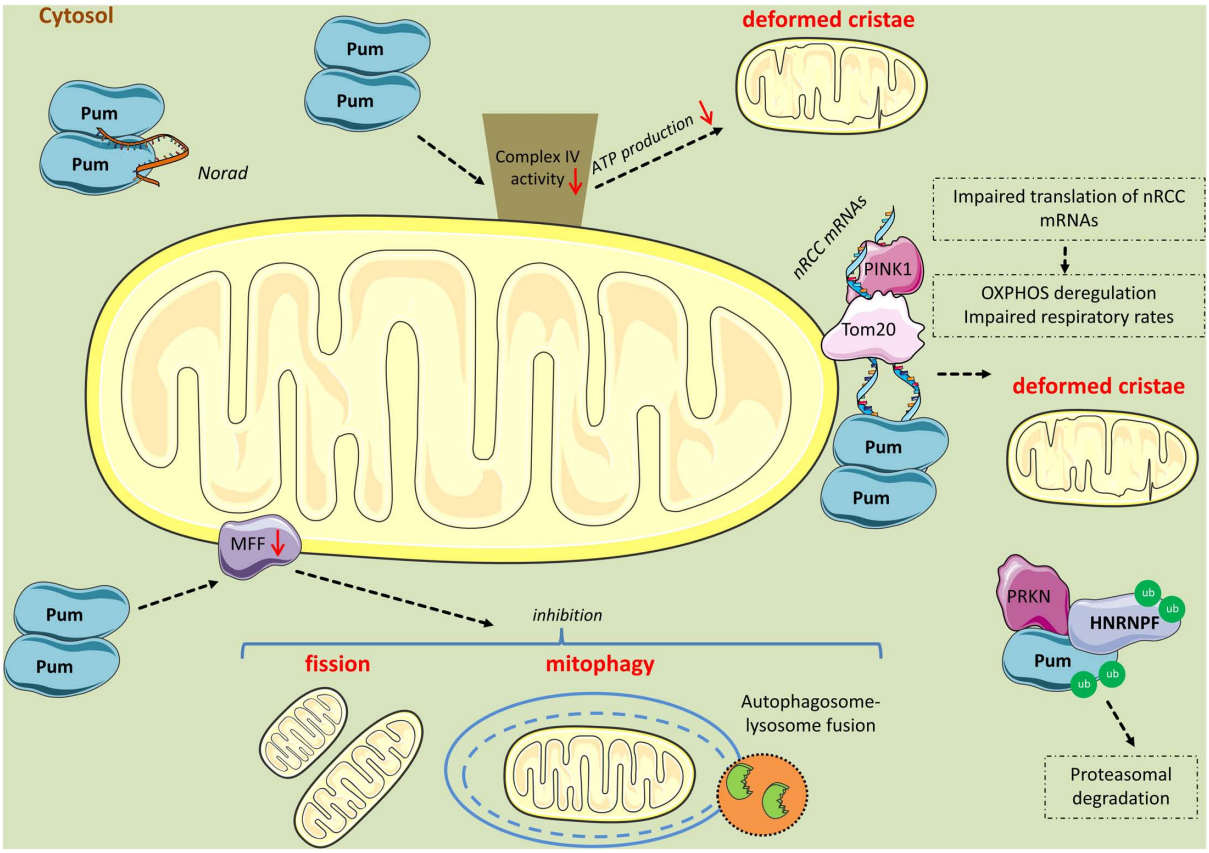

FIGURE 4 | Mitochondrial perturbations induced by PUM2. PUM2 reduces cytochrome c oxidase complex (Complex IV) activity, leading to impaired respiration and deformed cristae. Interestingly, the long non-coding RNA *NORAD* inhibits PUM2 function by sequestering PUM2 from binding to mitochondrial mRNA targets. Further, PINK1 in association with Tom20 promote the expression of nuclear-encoding mitochondrial (*nRCC*) mRNAs in the outer mitochondrial membrane by competing with PUM and other translation repressors. PINK1 competes with PUM for mRNA-binding, while PRKN mono-ubiquitinates PUM and HNRNPF lowering their affinity for *nRCC* mRNAs and possibly leading to their proteasomal degradation. However, when PUM is in excess, it binds to the *nRCC* mRNAs and represses their translation. Finally, PUM2 binds to *MFF* mRNA and represses its translation, leading to reduced fission and mitophagy.

PUM inhibition rescued ATP production, mitochondrial morphology, and neuromuscular-degeneration phenotypes of PINK1, but not PRKN mutant flies (Gehrke et al., 2015). Collectively, these findings revealed that besides its well-known serine/threonine kinase activity, PINK1 is also an RBP competing with PUM to control mitochondria biogenesis (Gehrke et al., 2015).

Electron microscopy of skeletal muscles from PUM2-overexpressing mice revealed the accumulation of subsarcolemmal, irregularly shaped and abnormally enlarged mitochondria lacking normal cristae (Kopp et al., 2019). Furthermore, a global reduction in cytochrome c oxidase (complex IV, COX) activity was observed. In addition, transient expression of PUM2 in MEFs or stable expression of either PUM1 or PUM2 in HCT116 cells significantly impaired respiration, providing compelling evidence that PUM hyperactivity results in mitochondrial dysfunction (Kopp et al., 2019). Interestingly, a non-coding RNA called NORAD acts as a guardian of the transcriptome by being a preferred target of PUM2, thereby inhibiting its translation suppressive functions (Kopp et al., 2019).

Research findings from D'Amico et al. (2019) associated PUM2 with aging defects via impaired mitochondrial dynamics. They reported that elevated levels of PUM2 are found in muscle and neocortex of aged mice (Edwards et al., 2007; Oberdoerffer et al., 2008; D'Amico et al., 2019) as well as muscle biopsies of aged humans (Yang et al., 2015). Additionally, *Pum2* levels in the brains of mice strains BXD and LXS are inversely correlated with longevity (Gelman et al., 1988; Liao et al., 2010). To experimentally validate this suggestive effect on lifespan, they used *Caenorhabditis elegans* to show that PUF8, ortholog of PUM2, knockdown was associated with increased lifespan (D'Amico et al., 2019). Consistently, knock-down of *puf8* and *Pum2* improved both basal and maximal respiration in old worms and mouse myoblasts, respectively (D'Amico et al., 2019). Like in previous studies, using multi-tissue gene set enrichment analysis (GSEA) in the human GTEx cohort, they found that PUM2 expression levels were inversely correlated with clusters

of genes responsible for mitochondrial function, including genes important for OXPHOS and cellular respiration (D'Amico et al., 2019). Furthermore, from CLIP-Seq data (Hafner et al., 2010), they identified a perfect PUM2 site in the 3′UTR of *MFF* mRNA and showed that PUM2 negatively regulated *MFF* translation in C2C12 and HeLa cells (D'Amico et al., 2019). Consequently, *Pum2* silencing increased the percentage of C2C12 cells undergoing fission and mitophagy, and this was reversed by simultaneously performing *Mff* RNAi. Similarly, *puf8* depletion improved mitochondrial homeostasis during nematode aging and canceled by *mff1* co-depletion (D'Amico et al., 2019). Lastly, *Pum2* depletion using CRISPR-Cas9 in the muscle of old mice increased MFF levels and mitophagy, and improved respiration. Collectively, these data suggest that PUM2 regulates mitochondrial dynamics and mitophagy via MFF.

CONCLUSION

Over the years, several lines of evidence have implicated mitochondrial dysfunctions in many diseases, particularly those associated with neurodegenerative disorders and aging. Following recent findings that mutations or misexpression of RBPs can cause neurological impairments, there has been tremendous interest in identifying their molecular pathogenetic mechanisms. Interestingly, it turned out that mitochondria are also direct and early targets of RBP misregulation reiterating their importance for cellular homeostasis. These findings suggest that pharmaceutical agents improving mitochondrial life cycle can be attractive therapeutics for easing mitochondrial dysfunction in these diseases.

AUTHOR CONTRIBUTIONS

SR and ED wrote and edited the manuscript.

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REVIEW
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Mitophagy, Mitochondrial Homeostasis, and Cell Fate

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Mitochondria are highly plastic and dynamic organelles that have graded responses to the changing cellular, environmental, and developmental cues. Mitochondria undergo constant mitochondrial fission and fusion, mitochondrial biogenesis, and mitophagy, which coordinately control mitochondrial morphology, quantity, quality, turnover, and inheritance. Mitophagy is a cellular process that selectively removes the aged and damaged mitochondria via the specific sequestration and engulfment of mitochondria for subsequent lysosomal degradation. It plays a pivotal role in reinstating cellular homeostasis in normal physiology and conditions of stress. Damaged mitochondria may either instigate innate immunity through the overproduction of ROS or the release of mtDNA, or trigger cell death through the release of cytochrome c and other apoptogenic factors when mitochondria damage is beyond repair. Distinct molecular machineries and signaling pathways are found to regulate these mitochondrial dynamics and behaviors. It is less clear how mitochondrial behaviors are coordinated at molecular levels. BCL2 family proteins interact within family members to regulate mitochondrial outer membrane permeabilization and apoptosis. They were also described as global regulators of mitochondrial homeostasis and mitochondrial fate through their interaction with distinct partners including Drp1, mitofusins, PGAM5, and even LC3 that involved mitochondrial dynamics and behaviors. In this review, we summarize recent findings on molecular pathways governing mitophagy and its coordination with other mitochondrial behaviors, which together determine cellular fate.

Keywords: mitophagy, mitochondrial dynamics, mitochondrial apoptosis, cell fate, mitophagy receptors

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INTRODUCTION

Mitochondria are organelles that govern energy transformation and ATP production through the tricarboxylic acid cycle (TCA) and oxidative phosphorylation (OXPHOS). Moreover, mitochondria control redox homeostasis, Ca^{2+} signaling, iron metabolism, innate immunity, and apoptotic cell death (Zorov et al., 2014; Zong et al., 2016; Paul et al., 2017; Pathak and Trebak, 2018). Mitochondria are both the major source and the main targets of reactive oxygen species (ROS). Under homeostatic conditions, mitochondrial ROS serve as retrograde signaling molecules for cell growth (Diebold and Chandel, 2016). However, in conditions of stress or aging, mitochondrial ROS elicit oxidative damage to mitochondrial proteins, lipids, and DNA (mtDNA), causing the malfunction of

mitochondria. Dysfunctional mitochondria may produce even more ROS via vicious cycle that further amplify the release of ROS and mtDNA into the cytosol, which in turn can act as instigators of inflammation (Nakahira et al., 2011; Zhou et al., 2011). Non-reparable and severe damage of mitochondria leads to the release from the intermembrane space into the cytosol of cytochrome c and other pro-death factors (Sinha et al., 2013) altogether triggering apoptosis, a specific form of programmed cell death. This process is governed by the BCL2 protein family that integrates apoptotic signals and controls mitochondrial outer membrane permeabilization (MOMP).

Mitochondria are highly dynamic organelles that undergo continuous fission and fusion, constant turnover through mitochondrial biogenesis and mitophagy to maintain mitochondrial morphology, homeostasis, and inheritance. When facing bioenergetic or oxidative challenges, mitochondria exhibit a graded response that involves changes in their morphology and dynamics through the activation of distinct molecular machineries that regulate mitochondrial fission, fusion, mitophagy, and mitochondrial biogenesis. Mitochondrial fission and fusion are tightly regulated by a complex protein machinery involving dynamin 1 like (DNM1L better known as Drp1), mitosfusin 1 (MFN1), mitosfusin 2 (MFN2), and Optic atrophy protein 1 (OPA1) in mammalian cells. Mitochondrial fission was found to contribute to mitochondrial apoptosis and was also suggested to be a prerequisite for mitophagy, while mitochondrial fusion is linked to an increase in mitochondrial metabolism. How these molecular machineries sense cellular stresses and how these complex mitochondrial behaviors are coordinated at the molecular level remains elusive. It is important to address these questions, as mitochondrial dynamics and homeostasis are tightly linked with cellular physiology and eventually cell fate.

John Lemasters first termed selective mitochondrial autophagy as "mitophagy" (Lemasters, 2005). Mitophagy is a process that selectively sequesters damaged or depolarized mitochondria into double-membraned autophagosomes for subsequent lysosomal degradation. The removal of damaged or unwanted mitochondria, mitophagy was found to be essential for maintaining cellular fitness. Both ubiquitin- and receptor-mediated mitophagy pathways have been described and extensively studied. Intriguingly, BCL2 family proteins were reported to participate in both mitochondrial dynamics and mitophagic processes, which puts them in the center of mitochondrial homeostasis. We recently have shown that PGAM5, a mitochondrial phosphatase, serves as a molecular switch for determining mitochondrial fate (apoptosis or mitophagy) by dephosphorylating BCL-xL, a key apoptosis inhibitor and FUNDC1, a mitophagy receptor. These results demonstrated the integration of stress signals and the coordinated execution of graded responses in response to mitochondrial stress conditions (Ma K. et al., 2019). Here, we provide a focused overview on the molecular mechanisms of mitophagy and its interplay with mitochondrial dynamics and behaviors, thus contributing to aging and aging-related diseases.

MOLECULAR REGULATION OF MITOPHAGY

Mitophagy in Yeast

Electron microscopy has revealed that, in *Saccharomyces cerevisiae*, mitochondria can be specifically sequestered by autophagosomes, or be engulfed together with cytosolic material (Kissova et al., 2007). This process depends on the complete set of Atg-proteins such as Atg11, Atg17, and Atg29, as well as specific adaptor proteins (Farre et al., 2009). The mitochondrial outer membrane protein, Uth1, and mitochondrial protein phosphatase homolog, Aup1, have both been implicated in mitophagy (Kissova et al., 2007; Tal et al., 2007). Pioneering work from Ohsumi's and Klionsky's laboratories have identified that, Atg32, a mitochondria-anchored protein, is essential for mitophagy in yeast. It acts as a mitophagy-specific receptor and interacts with autophagy key proteins such as Atg8 via an Atg8 interacting motif (AIM) and Atg11 to recruit autophagosomes to mitochondria for their engulfment and final degradation (Kanki et al., 2009; Okamoto et al., 2009). Atg32 undergoes both transcriptional and post-translational regulation in response to mitophagy induction. Expression of *Pichia pastoris* Atg32 (PpAtg32, Atg32 homolog in *P. pastoris*) is highly suppressed in nutrient-rich media caused by the DNA-binding protein Ume6 and the histone deacetylase complex Sin3–Rpd3, which interact with the promoter region of the gene encoding PpAtg32 to repress its transcription (Aihara et al., 2014). Kang's group provided evidence that the kinase CK2 could phosphorylate N-terminal cytosolic region of Atg32 at serine 114 and serine 119 to promote the Atg32–Atg11 interaction and further accelerate the mitophagic process (Kanki et al., 2013), but how CK2-dependent phosphorylation takes place during starvation remains elusive. The C-terminal intermembrane space domain of Atg32 was found to be proteolytically processed by inner membrane i-AAA (ATPases associated with various cellular activities) protease Yme1 during mitophagy induction (Wang et al., 2013).

Mitophagy in Mammalian System

It has become clear that the regulation of mitophagy in mammalian cells appears to be more complex. Thus, both ubiquitin-mediated and receptor-mediated pathways have been described to facilitate mitophagy in response to cellular, developmental, and environmental cues in mammalian systems.

Ubiquitin Pathways

In mammalian cells, the PTEN-induced putative kinase protein 1 (PINK1) and Parkin-mediated ubiquitination pathway is one of the most-studied mitophagy mechanisms so far. Two key factors, the serine/threonine kinase PINK1 and the E3 ubiquitin ligase Parkin, cooperatively sense cellular stress and mediate the removal of damaged mitochondria. Under physiological conditions with normal mitochondrial membrane potential, PINK1 is continuously imported into mitochondria where it is cleaved by the intramembrane protease presenilin associated rhomboid like (PARL), leading to its retro-translocation into

the cytosol and rapid proteasomal degradation (Sekine and Youle, 2018). When mitochondrial membrane potential drops, PINK1 escapes from PARL-dependent cleavage and aggregates on the outer mitochondrial membrane to exert its pro-mitophagic function. Stabilized PINK1 phosphorylates both Parkin and ubiquitin (at Ser65) to promote ubiquitination of outer mitochondrial membrane proteins (Kane et al., 2014; Koyano et al., 2014). Phosphorylated ubiquitin binding to Parkin further unleashes Parkin from its autoinhibited state (Kazlauskaite et al., 2015). Activated Parkin appends ubiquitin moieties on specific mitochondrial outer membrane proteins such as MFN1, MFN2, FIS1, and translocase of outer mitochondrial membrane (TOMM) proteins, thus inducing their proteasomal degradation, which in turn promotes mitochondrial fission and mitophagy (Tanaka et al., 2010; Desai et al., 2018). The phosphatase and tensin homolog (PTEN)-long (PTEN-L) is able to dephosphorylate (Ser65 of) both ubiquitin and Parkin, which reduces the mitochondrial translocation of Parkin and negatively regulates mitophagy (Wang et al., 2018). On the other hand, the Parkin-mediated formation of ubiquitin chains on mitochondrial outer membrane proteins or even PINK1 itself can recruit ubiquitin-binding adaptor proteins such as optineurin (OPTN) and Calcium Binding And Coiled-Coil Domain 2 (CALCOCO2, better known as NDP52) onto mitochondrial surfaces, followed by the assembly of autophagy factors on Parkin and ubiquitin-marked mitochondria (Wong and Holzbaur, 2014; Lazarou et al., 2015). Ubiquitination of sperm mitochondria in both *Caenorhabditis elegans* and mammalian systems serves as "eat me" signal for their elimination by receptor-mediated mitochondrial degradation (Sutovsky et al., 1999; Molina et al., 2019). Both mitochondrial E3 ubiquitin protein ligase 1 (MUL1) and Parkin are necessary to remove paternal mitochondria from mouse embryos via mitophagy to ensure maternal mitochondrial inheritance (Rojansky et al., 2016).

Moreover, deubiquitinases play a crucial role in modulating the efficiency of PINK1 and Parkin-mediated mitophagy. Thus, ubiquitin-specific peptidase 8 (USP8) directly deubiquitinates Parkin and removes non-canonical Lys6-linked ubiquitin chains from Parkin, thereby promoting its translocation to depolarized mitochondria. In contrast to USP8 (Durcan et al., 2014), USP15 deubiquitinates the mitochondrial substrates of Parkin to inhibit mitophagy (Cornelissen et al., 2014). Recently, several deubiquitinases such as USP30, USP35, and USP33 were reported to antagonize Parkin-mediated ubiquitination and thus oppose Parkin-mediated mitophagy (Bingol et al., 2014; Wang et al., 2015; Niu et al., 2019). In addition, PINK1 and Parkin have been suggested to be required for mitochondria-derived vesicle (MDV)-dependent mitophagy such that vesicles budding from mitochondria under oxidative stress can be delivered to the lysosomes independent of LC3 (Soubannier et al., 2012; McLelland et al., 2014).

Mitophagy Receptor Pathway

Several mitophagy receptors have been identified in mammalian cells, significantly advancing the field of both mitochondrial and selective autophagy. Mitophagy receptors in mammalian cells are characterized by the presence of at least one LC3 interacting region (LIR) that can directly bind to the autophagy mediator LC3 to recruit autophagosomes to mitochondria.

BCL2 interacting protein 3 like (BNIP3L, better known as NIX) was identified as an essential mitophagy receptor for the autophagic clearance of mitochondria during the maturation of erythroid cells (Sandoval et al., 2008). Recently, the phosphorylation of the LIR domain of NIX was shown to further enhance the affinity of the interaction between NIX and LC3 (Rogov et al., 2017). Moreover, BCL2 interacting protein 3 (BNIP3), a homolog of NIX, was found to mediate mitophagy in conditions of hypoxia (Quinsay et al., 2010).

We have discovered that FUNDC1 acts as an important mitophagy receptor, whose function is regulated by its phosphorylation state (Liu et al., 2012). Structural analysis revealed the functional importance of the close proximity of Tyr18 of FUNDC1 with Asp19 of LC3. Consistently, phosphorylation of Tyr18 of FUNDC1 via SRC proto-oncogene, non-receptor tyrosine kinase (SRC) kinase significantly weakens its binding affinity for LC3 due to electrostatic repulsion *in vitro* (Kuang et al., 2016). The dephosphorylation (of Ser13) of FUNDC1 can promote mitophagy by recruiting Drp1 while dissociating it from OPA1, thus inducing mitochondrial fission (Chen et al., 2016).

Other mitophagy receptors have been reported such as BCL2 Like 13 (BCL2L13) (the functional homolog of ATG32 in mammals) (Otsu et al., 2015), FKBP prolyl isomerase 8 (FKBP8) (Bhujabal et al., 2017), NLR family member X1 (NLRX1) (Zhang Y. et al., 2019), autophagy and Beclin 1 regulator 1 AMBRA1 (Strappazzon et al., 2015), as well as the mitochondria inner membrane protein prohibitin 2 (PHB2) (Wei et al., 2017). All of them were found to interact with LC3 via the conserved LIR motif to mediate mitophagy when mitochondria become damaged. However, the molecular regulation and their cooperation in response to mitochondrial stresses are not completely understood. Moreover, mitophagy receptors are not limited to proteins, as certain types of lipids such as cardiolipin and ceramide have been reported to interact with LC3 and to mediate mitophagy (Sentelle et al., 2012; Chu et al., 2013).

The Interplay Between Mitochondrial Dynamics and Mitophagy

Distinct molecular machineries have been identified to regulate mitochondrial fission and fusion. In mammalian cells, the GTPase MFN1, MFN2, and OPA1 mediate the fusion of the outer and inner membranes of mitochondria, respectively. Mitochondrial fission is regulated by Drp1 that normally resides in the cytosol and is recruited to mitochondria by mitochondrial fission factors such as FIS1, MFF, MIEF1, or MIEF2 (Mishra and Chan, 2014). ER tubules, which are in contact with mitochondria, play an active role in the initial step of mitochondrial division and mediate mitochondrial constriction before Drp1 recruitment (Friedman et al., 2011). At the final step of mitochondrial division, the Drp1-mediated constriction promotes dynamin-2 (DNM2) assembly, which can induce membrane fission to complete division (Lee et al., 2016). In response to bioenergetic crisis and oxidative stress, these

mediators of mitochondrial dynamics are posttranslationally modified to fine-tune their activities. Phosphorylation of Drp1 by protein kinase A (PKA, also known as cAMP-dependent protein kinase) at Ser637 (Chang and Blackstone, 2007) and Ser656 (Cribbs and Strack, 2007) inhibits Drp1, resulting in mitochondrial elongation, while dephosphorylation of Drp1 at Ser65 by the calcium-dependent protein phosphatase calcineurin or by protein phosphatase 2A (PP2A) enhances mitochondrial fragmentation (Cribbs and Strack, 2007). Another report suggested that Drp1 is phosphorylated at Ser616 by the cyclin-dependent kinase 1 (CDK1)/cyclin B complex during mitosis (Taguchi et al., 2007; Marsboom et al., 2012). The phosphorylation of the Drp1 receptor MFF by energy-sensing adenosine monophosphate (AMP)-activated protein kinase (AMPK) results in the recruitment of Drp1 and final mitochondrial fragmentation (Toyama et al., 2016). Other Drp1 modifications include *S*-nitrosylation (Cho et al., 2009) and ubiquitination by MARCH5 to mediate mitochondrial division (Karbowski et al., 2007) or by Parkin to inhibit mitochondrial fission (Wang et al., 2011).

The mitochondrial fusion molecule MFN1 can be phosphorylated by extracellular regulated kinase (ERK) at Thr562 to inhibit fusion (Pyakurel et al., 2015), ubiquitinated by MARCH5 (Park et al., 2014), and deubiquitinated by USP30 (Yue et al., 2014) to regulate protein stability and fusion activity, while MFN2 can be phosphorylated by mitogen-activated protein kinase 8 (MAPK8 better known as JNK) at Ser27 and ubiquitinated for degradation by HUWE1 (Leboucher et al., 2012), Parkin (Gegg et al., 2010), and MARCH5 (Sugiura et al., 2013), and deubiquitinated by USP30 (Yue et al., 2014). During mitophagy, MFN2 also functions as a mitochondrial receptor for the PINK1-dependent recruitment of Parkin. PINK1 phosphorylates MFN2 at Thr111 and Ser442 to promote the recruitment of Parkin to depolarized mitochondria (Chen and Dorn, 2013). OPA1 can be proteolytically processed by mitochondria-resident proteases, including YME1-like 1 ATPase (YME1L) (Griparic et al., 2007) and zinc metallopeptidase (OMA1) (Head et al., 2009), in response to intra-mitochondrial signals, to regulate fusion of the inner mitochondrial membrane.

It was suggested that mitochondrial fission is necessary for mitochondrial degradation by mitophagy because fission enables the separation of depolarized mitochondria from the mitochondrial network and allows their engulfment by autophagosomes. Mitochondrial stress-induced mitophagy is accompanied by enhanced mitochondrial fission. The inhibition of mitochondrial fission processes by overexpression of dominant negative $Drp1^{K38A}$ or knockdown of FIS1 decreases mitophagy and leads to the accumulation of oxidized mitochondrial proteins (Twig et al., 2008). In agreement with this, mitophagic players were found to regulate mitochondrial dynamics. Thus, Parkin is able to ubiquitinate MFN1 and MFN2 to promote their degradation, leading to increased fragmentation of mitochondria (Gegg et al., 2010). Our early work showed that Parkin also ubiquitinates and degrades Drp1 (Wang et al., 2011). This may be counterintuitive, as degradation of Drp1 prevents mitochondrial fragmentation. It is possible that under homeostatic conditions, Parkin monitors the molecular status of Drp1 to prevent mitochondrial fragmentation, and upon stress conditions, Parkin translocates to mitochondria to promote mitochondrial fragmentation and mitophagy.

Mitophagy receptors such as FUNDC1 and BNIP3 were found to promote mitochondrial fission in response to stress (Landes et al., 2010; Chen et al., 2016). FUNDC1 directly interacts and recruits Drp1 toward mitochondria for mitochondrial fission. Interestingly, FUNDC1 is a transmembrane protein with a motif that faces the mitochondrial intermembrane space and directly interacts with OPA1 to promote mitochondrial fission.

It was noted that mitochondrial fission is necessary, but not sufficient for mitophagy. Reports suggested that Drp1-mediated mitochondrial fission was dispensable for mitophagy (Song et al., 2015; Yamashita et al., 2016). We have found that mitochondrial targeting of the LIR-containing cytosolic portion of FUNDC1 is sufficient to induce mitophagy even in the absence of mitochondrial fragmentation, when phosphorylation of Tyr18 is blocked (Kuang et al., 2016). Recently, by using structure illumination microscopy (SR-SIM), Xian et al. (2019) observed that the overexpression of the SNARE protein syntaxin 17 (STX17) initiated mitophagy in FIS1-depleted cells but not in other mitochondria dynamic factors-silenced cells. They further demonstrated that FIS1 negatively regulated STX17 by inhibiting its trafficking to mitochondria-associated membranes (MAMs) and mitochondria independent of mitochondrial dynamics (Xian et al., 2019). In summary, a sensitive reaction to various types of stress mitochondrial fragmentation at early stages facilitates segregation and clearance of dysfunctional mitochondria from the mitochondrial network for maintaining mitochondrial and cellular homeostasis.

Mitochondrial Dynamics and Cell Death

Mitochondria in mammalian cells sense apoptotic stress, mainly through BCL2 and its family proteins, ultimately leading to MOMP and the subsequent release of cytochrome c and other apoptogenic factors for the activation of the caspase cascade governing apoptotic cellular disintegration. The BCL2 protein family is composed of antiapoptotic molecules including BCL2, BCL-xL, MCL1, and proapoptotic molecules such as BCL2 associated X (BAX), BCL2 antagonist/killer 1 (BAK), and BH-3-only subfamily proteins such as such as BIM, BAD, NOXA, and BID (Doerflinger et al., 2015). In healthy cells, BAX and BAK1 are blocked by antiapoptotic proteins such as BCL2, BCL-xL, and MCL1, which contain four BH motifs (BH1–4). The BH3-only proteins can induce apoptosis by direct interaction with BAX and BAK or by binding to antiapoptotic members and thus neutralizing the inhibitory sequestration of BAX and BAK (Chen et al., 2005; Chipuk et al., 2010). The antiapoptotic protein BCL-xL interacts with BAX to continuously retro-translocate mitochondrial BAX into the cytosol and keep it from integrating into the mitochondrial outer membrane (Edlich et al., 2011). In apoptotic cells, BAX and BAK oligomerization triggers MOMP and initiates the caspase cascade ultimately leading to cell death (Tait and Green, 2010).

Emerging evidence indicates that the mechanisms governing mitochondrial dynamics are also involved in the regulation of apoptotic processes. Inhibition of mitochondrial fission reduces

cytochrome c release and apoptosis (Frank et al., 2001; Cereghetti et al., 2010). On the contrary, the dephosphorylation of Drp1 at Ser637 by the phosphatase calcineurin promotes Drp1-mediated mitochondrial fragmentation and leads to apoptosis (Cereghetti et al., 2010). Drp1-dependent mitochondrial fission through MIEF2 facilitates apoptotic cristae remodeling during the early phase of intrinsic apoptosis (Otera et al., 2016), and moreover, Drp1 can stimulate truncated Bid (tBID)-induced Bax oligomerization and cytochrome c release by promoting tethering and hemifusion of membranes. Dephosphorylation of Drp1 by the mitochondrial phosphatase PGAM5 can facilitate necroptosis by enhancing mitochondrial fission (Wang et al., 2012). However, other reports have shown that blocking mitochondrial fission can just delay but does not block apoptosis (Parone et al., 2006; Rolland and Conradt, 2010; Clerc et al., 2014).

On the other hand, BCL2 family members can affect the morphology of mitochondria. In healthy cells, BAX and BAK are required for mitochondrial fusion (Karbowski et al., 2006). Mitochondria are fragmented and have less network continuity in cells lacking BAX and BAK. The interaction between BAX and MFN2 activates the assembly of the MFN2 complex and changes its membrane mobility and distribution. However, the activation of pro-apoptotic BAX and BAK promotes the fragmentation of the mitochondrial network during apoptosis (Autret and Martin, 2009; Montessuit et al., 2010), which is not inhibited by the expression of BCL-xL, MCL1, or other members of the BCL2 subfamily (Sheridan et al., 2008). BAX and BAK form foci that colocalize with ectopic MFN2 and Drp1 at the sites of mitochondrial division to promote mitochondrial fission during apoptosis (Karbowski et al., 2002). Furthermore, BCL-w, an antiapoptotic BCL2 family member, was proposed to regulate mitochondrial fission in Purkinje cell dendrites (Liu and Shio, 2008). BCL-xL overexpression induces the remodeling of the mitochondrial network by altering the relative rates of mitochondrial fusion and fission (Delivani et al., 2006; Li et al., 2008). In neuronal cells, overexpression Bcl-xL can increase the rates of both fission and fusion and mitochondrial biomass (Berman et al., 2009). Other studies found that, in hippocampal neurons, BCL-xL increases synapse dynamics and the localization of mitochondria to synapses and vesicle clusters via a Drp1-dependent manner (Li et al., 2013). Alternatively BCL-xL can interact with Drp1 to function in mitochondrial fission during neuronal development (Li et al., 2008). Although the mechanisms of action require further clarification, these findings demonstrate that the BCL2 protein family indeed orchestrates mitochondrial morphology and apoptosis.

Mitophagy and Cell Death

The BCL2 family was initially recognized for their function in apoptosis, and is now widely proven to also have other roles in cellular function involving mitochondrial dynamics, autophagy/mitophagy, and cellular metabolism. Early studies have shown that the antiapoptotic protein BCL2 can interact with Beclin 1 (BECN1) to inhibit autophagy (Pattingre et al., 2005). Further analysis reveals that depending on its phosphorylation status, BCL2 has dual roles in regulating autophagy and apoptosis. It suggests that initial JNK1-mediated BCL2 phosphorylation may promote cellular survival by disrupting BCL2–BECN1 complexes and activating autophagy (Wei et al., 2008a). At a point when autophagy is no longer able to maintain survival, the phosphorylation of BCL2 serves to inactivate its antiapoptotic function for progression of regulated cell death (Wei et al., 2008b). Parkin-dependent mitophagy is antagonized by BCL-xL and MCL1 in a BECN1-independent manner. Specifically, BCL2 and BCL-xL suppress Parkin translocation to depolarized mitochondria, while BH3-only proteins (or BH3-only mimetics) can promote this process (Hollville et al., 2014).

Several mitophagy receptors including BNIP3, NIX, and BCL2L13 belong to the BCL2 family (Novak et al., 2010; Hanna et al., 2012; Murakawa et al., 2015), highlighting an intrinsic link of mitophagy with apoptosis. Apparently, these BCL2 family proteins have dual roles in both apoptosis and mitophagy. For example, BNIP3 and NIX can directly interact with antiapoptotic BCL2 or BCL-xL, which antagonizes the activation of proapoptotic BAX and BAK, to promote apoptosis (Imazu et al., 1999; Dorn, 2010). As discussed above, NIX also induces mitophagy via its interaction with LC-3, and enhanced interaction of BNIP3 with Atg8 family members promotes pro-survival mitophagy prior to cytochrome c release and apoptosis (Zhu et al., 2013). It was also found that the mitochondrial fragmentation is a prerequisite for BNIP3-induced mitophagy in cardiac myocytes, and dominant negative $Drp1^{K38E}$ mutant, or MFN1 overexpression inhibit BNIP3-induced mitochondrial division and mitophagy (Lee et al., 2011). Similar to NIX, BNIP3 induces the disintegration of elongated mitochondria into numerous spherical particles, accompanied by the recruitment of Drp1 to fragmented mitochondria in adult myocytes (Lee et al., 2011). Moreover, BNIP3 can directly interact with OPA1, promote the disassembly of OPA1 oligomers, and thus antagonize its fusion activity in HeLa cells (Landes et al., 2010). Thus, BCL2 family proteins act as general regulators of mitochondrial dynamics and homeostasis, in addition to their role in apoptosis-associated mitochondrial permeabilization.

Mitophagy was suggested to play a protective role in stress-induced cell death and early studies showed that Parkin strongly inhibits the translocation of BAX to mitochondria, thus preventing apoptosis (Darios et al., 2003; Johnson et al., 2012). Further studies revealed that Parkin is able to directly ubiquitinate the apoptotic effector proteins such as BAX and BAK, and the ubiquitination of BAK by Parkin impairs its activation and the formation of oligomers to suppress errant apoptosis (Bernardini et al., 2019). Parkin suppression of BAX-dependent apoptosis will allow the effective clearance of apoptotic mitochondria to limit their potential pro-inflammatory effect (Bernardini et al., 2019). Parkin suppression of apoptosis is likely the cellular context and apoptosis inducer dependent. Studies from Seamus Martin's laboratory showed that upon mitochondrial depolarization, the BCL2 family member MCL1 underwent rapid PINK1- and Parkin-dependent polyubiquitination and degradation, which sensitized cells toward apoptosis via opening of the BAX and BAK-dependent pathway. Knockdown of BAX is able to suppress Parkin-dependent apoptosis in HeLa cells

(Carroll et al., 2014). It was also reported that NIX-mediated mitophagy protects glioblastoma cells against hypoxia (Jung et al., 2019). Furthermore, abrogating NIX- and FUNDC1-mediated mitophagy during adult cardiac progenitor cells (CPCs), differentiation leads to increased susceptibility to cell death (Lampert et al., 2019).

In addition, there is certain evidence showing that mitophagy plays an accelerative role in programmed cell death. Thus, LC3–ceramide interactions provoked by ceramide treatment induce mitophagy and can progress to autophagic cell death in human cancer cells (Sentelle et al., 2012). Moreover, inhibition of mitophagy and mitochondrial fission reduces cigarette smoke-induced necroptosis in mice epithelial cells *in vitro*, and in chronic obstructive pulmonary disease (COPD) *in vivo* (Mizumura et al., 2014). Furthermore, in hippocampal neural stem cells deprived of insulin, Parkin-mediated mitophagy is necessary for autophagy-dependent cell death (Park et al., 2019). Moreover, drug-induced mitochondrial dysfunction and heme oxygenase 1 (HMOX1) overactivation synergize to trigger lethal mitophagy in glioma cells, which is significantly blocked by silencing of the mitophagy receptors BNIP3 and NIX (Meyer et al., 2018).

We have found that BCL-xL, but not BCL2, strongly suppresses FUNDC1-mediated mitophagy. BCL-xL interacts with and inhibits the mitochondrial Ser/Thr phosphatase PGAM5 to prevent the dephosphorylation of FUNDC1 (at Ser13), thus further blocking hypoxia-induced mitophagy (Wu et al., 2014). The functions of PGAM5 not only are limited to the induction of mitophagy but also involve the regulation of mitochondrial homeostasis (**Figure 1**). PGAM5 exists in an equilibrium between a dimeric and a multimeric state, which is sensitive to oxidative stress. Dimeric PGAM5 binds with and dephosphorylates BCL-xL in mitotically arrested cells, thus exerting its antiapoptotic function *in vitro* and *in vivo*. Mitochondrial oxidative stress enhances the multimerization of PGAM5, resulting in its dissociation from BCL-xL. Liberated multimeric PGAM5 dephosphorylates FUNDC1 to initiate mitochondrial fission and mitophagy. When FUNDC1-mediated mitophagy is blocked by the microtubule inhibitor vinblastine, PGAM5 dephosphorylates FUNDC1 and mediates mitochondrial fission that aggravates vinblastine-induced cell death (Ma K. et al., 2019).

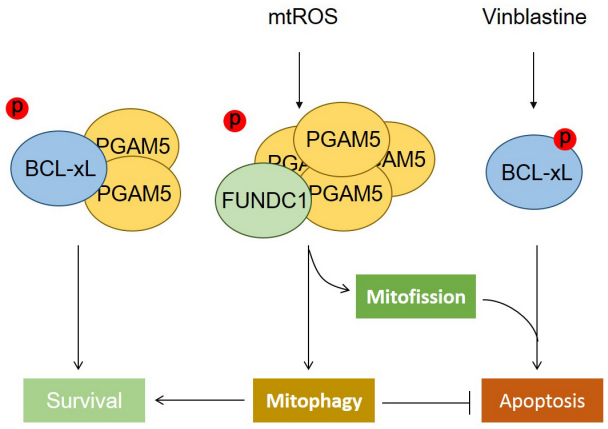

FIGURE 1 | The phosphatase PGAM5 regulates mitochondrial fate. The phosphatase PGAM5 is a dimeric protein that can bind with and dephosphorylate BCL-xL at Ser62, which increases its antiapoptotic function and thus inhibits apoptotic cell death. Mitochondrial oxidative stress causes the transformation of dimeric PGAM5 into a multimeric state that fails to bind with BCL-xL, but instead interacts with and dephosphorylates FUNDC1 at Ser13 to mediate mitochondrial fission and mitophagy. The dephosphorylation of FUNDC1 cooperates with the phosphorylation of BCL-xL, aggravating cell death when mitophagy is blocked.

Mitophagy Is Balanced With Mitochondrial Biogenesis for Mitochondria Homeostasis

Mitophagy is balanced with mitochondrial biogenesis, together defining mitochondrial turnover. Mitochondrial biogenesis is a cellular process in which "new" mitochondria are produced, depending on the cooperation of nuclear and mitochondrial genome (Zhang and Xu, 2016). Mitochondrial biogenesis preserves mitochondrial function and cellular homeostasis (Rasbach and Schnellmann, 2007; Miwa et al., 2008; Gottlieb and Carreira, 2010), while mitophagy mitigates the source of oxidative stress that reduces the risk of apoptosis (Hickson-Bick et al., 2008). The crosstalk between mitophagy and mitochondrial biogenesis also allows cells to undergo metabolic reprogramming during development and differentiation. Similar to mitophagy, mitochondrial biogenesis is highly variable and tightly regulated in response to diverse stimuli such as energy demand, cell cycle, and intracellular stress (Zhang and Xu, 2016). Many cellular signaling pathways converge on the regulation of both mitophagy and mitochondrial biogenesis such as the mammalian target of rapamycin (mTOR), which regulates cellular growth and energy homeostasis in conditions of nutrient stress (Dibble and Cantley, 2015; Vyas et al., 2016). The mTOR signal pathway can transcriptionally and translationally regulate mitochondrial biogenesis. Thus, mTOR controls mitochondrial function through the modulation of PPARG coactivator 1 alpha (PGC1α) transcriptional activity (Cunningham et al., 2007) and ablation of PPARGC1B (PGC1β) is associated with the constitutive activation of mTORC1 (Camacho et al., 2012). At the transcriptional level, it can mediate the activation of PGC1α, which is a key transcriptional co-activator regulating mitochondrial biogenesis via its interaction with a variety of transcription factors (Morita et al., 2015). Similar to mTOR, the hypoxia-inducible factor 1 subunit alpha (HIF1A) signaling in response to limited oxygen availability can impinge on mitochondrial biogenesis, mitophagy, and mitochondrial metabolism through regulation of PGC1α (LaGory et al., 2015).

mTORC1 phosphorylates UNC-51 like autophagy activating kinase 1 (ULK1) and 2 (ULK2) and disrupts the interaction between ULK1 and protein kinase AMP (AMPK), leading to the inhibition of autophagy and mitophagy when nutrient levels are sufficient. Under nutrient starvation, ATP depletion leads to serine/threonine kinase 11 (LKB1)-mediated AMPK activation (Garcia and Shaw, 2017), which in turn leads to the

phosphorylation of ULK1 to initiate autophagy and mitophagy (Kim et al., 2011; Tian et al., 2015). Tuberous sclerosis complex (TSC1/2) is an inhibitor of the mTOR signaling pathway. In TSC1/2-deficient neurons, axonal and global mitophagy is impaired and mitochondrial homeostasis can be restored by blocking mTORC1 (Ebrahimi-Fakhari et al., 2016). Moreover, TSC2-deficient cells exhibit constitutive mTOR activation, impaired autophagic flux, and accumulation of damaged mitochondria, which associates with reduced PINK1 expression and Parkin mitochondrial translocation to mitochondria. These data link mTOR signaling to PINK1-Parkin-mediated mitophagy (Bartolome et al., 2017).

Hypoxia-induced mtROS can activate HIFs leading to the upregulation of target genes, including the mitophagy receptor BNIP3 (Bell et al., 2007; Chourasia and Macleod, 2015). As a feedback mechanism, BNIP3-mediated mitophagy reduces the generation of mtROS, which in turn can stabilize HIF1A (Chourasia and Macleod, 2015). Nevertheless, ROS can also activate JNK–PGC1α signaling pathway to promote mitochondrial biogenesis and the expression of genes involved in OXPHOS (Chae et al., 2013). Increased mtROS was also found to promote cellular proliferation by activating NF-κB (Guha et al., 2010). Recently, we found that a hypoxic microenvironment can induce siah E3 ubiquitin protein ligase 2 (SIAH2)-dependent ubiquitination and subsequent proteasomal degradation of nuclear respiratory factor 1 (NRF1), a transcription factor crucial for mitochondrial biogenesis. In conditions of cancer, this signaling axis alters the level of mitochondrial biogenesis and is involved in metabolic adaptations finally maintaining tumor progression (Ma B. et al., 2019).

MITOCHONDRIAL QUALITY CONTROL IN IMMUNE RESPONSES

The innate and adaptive immune systems are able to sense pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) arising from exogenous clues including bacteria, virus, fungi, and parasites, as well as endogenous entities such as cancer cells and to mount defensive immune responses. Mitochondria have emerged as central organelles contributing to immune response at multiple levels, such as adaptations of mitochondrial metabolism, dynamics, biogenesis, and mitophagic turnover. Mitochondrial DNA (mtDNA), once released into the cytoplasm, acts as intrinsic DAMP, which can be sensed by toll-like receptor 9 (TLR9) and triggers nuclear factor kappa B (NF-κB) signaling in human polymorphonuclear neutrophils (Zhang et al., 2010). Moreover, mtDNA activates the NLRP3 inflammasome, which in turn boosts the production of cytokines such as IL18 and IL1β induces pyroptosis in immune cells (Liu et al., 2018). Furthermore, cellular mtDNA activates the STING pathway via cGAS and leads to the expression of IRF3-dependent genes such as type I interferons and participates to antiviral immune responses (West et al., 2015). Interestingly, live-cell lattice light-sheet microscopy observed mouse embryonic fibroblasts result has shown that BAK/BAX form macropores after activation and allowed mitochondrial matrix components, including the mtDNA releasing into the cytosol (McArthur et al., 2018). In addition, evidence has shown that mtROS enhances the NLRP3 inflammasome activation and upregulates NF-κB signaling. Mitophagy counteracts chronic inflammation via the elimination of damaged mitochondria, which are the major sources of mtDNA and ROS.

Parkin-mediated mitophagy restrains excess ROS and cytosolic mtDNA, and inhibits NLRP3 inflammasome activity in macrophages and favors tissue repair via a NF-κB and sequestosome 1 (SQSTM1, better known as p62)-dependent mitophagic pathway (Zhong et al., 2016). Intriguingly, Parkin is cleaved by caspase-1 to limit mitophagy and resultant excess inflammation (Yu et al., 2014). Defective mitophagy leads to the upregulation mRNA levels of inflammasome-related proteins in primary hepatocytes under palmitic acid treatment and in a murine model of NASH (Zhang N. P. et al., 2019). Defects in mitophagy arising from deletion, mutation, or silencing of mitophagy receptors, such as NIX, Parkin, and p62, lead to mitochondrial dysfunctions and have been linked to inflammasome activation and cancer (Drake et al., 2017). Likewise, ablation of FUNDC1 causes the inhibition of mitophagy and increases the accumulation of dysfunctional mitochondria, which in turn results in inflammasome activation and inflammatory responses that can promote hepatocyte tumorigenesis *in vivo* (Li et al., 2019). *Listeria monocytogenes* can induce mitophagy in macrophages to evade host immune response. Mechanistically, Nod-like receptor (NLR) family member X1 (NLRX1), a novel mitophagy receptor located at the mitochondria, directly interacts with LC3 via its LIR motif, thus contributing to the induction of mitophagy for the elimination of ROS, and maintains the survival of *L. monocytogenes* (Zhang Y. et al., 2019). Furthermore, interleukin 10 (IL-10), an anti-inflammatory cytokine that promotes mitophagy, leads to a decrease in the activation of the NLRP3 inflammasome and the production of IL-1β in macrophages (Ip et al., 2017).

The mitochondrial outer membrane is a platform for MAVS-mediated innate immune responses, which are activated by the viral RNA sensors RIG-I-mediated signaling cascade culminating in the activation by NF-κB and IRF3 (Seth et al., 2005). Alternatively, MAVS can oligomerize upon sensing mtROS independent of RIG-I to facilitate the production of type I interferon (Buskiewicz et al., 2016). Intriguingly, MAVS has been found to contain a LIR motif and act as a potential receptor for mitophagy (Sun et al., 2016). Furthermore, the ubiquitination of MAVS by ring finger protein 34 (RNF34) causes NDP52-associated mitophagy to mitigate innate immune response upon viral infection (He et al., 2019).

BCL2 family regulated mitochondria-dependent cell death has also been reported to play an important role in innate and adaptive immune responses. One of the therapeutic strategies to Legionnaires' disease is the pharmacological inhibition of BCL-xL. Inhibition of BCL-xL can induce the apoptosis of macrophages infected with virulent Legionella and thus abrogate Legionella replication and disease progression in mice (Speir et al., 2016). Additionally, it is well known that activated T cells will undergo cell death once the antigen has disappeared. This

mechanism is triggered by the BCL-xL- and BCL2-mediated release of pro-apoptotic BAX and BAK or the fact that BCL-xL without its unstructured loop, which cannot bind to any form of BAX and BAK, binds BIM less well than wild-type BCL-xL and thus sensitizes T cells to the induction of regulated cell death (Liu et al., 2006). Overall, more and more evidence demonstrates that mitochondrial quality control mechanisms regulate essential functions in immune cell and are important in controlling immune responses. Mitophagy plays a protective role in cellular homeostasis to negatively regulate innate immune response, but a systematic drawing of this intricate relationship has not been completed yet due to its complexity.

DYSREGULATION OF MITOCHONDRIAL HOMEOSTASIS IN AGING AND AGING-RELATED DISEASES

Aging increases the risk for the onset of various chronic diseases often associated with the accumulation of mtDNA mutations, altered mitochondrial mass, compromised mitochondrial functions, chronic immune activation, and accelerated cell death. These pathogenic manifestations are likely due to dysregulated mitochondrial dynamics and mitochondrial quality control mechanisms, leading to the accumulation of dysfunctional mitochondria, which enhances both chronic immune activation (through the release of mtDNA and ROS) and mitochondrial apoptosis (through the liberation of apoptogenic factors). There is emerging evidence that compromised mitophagy causes aging, while enhancing mitophagy by caloric restriction and physical exercises increases healthy life span. Thus, the reduction of mitophagy has been suggested as areas on for the accumulation of mitochondria in aged *C. elegans* (Palikaras et al., 2015), and the induction of mitophagy results in life span extension in this model (Ryu et al., 2016). Overexpression of the *Drosophila* PGC-1 homolog (dPGC-1/spargel) increases mitochondrial activity, and intestinal stem cell (ISC) lineage-specific expression of dPGC-1 leads to an extended life span in *Drosophila melanogaster* (Rera et al., 2011). Physical exercise caused the activation of AMPK that leads to ULK1 phosphorylation and enhanced mitophagy in skeletal muscle, which in turn promotes mitochondrial turnover and improves general health in murine models (Laker et al., 2017).

Mitochondrial dysfunction is a common pathogenic factor for neurodegenerative disorders. Mitochondria supply ATP, generate mtROS, and regulate calcium homeostasis, all of which affect neuronal cell physiology. Aberrations in mitochondrial ROS and Ca^{2+} homeostasis have been implicated in Parkinson's disease (PD) (Ludtmann and Abramov, 2018). Both elevated cytosolic Ca^{2+} levels and mitochondrial ROS are pathological hallmarks of PD. Thus, PINK-1 deficiency in midbrain neurons leads to mitochondrial Ca^{2+} overload in response to dopamine, which further promotes ROS production and neuronal cell death (Gandhi et al., 2009). The loss of mitochondrial fission factor (MFF) increases mitochondrial size and mitochondrial Ca^{2+} uptake during neurotransmission, thus affecting neurotransmitter release and neuronal fitness (Lewis et al., 2018). Production of mtROS caused by damaged mitochondria in mice microglia promotes the secretion of pro-inflammatory cytokines and results in neurodegeneration (von Bernhardi et al., 2015). Furthermore, the accumulation of Ca^{2+} and ROS in the mitochondria triggers mitochondrial permeability transition pore (mPTP) opening, subsequently releasing cytochrome c and other pro-apoptotic intermembrane space proteins into the cytosol (Hunter and Haworth, 1979). It needs to be noted that BCL2 family proteins are involved in the regulation of Ca^{2+} dynamics of the ER and the mitochondria (Vervliet et al., 2016). For instance, a fraction of NIX is localized at the conjunction between mitochondria and the ER to regulate ER and mitochondrial Ca^{2+} homeostasis (Diwan et al., 2009). In addition, the upregulation of NIX in cardiac hypertrophy was associated with the apoptotic death of cardiomyocytes (Yussman et al., 2002). Altogether, mitochondrial quality control appears crucial for protecting neurons from damage and death.

It has been well established that compromised mitophagy contributes to the pathogenesis of Parkinson's disease, and enlarged or swollen mitochondria have been observed in several disease models and in the brains of Parkinsonian patients. Mutations in PINK1 and Parkin are involved in rare familial cases of Parkinson's disease (PD) (Kitada et al., 1998; Valente et al., 2004). PGAM5 deficiency disables PINK1-mediated mitophagy *in vitro* and causes a Parkinson's-like phenotypes in mice model (Lu et al., 2014; Sekine et al., 2016). It is still puzzling that *Parkin* knockout mouse does not completely recapitulate PD phenotype. When Parkin knockout mouse was crossed with mouse that harbors high mtDNA mutation, the accumulation of

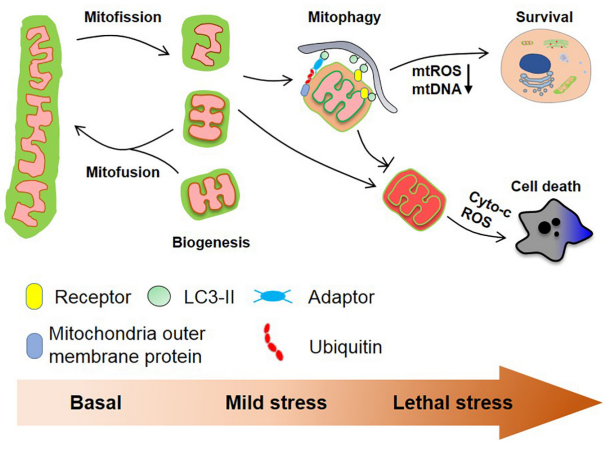

FIGURE 2 | Mitochondrial homeostasis and cell fate. Mitochondria are dynamic organelles that constantly divide and fuse in healthy cells and mitochondrial biogenesis and mitophagy cooperate to maintain mitochondrial quality. Mitochondrial fission usually is a prerequisite for ubiquitin- and receptor-mediated mitophagy facilitating the removal of aged and damaged mitochondria. Severe stress-induced mitochondrial damage can lead to mitochondria outer membrane permeabilization (MOMP) that in turn triggers apoptosis. The clearance of depolarized mitochondria by mitophagy (before MOMP occurs) mitigates this process. Besides, mitophagy reduces the production of mtROS and the cytosolic secretion of mtDNA, which further prevents excess immune response.

mutated mtDNA in neuronal cells was observed, but these mice does not have much increase of mitochondrial mass (Gautier et al., 2008; Stevens et al., 2015; Pinto et al., 2018). Studies using the recently developed mitophagy reporter mice and Drosophila also show that mitophagy is rather constitutive and is minimally impacted by loss of PINK1 or Parkin (McWilliams et al., 2016; Whitworth and Pallanck, 2017; Lee et al., 2018; McWilliams et al., 2018), suggesting that additional factors are required in the absence of PINK1 or Parkin. Mitophagy has been implicated with disease progression in Alzheimer's disease (Witte et al., 2009; Wilhelmus et al., 2011). Thus, Alzheimer's disease phenotype-related accumulation of mutant amyloid beta precursor protein (APP) induces Parkin-dependent mitophagy in cultured human neurons and in the brain of Alzheimer's patients (Ye et al., 2015). Recent studies showed that Tau pathology, another hallmark of Alzheimer's disease, impairs mitophagy by inhibiting Parkin translocation to mitochondria (Cummins et al., 2019). These studies indicate that insufficient mitophagy might be the cause for the accumulation of damaged mitochondria in Alzheimer's disease-affected neurons. Conversely, there are arguments that mitophagy improves the neuropathology of Alzheimer's disease and reverses cognitive deficits in animal models (Kerr et al., 2017).

Moreover, recent studies have suggested that the loss of mitophagy regulators is closely linked to cardiovascular disease. Thus, Parkin-mediated mitophagy is required for the metabolic transition in the perinatal murine heart (Gong et al., 2015). Deletion of PINK1, Parkin, or other mitophagy receptors such as FUNDC1, BNIP3, or NIX leads to the accumulation of dysfunctional mitochondria and results in various heart defects involved in exacerbated ischemia/reperfusion injury and cardiomyopathy (Dorn, 2010; Kubli et al., 2013; Zhang et al., 2016). Consistently, impaired PINK1 and Parkin-mediated mitophagy affected by Parkin deficiency or mutations in MFN2 results in the retention of fetal cardiac mitochondria, reduced oxidative metabolism, heart failure, and premature death (Chen and Dorn, 2013; Gong et al., 2015), highlighting that mitophagy underlies mitochondrial plasticity and metabolic transitioning in developing cardiomyocytes.

SUMMARY AND FUTURE PERSPECTIVES

Mitochondria are highly plastic organelles that adapt to cellular and environmental stress and developmental cues by changes in their morphology and their overall mass. Changes in the mitochondrial behaviors are regulated by distinct but interlinked molecular machineries that control mitochondrial dynamics (fission, fusion) and mitochondrial homeostasis (mainly through biogenesis and mitophagy), collectively allowing the graded response to stress (**Figure 2**). BCL2 family proteins, and in particular BCL-xL, act as global regulators of mitochondrial homeostasis and quality control through their interaction with various partners including DRP1, MFN1/2, and PGAM5, that are tightly controlled by reversible phosphorylation, acetylation, and ubiquitination, thereby modulating mitochondrial behaviors and cell fate. This is further exemplified by our recent finding that the mitochondrial phosphatase, PGAM5 exists in an equilibrium between a dimeric and a multimeric state to dephosphorylate FUNDC1 and BCL-xL, respectively, to switch on/off mitophagy and apoptosis. Further research is needed to explore the (patho-) physiological roles of this molecular switch in response to environmental and cellular stresses.

By removing the damaged and unwanted mitochondria, mitophagy is essential for mitochondrial quality control and homeostasis. As discussed above, almost all mitophagy players including both receptor-dependent pathway and PINK1/Parkin pathway are found to regulate mitochondrial dynamics and apoptosis. Furthermore, mitophagy not only governs the mitochondrial quality and quantity, but also controls mitochondrial dynamics and behaviors. Enhanced mitophagy and mitochondrial turnover contributes to increased mitochondrial function and cellular activity. Conversely, the inhibition of mitophagy leads to accelerated aging and the manifestation of aging-associated diseases. Moreover, the accumulation of dysfunctional mitochondria, and the associated release of mtDNA, the overproduction of mtROS, and mitochondria-controlled apoptosis result in a chronic state of immune activation, which is also the common etiology for aging-associated neurodegenerative disease. We further suggest that targeting mitophagy is an important strategy to fight aging and aging-associated disease, which needs to be further explored in the future.

AUTHOR CONTRIBUTIONS

KM, GC, QC, and WL wrote the manuscript. OK and YZ revised the manuscript. All authors provided intellectual input and read the manuscript.

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REVIEW
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Role of Mitofusins and Mitophagy in Life or Death Decisions

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Mitochondria entail an incredible dynamism in their morphology, impacting death signaling and selective elimination of the damaged organelles. In turn, by recycling the superfluous or malfunctioning mitochondria, mostly prevalent during aging, mitophagy contributes to maintain a healthy mitochondrial network. Mitofusins locate at the outer mitochondrial membrane and control the plastic behavior of mitochondria, by mediating fusion events. Besides deciding on mitochondrial interconnectivity, mitofusin 2 regulates physical contacts between mitochondria and the endoplasmic reticulum, but also serves as a decisive docking platform for mitophagy and apoptosis effectors. Thus, mitofusins integrate multiple bidirectional inputs from and into mitochondria and ensure proper energetic and metabolic cellular performance. Here, we review the role of mitofusins and mitophagy at the cross-road between life and apoptotic death decisions. Furthermore, we highlight the impact of this interplay on disease, focusing on how mitofusin 2 and mitophagy affect non-alcoholic fatty liver disease.

Keywords: mitochondria, mitofusins, MFN2, mitophagy, apoptosis, NAFLD

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INTRODUCTION

Mitochondrial biology has raised extensive research interest, thanks to its expanding roles in tailored metabolic performance, in quality control responses, but also in inflammatory processes and in cell death (Westermann, 2010; Nunnari and Suomalainen, 2012; Xiong et al., 2014; Angajala et al., 2018; Pickles et al., 2018; Spinelli and Haigis, 2018; **Figure 1**). Mitochondria are double membrane organelles, being its structure and biogenesis extensively described (Pfanner et al., 2019). The outer membrane (OM) provides the first semi-permeable barrier to the cytoplasm. It contains the protein and lipid receptors of mitophagy and is a critical determinant of cell death triggers (Harper et al., 2018; Pickles et al., 2018; Xie et al., 2018; Sedlackova and Korolchuk, 2019). The OM also anchors the effectors of mitochondrial intraorganellar fusion and protein complexes forming interorganellar contact sites. These sustain, for example, mitochondria and endoplasmic reticulum (ER) exchanges, which are determinant for calcium (Ca^{2++}) buffering and phospholipid transfer (Marchi et al., 2017; **Figure 1A**). The inner membrane (IM), thanks to its impermeability, maintains the proton motive force necessary for mitochondrial biogenesis and energy conversion. By folding on itself, the IM creates invaginations called cristae, where the mitochondrial oxidative phosphorylation system (OXPHOS) is located (Frey et al., 2002). Their electron shuttling and proton pumping capacity sustains the mitochondrial membrane potential, enabling the production of energy (Zhao et al., 2019). In addition to ATP production, the IM is also in charge of phospholipid synthesis (Tatsuta and Langer, 2017). The different cristae are spaced by IM portions lining parallel to the OM, called inner boundary membrane, being the

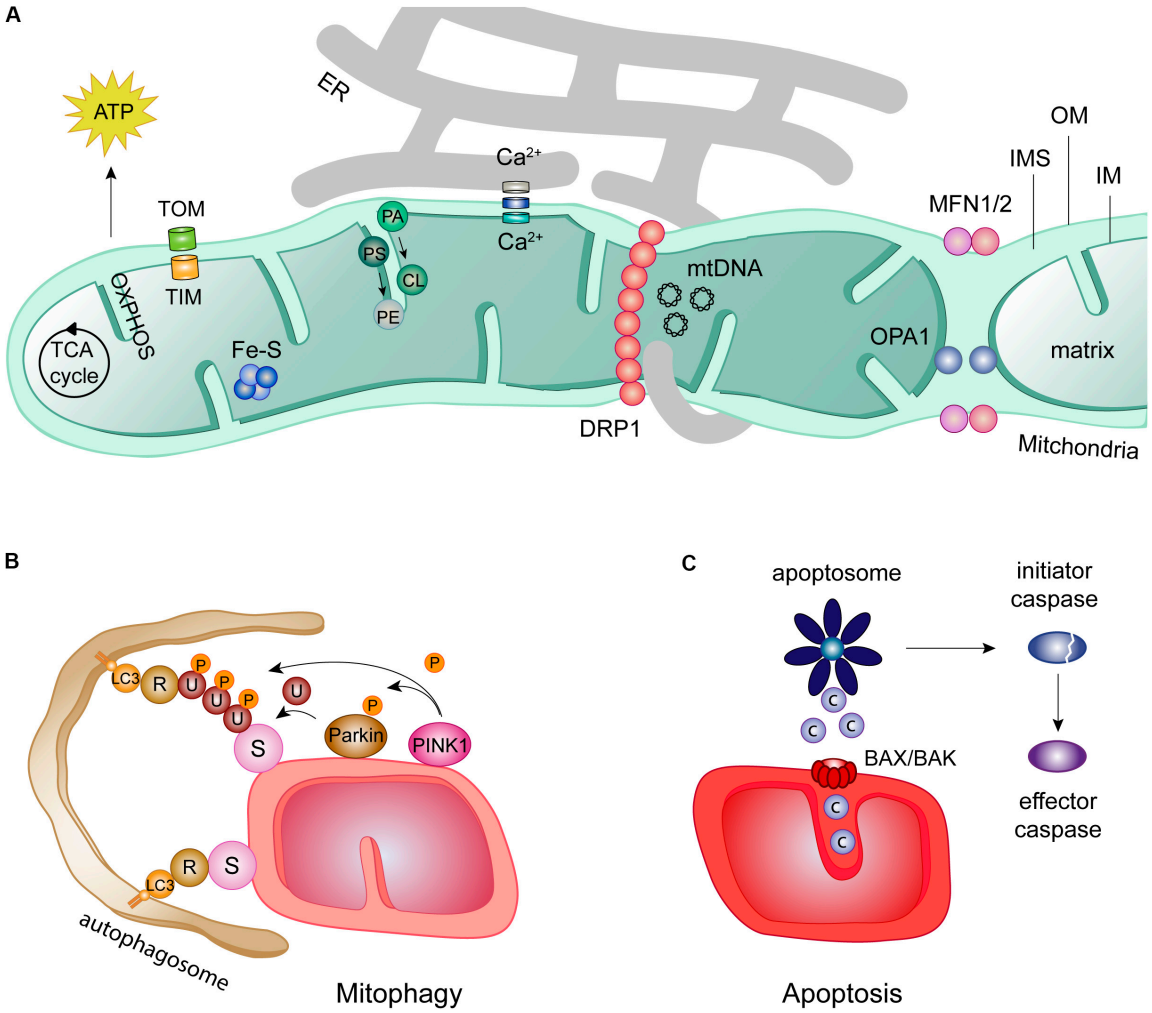

FIGURE 1 | Mitochondrial roles in health and disease. **(A)** Despite the presence of an own DNA in the mitochondrial matrix (mtDNA), most mitochondrial proteins are imported from the cytosol by two translocase complexes: the translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM). Mitochondria are hubs for several cellular processes, such as iron-sulfur clusters (Fe-S) assembly, metabolite oxidation by the tricarboxylic acid (TCA) cycle and ATP production via the oxidative phosphorylation chain (OXPHOS). Further, mitochondrial proximity to the endoplasmic reticulum (ER) regulates calcium (Ca^{2+}) buffering and phospholipid synthesis, e.g., cardiolipin (CL) and phosphatidylethanolamine (PE) from their ER precursors phosphatidic acid (PA), and phosphatidylserine (PS), respectively. ER-mitochondrial contacts also regulate mitochondrial fission, by facilitating the recruitment of DRP1 to the mitochondria. In turn, mitochondrial fusion requires the mitofusins MFN1 and MFN2 at the OM and OPA1 at the IM. **(B)** Mitochondrial function is kept in check by mitophagy, a quality control mechanism. Mitophagy can occur dependently or independently of ubiquitin. The canonical ubiquitin-dependent PINK1/Parkin pathway initiates with the accumulation of the kinase PINK1 at the OM, which recruits the E3 ligase Parkin. Ubiquitylation (U) of several OMM substrates (S) by Parkin and additional phosphorylation (P) of ubiquitin and Parkin generates a positive feedback loop increasing Parkin activity. The ubiquitin chains formed on OM substrates bind to the lipidated autophagosome receptor LC3, via receptors proteins (R). Mitochondria are then surrounded and engulfed by the autophagosome, which finally fuses with the lysosome for degradation. The ubiquitin-independent mitophagy only requires the recognition of OM substrates (S) by lipidated LC3 directly via mitochondrial receptors (R). **(C)** Mitochondria are directly involved in the initiation of apoptosis via the intrinsic apoptotic pathway. In this pathway, intrinsic death stimuli induce permeabilization of the OM by oligomerization of the pro-apoptotic BCL-2 proteins BAX and BAK. Apoptotic molecules such as cytochrome c (c) are release form the IMS, activating the apoptotic complex apoptosome. This complex is able to cleaved and thereby activate initiator caspases which, in turn, activate effector caspases.

connection points defined as cristae junctions. The inter membrane space (IMS)–the small aqueous compartment confined between both mitochondrial membrane-s is a signaling hub reservoir, in both pro-survival and cell death responses. It allows the accumulation of the protons released by the electron transport chain and comprises cytochrome c (CytC) the high-temperature requirement protein A2 (Htr2/Omi), the apoptosis inducing factor (AIF), the second-mitochondria-derived activator of caspases (Smac), the direct IAP-binding protein with low PI (Diablo) and Endonuclease G, which differently support mitochondrial metabolism (Herrmann and Riemer, 2010). However, once released from the mitochondria into the cytoplasm, upon cristae opening, these pleiotropic proteins convert into apoptosis initiators, pushing the cell toward a deadly end (Snigirevskaya and Komissarchik, 2019). Besides apoptosis, mitochondria are also recognized by regulating other

types of cell death such as necroptosis, ferroptosis, pyroptosis, and mitochondrial-mediated necrosis (Baines, 2010; Baker et al., 2014; Marshall and Baines, 2014; Battaglia et al., 2020; Kai et al., 2020; Wang et al., 2020; Wei et al., 2020). Finally, the IM encloses the mitochondrial matrix, where essential reactions take place, such as iron-sulfur cluster assembly (Cardenas et al., 2018; Lill, 2020) or the tricarboxylic acid (TCA) cycle, which feeds electrons to the respiratory chain and provides amino acid precursors (Mailloux et al., 2007; Martínez-Reyes and Chandel, 2020). Finally, the matrix harbors the mitochondrial DNA and respective transcription and translation machineries (Mazunin et al., 2015; **Figure 1A**).

Mitochondria are constantly reshaped, by fusion and fission events of the whole organelle, but also by alterations in cristae organization, altering access of the IMS content to the OM (Rampelt et al., 2017; Giacomello et al., 2020). Mitochondrial adaptive morphology, through the shift in activity of its fission and fusion machineries, is essential for their correct functioning (Chan, 2012; Buck et al., 2016; Cantó, 2018; Tilokani et al., 2018; Dorn, 2019; Whitley et al., 2019). It capacitates mitochondria to respond to cellular cues both in healthy and stress situations (Liesa and Shirihai, 2013; Mishra and Chan, 2016; Schrepfer and Scorrano, 2016; Chen and Chan, 2017; Eisner et al., 2018; Zemirli et al., 2018). While, for example, nutrient starvation shifts the balance toward a tubular mitochondrial network (Tondera et al., 2009; Gomes et al., 2011; Rambold et al., 2011), loss of membrane potential and nutrient excess were shown to induce mitochondrial fragmentation (Yu et al., 2006). The mitochondrial morphology machinery is composed by DRP1 (Dnm1 in yeast), responsible for fission, Mitofusin 1 and Mitofusin 2 (MFN1/MFN2) (Fzo1 in yeast), responsible for OM fusion and OPA1 (Mgm1 in yeast), responsible for IM fusion (Youle and van der Bliek, 2012; Tilokani et al., 2018; **Figure 1A**). Recent developments highlighted novel molecular determinants of mitochondrial ultrastructure dynamics (Xie et al., 2018; Kondadi et al., 2019; Rastogi et al., 2019; Snigirevskaya and Komissarchik, 2019). The fascinating plasticity of mitochondrial morphology is also brought about by post-translational modifications of the fusion and fission components, including ubiquitylation, phosphorylation, sumoylation, and proteolytic processing (Escobar-Henriques and Langer, 2014; Hofer and Wenz, 2014; Macvicar and Langer, 2016; Mishra and Chan, 2016). The mitofusins MFN1 and MFN2 are ubiquitylated by different E3 ligases, in response to a big variety of stimuli, which tightly regulate their fusion properties, mitochondria-ER contact sites, mitophagy and apoptosis (Escobar-Henriques and Joaquim, 2019).

In sum, mitochondria are docking stations for cellular fitness (Abate et al., 2019). By integrating external signals, which change their metabolism, shape and signaling response properties, mitochondria dictate life and death decisions. In turn, by sequestering pro-apoptotic molecules that are only to be released in the presence of death stimuli, mitochondria themselves must be tightly regulated, to prevent undesired cell death. Thus, elimination of dysfunctional mitochondria by mitophagy is critical for cellular survival (Pickles et al., 2018; Allen and Baehrecke, 2020; Markaki and Tavernarakis, 2020). Despite being mostly pro-survival, mitophagy can also synergize with apoptosis to instead promote cell death (Yee et al., 2010; Panda et al., 2018; Ding et al., 2019; Han et al., 2019). Here, we discuss how the mitochondrial fusion factors, mitofusins, impact on mitophagy and apoptosis, present pro-survival and pro-apoptotic roles of mitophagy and detail the roles of mitophagy and mitofusin 2 in non-alcoholic fatty liver disease (NAFLD).

THE MITOFUSIN PROTEINS, MFN1 AND MFN2

Mitofusin 1 and Mitofusin 2 are homologous conserved transmembrane proteins, being mainly exposed to the cytosol (Zorzano and Pich, 2006). They possess a GTPase domain at the N-terminal and two hydrophobic heptad repeat domains, separated by transmembrane anchor(s) (Hales and Fuller, 1997; Rojo et al., 2002; Low et al., 2009; Qi et al., 2016; Cao et al., 2017; Daste et al., 2018; Mattie et al., 2018; Yan L. et al., 2018; Li et al., 2019). Despite having 77% of similarity, their deletion differentially affects mitochondrial morphology (Chen et al., 2003). While depletion of *MFN1* leads to highly fragmented mitochondria, organized in small fragments dispersed throughout the cytosol, depletion of its homolog *MFN2* leads to bigger mitochondrial fragments that cluster perinuclearly. However, overexpression of either Mfn1 or Mfn2 in single and double mitofusins knockout murine fibroblasts leads to complete rescue of the mitochondrial morphology phenotypes (Detmer and Chan, 2007). Although ubiquitously expressed, *MFN1* is mainly present in heart and testis, while *MFN2* is predominant in the brain and muscle tissues; in other tissues, mitofusins present similar expression levels (Eura et al., 2003; Santel et al., 2003). Murine homozygous deletion of *Mfn1* or *Mfn2* is lethal and double-knockout mice die even earlier than single knockouts (Chen et al., 2003), suggesting that they play non-redundant roles. Additionally, mice depleted for *Mfn2* (but not for *Mfn1*) present placental defects within the giant cell layer (Chen et al., 2007). If *Mfn2* is only depleted after placentation, it leads to cerebellar neurodegeneration (Chen et al., 2007), highlighting different impact of these proteins according to the developmental stage.

Although both mitofusins regulate mitochondrial fusion, additional roles have been attributed to MFN2 (Chen and Chan, 2017; Filadi et al., 2018; Mattie et al., 2019; Dorn, 2020). MFN2 was proposed to regulate tethering and distance between mitochondria and ER (De Brito and Scorrano, 2008; Cosson et al., 2012; Filadi et al., 2015; Naon et al., 2016), controlling the Ca^{2+} exchange between both organelles (Rizzuto et al., 1998; Szabadkai et al., 2006). Consistently, Mfn2 and ER-mitochondrial contacts modulated Ca^{2+}-dependent roles in vascular remodeling (Zhu et al., 2017; Göbel et al., 2020). Moreover, mitochondria-ER contact sites are essential for phospholipid transfer between the two organelles (Kojima et al., 2016), being a direct role of MFN2 in lipid transfer recently proposed (Hernández-Alvarez et al., 2019). However, it is still under debate under which conditions Mfn2 acts as a spacer between both organelles, consistent with decreased distance observed in its absence (Cosson et al., 2012;

Filadi et al., 2015; Wang et al., 2015) or rather acts as an ER-mitochondria tether (De Brito and Scorrano, 2008; Naon et al., 2016; Basso et al., 2018; McLelland et al., 2018). MFN2 has also been extensively linked to mitophagy (Gegg et al., 2010; Poole et al., 2010; Ziviani et al., 2010), to apoptosis (Karbowski et al., 2002; Brooks et al., 2007; Hoppins et al., 2011), as detailed below, and recently also to pyroptosis (Kai et al., 2020) and ferroptosis (Wei et al., 2020). Furthermore, MFN2 is believed to play multiple roles in metabolism, explaining its involvement in metabolic disorders such as obesity and diabetes mellitus (Bach et al., 2005; Toledo et al., 2006; Sebastián et al., 2012; Schneeberger et al., 2014; Boutant et al., 2017; Ramiréz et al., 2017; Bell et al., 2019). *MFN2* depletion leads to reduced mitochondrial membrane potential, oxygen consumption rate and mitochondrial proton leakage and impairs glucose, pyruvate and fatty acid oxidation (Bach et al., 2003; Chen et al., 2005; Pich et al., 2005; Mourier et al., 2015). Importantly, *Mfn2* loss-of-function represses nuclear-encoded subunits of OXPHOS complexes I, II, III and V (Pich et al., 2005), independently of its fusogenic role (Pich et al., 2005; Loiseau et al., 2007). Moreover, mitofusins were shown to be required for mitochondrial DNA (mtDNA) replication and nucleoid distribution (Chen et al., 2010; Ramos et al., 2019). Both mitofusins have also been associated with female fertility (Liu et al., 2016; Zhang et al., 2019a,b). Mfn2 is important for oocyte and follicle development (Liu et al., 2016) and both mitofusins are required for the maintenance of the ovarian follicular reserve (Zhang et al., 2019a,b). Regarding cell cycle progression, *MFN2* overexpression supressed cellular proliferation (Cheng et al., 2013) and MFN2 depletion increased it, dependent on the Ras-Raf-ERK signaling pathway (Chen et al., 2014). The role of mitofusin 2 in controlling cell proliferation possibly explains its link with cancer. Indeed, *MFN2* overexpression is able to slow the growth of different cancer cell lines (Xie et al., 2015; Xu et al., 2017). Furthermore and quite controversially, lack of *MFN2* seems to equally impair both stem cell self-renewal and differentiation capacity (Kasahara et al., 2013; Fang et al., 2016; Khacho et al., 2016).

Mitofusin 1 and Mitofusin 2 have a clearly different biological impact, perhaps explaining the inexistence of MFN1 mutations causing human diseases. In contrast, more than a hundred *MFN2* mutations are known to cause the Charcot-Marie-Tooth Type 2A (CMT2A) disorder (Stojkovic, 2016; Dohrn et al., 2017), a subtype of the incurable peripheral neuropathy Charcot-Marie-Tooth (CMT). CMT affects about 1 in 2500 people, being the most common inherited neurological disease and is characterized by progressive distal weakness, muscular atrophy, and sensory abnormalities (Tazir et al., 2013; El-abassi et al., 2014; Stuppia et al., 2015; Stojkovic, 2016; Barbullushi et al., 2019). The restoration of mitochondrial fusion by either transgenic overexpression of Mfn1 or by Mfn2 agonist molecules in murine models led to reversion of some of the CMT2A defects (Rocha et al., 2018; Zhou Y. et al., 2019). However, to date, the disease-underlying functions of MFN2 in CMT2A remain elusive. So far, reports have pointed to apoptosis resistance and increased mitophagy, observed in iPSCs-derived CMT2A motor neurons lines (Rizzo et al., 2016). Importantly, different CMT2A disease mutant cell lines have displayed impaired ER-mitochondria contacts, as well as ER stress, defective Ca^{2+} uptake and phospholipid synthesis and transfer (Bernard-Marissal et al., 2018; Larrea et al., 2019), pointing to a possible role of these contact sites at the basis of CMT2A disease. Besides CMT2A, MFN2 has been linked to a variety of diseases (Chandhok et al., 2018; Filadi et al., 2018). The most described links are with prevalent neuropathies such as Parkinson's and Alzheimer's disease (Han et al., 2011; Lee et al., 2012; Stuppia et al., 2015; Gao et al., 2017), cardiac dysfunction (Hall et al., 2014; Nan et al., 2017; Dorn, 2018; Hernandez-Resendiz et al., 2020), type 2 diabetes, obesity and insulin resistance (Zorzano et al., 2009; Dai and Jiang, 2019) and cancer (Allegra et al., 2019). Finally, MFN2 has also been associated with progression of liver diseases such as acute-on-chronic liver failure (ACLF) and NAFLD (Wang et al., 2013; Hernández-Alvarez et al., 2019; Xue et al., 2019a,b) and proposed as a possible therapeutic target for hepatic inflammation and fibrosis (Zhu et al., 2020).

REGULATION OF MITOPHAGY BY MITOFUSINS

Mitochondrial homeostasis is ensured by the coordination between its biogenesis rate, enabling the replenishment of novel healthy organelles, and the elimination of the superfluous or damaged mitochondria by selective self-digestion, via mitophagy (Harper et al., 2018; Pickles et al., 2018; Pfanner et al., 2019; Allen and Baehrecke, 2020; Markaki and Tavernarakis, 2020). Mitophagy requires the recognition of mitochondrial adaptors by receptors on the autophagosome, the double membrane autophagic vacuole responsible for engulfment of the material to be degraded (Lahiri et al., 2019; Allen and Baehrecke, 2020; **Figure 1B**). Moreover, mitochondrial elimination requires the ubiquitin-like modifier LC3 (Atg8), whose lipidated and active form integrates into the autophagosome membrane. LC3 is recognized by specific receptors, either present at the mitochondrial OM, like Atg32, NIX and BNIP3, or instead soluble at the cytoplasm and being recruited to the OM, like Optineurin, NDP52, p62, NBR1, and TAX1BP1 (Geisler et al., 2010; Narendra et al., 2010; Lazarou et al., 2015; Khaminets et al., 2016; Mcwilliams and Muqit, 2017). In fact, these receptors interact with both ubiquitin and LC3 interacting (LIR) motifs, being therefore recruited to mitochondria by ubiquitylated OM proteins. Once loaded with damaged mitochondria, the autophagosome then fuses with the lysosome, forming the autolysosome, where mitochondrial degradation takes place (Allen and Baehrecke, 2020). Upon acute stress conditions, fission and selective fusion were shown to facilitate segregation and subsequent turnover of the damaged pieces (Twig et al., 2008; Burman et al., 2017). Mitofusins have been extensively implicated in mitochondrial quality control, mainly attributed to their decisive role in mitochondrial length and their receptor property for mitophagy effectors (Dorn, 2020).

The general ubiquitylation of OM proteins is one of the early steps and a hallmark in mitophagy (Palikaras et al., 2018; Wang Y. et al., 2019; **Figure 1B**). The most extensively studied ubiquitin-dependent pathway is undertaken by the

serine/threonine kinase PINK1 and by Parkin, a RING-between-RING E3 ubiquitin ligase (Pickles et al., 2018). Under healthy conditions, PINK1 is inactivated by proteasomal turnover. First, PINK1 is imported through the TOM and TIM23 translocator complexes (Lazarou et al., 2012; Sim et al., 2012; Sekine and Youle, 2018). PINK1 is then constitutively cleaved by the IM residing protease PARL, being its truncated soluble form extracted back to the cytosol and degraded by the proteasome (Yamano and Youle, 2013). However, upon mitochondrial stress, decreased mitochondrial membrane potential prevents PINK1 import, consequently accumulating its full-length form at the OM, exposing its kinase domain to the cytosol (Matsuda et al., 2010; Vives-Bauza et al., 2010). Mitophagy is then initiated by phosphorylation of ubiquitin molecules at serine 65 (Kane et al., 2014; Koyano et al., 2014) and of Parkin (Shiba-Fukushima et al., 2012; **Figure 1A**). This shifts Parkin to an active conformation, fostering its recruitment to mitochondria and potentiating ubiquitylation of OM proteins (Gegg et al., 2010; Poole et al., 2010; Tanaka et al., 2010; Ziviani et al., 2010; Chan et al., 2011; Glauser et al., 2011; Rakovic et al., 2011; Riley et al., 2013; Spratt et al., 2013; Wauer and Komander, 2013; Caulfield et al., 2014; Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014; Swatek and Komander, 2016; Wauer et al., 2016; Kumar et al., 2017; Mcwilliams et al., 2018; Gladkova et al., 2019). The continuous phosphorylation of the poly-ubiquitin chains by PINK1 creates a positive feed-forward cycle, which massively increases Parkin recruitment to mitochondria and ubiquitylation of OM proteins (Ordureau et al., 2014). Besides Parkin, other E3 ligases have been shown to ubiquitylate OM proteins and thus signal mitophagy, like MARCH5, Gp78 and MGRN1, HUWE1, MUL1, and SIAH-1 (Liu et al., 2012; Fu et al., 2013; Cilenti et al., 2014; Yun et al., 2014; Tang et al., 2015; Mukherjee and Chakrabarti, 2016a,b; Szargel et al., 2016; Chen et al., 2017; Daskalaki et al., 2018; Di Rita et al., 2018; Ferrucci et al., 2018; Lampert et al., 2019; Shefa et al., 2019).

Mitofusins are preferred targets at the OM, being ubiquitylated by all above-mentioned E3 ligases (extensively reviewed in Escobar-Henriques and Joaquim, 2019). They are among the first substrates to be ubiquitylated by Parkin, mostly observed upon Parkin overexpression (Chan et al., 2011; Sarraf et al., 2013; McLelland et al., 2018) and therefore possibly reflecting experimental artifacts. Nevertheless, the ubiquitylation of mitofusins by Parkin was equally demonstrated to occur and be important for mitophagy in the absence of overexpression, with endogenous PINK1 and Parkin, in reprogrammed induced neuron cells (Ordureau et al., 2020). Upon mitophagy induction, ubiquitylation of mitofusins 1 and 2 targets them for degradation by the proteasome, quickly leading to abrogation of mitochondrial fusion events, resulting in mitochondrial fragmentation (Geisler et al., 2010; Narendra et al., 2010; Lazarou et al., 2015; Khaminets et al., 2016; Mcwilliams and Muqit, 2017). However, in the above-mentioned induced neurons -a form of not fully differentiated immature neuronal cells converted from human embryonic stem cells- extraction from the OM and proteasomal degradation of mitofusins was not required for mitophagy (Ordureau et al., 2020). This favors the previously suggested pro-mitophagy role of mitofusins as autophagic receptors (Chen and Dorn, 2013; Song et al., 2015). Loss of *MFN2*/*Mfn2* has also been connected with a decrease in autophagosome formation and/or defects in autophagosome-lysosome fusion, two events of mandatory nature for mitophagy to occur (Zhao et al., 2012; Sebastián et al., 2016; Peng et al., 2018). Consistently, depletion of both *Mfn1* and *Mfn2* in murine cardiomyocytes caused accumulation of defective mitochondria (Song et al., 2014, 2015). Equally supporting a pro-mitophagic role of mitofusins, knockdown of *Mfn1* led to mitophagy inhibition caused by overexpression of the E3-ligase Gp78 (Fu et al., 2013). In contrast, an active role of MFN2/Mfn2 in preventing mitophagy was also proposed, connected to the ER-mitochondrial tether function of MFN2 (Basso et al., 2018; McLelland et al., 2018). Consistently, CMT2A-linked *MFN2* mutants caused increased autophagic flux, whereas MFN2 overexpression prevented autophagy (Rizzo et al., 2016; Ying et al., 2017). Finally, as detailled bellow, upon peripheral nerve injury *Mfn1* depletion induced mitophagy and apoptosis (Yang et al., 2020). Finaly, mitophagy progression was even proposed not to depend on mitofusins (Narendra et al., 2008; Chan et al., 2011). In brief, how mitofusins affect mitophagy might be context-dependent, which is perhaps not so surprising considering the multi-functionality of MFN2 and its responsiveness to many different stress conditions.

MITOFUSINS AND APOPTOSIS

Cell death by apoptosis is an essential mechanism for cellular turnover, which occurs via a programmed and tightly regulated way (Kerr et al., 1972; Adams and Cory, 2007; Nair et al., 2014). It is important in physiological conditions, e.g., during embryonic development or neuronal network formation, but also under pathological conditions, e.g., for tissue homeostasis in response to stress (Elmore, 2007). Apoptosis can be induced via two distinct pathways, extrinsic or intrinsic, culminating in the activation of different cysteine-dependent aspartate-directed proteases (caspases), the final effectors of apoptotic cell death (Meier and Vousden, 2007). The extrinsic apoptotic pathway is initiated by external cellular signals, followed by binding of a death ligand to a cell-surface receptor (Jin and El-Deiry, 2005; Nair et al., 2014). The intrinsic apoptotic pathway, known as mitochondrial apoptotic pathway, is initiated by the release of AIFs from the IMS to the cytosol (Bock and Tait, 2020), such as CytC, SMAC/DIABLO and HtrA2/Omi (Griffiths et al., 1999; Antonsson et al., 2001; Wang and Youle, 2009; **Figure 1C**). This requires mitochondrial OM permeabilization (MOMP) (Edlich et al., 2011; Todt et al., 2015). MOMP is mediated by oligomers of BAX and BAK, the two main regulators of mitochondrial apoptosis, which in the absence of stress are constantly translocated between the cytosol and mitochondria (Todt et al., 2015). Upon death stimuli, BAX and BAK accumulate at the mitochondrial OM, bind to death signal sensors (BH3 domain-only proteins), undergo conformational changes and oligomerize, enabling MOMP. Once in the cytosol, CytC binds to the apoptotic protease activating factor-1 (APAF-1), activating its nucleotide exchange factor activity and forming

a homoheptameric APAF-1 complex, named apoptosome (Liu et al., 1996; Li et al., 1997). Finally, the apoptosome cleaves and activates pro-caspase-9, necessary for activation of downstream effector caspases (Xiong et al., 2014). In general lines, effector caspases induce endonucleases and proteases to degrade nuclear material and key structural proteins (Hengartner, 2000; Slee et al., 2001). The action of the effector caspases over their targets leads to apoptotic morphological traces, such as cytoplasmic disorganization, cell shrinkage, chromatin condensation, DNA fragmentation and apoptotic body formation.

The key proteins mediating mitochondrial shape–OPA1, mitofusins and DRP1- were also implicated in the regulation of apoptosis (Campello and Scorrano, 2010; Karbowski, 2010; Xie et al., 2018). The cristae remodeling events necessary for release of CytC from cristae junctions were proposed to be mediated by OPA1 (Scorrano et al., 2002; Cipolat et al., 2006; Frezza et al., 2006). In turn, DRP1 and mitofusins have been shown to interact with BAX and BAK (Karbowski et al., 2002, 2006; Brooks et al., 2007). Upon apoptosis induction, activated BAX is recruited to MFN2-containing puncta (Neuspiel et al., 2005), suggesting a synergistic relation between MFN2 and pro-apoptotic proteins. Indeed, overexpression of MFN2, and of its yeast homolog Fzo1, led to apoptosis induction (Sugioka et al., 2004; Neuspiel et al., 2005). Further supporting a positive role of mitofusins in apoptosis, MFN2 protein levels are decreased in urinary bladder carcinoma tissues and its overexpression in a cellular model of the disease decreased cell viability via apoptosis induction (Jin et al., 2011). Similarly, in atherosclerosis or restenosis, MFN2 expression is downregulated. In vascular smooth muscle cells, overexpression of Mfn2 induces growth arrest (Guo et al., 2007). In fact, adenoviral expression of MFN2 showed an anti-tumor effect *in vitro* in a wide range of different cancer cell lines (Wu et al., 2008). MFN1 overexpression was also shown to induce apoptotic cell death of osteosarcoma cells, reducing malignancy, while the microRNA miR-19b, predicted to downregulate MFN1, had the opposite effect (Li X. et al., 2014). However, apoptosis is generally accompanied by mitochondrial fragmentation and, indeed, MFN2 was shown to be phosphorylated and proteasomally degraded upon genotoxic stress, which also induced apoptosis (Leboucher et al., 2012). Besides, *Mfn2* depletion in murine liver was shown to aggravate apoptosis provoked by bavachin, a flavonoid causing ER-stress, and *Mfn2* depletion in the hippocampus led to neuronal death (Yang et al., 2018; Han et al., 2020). Other than leading to mitofusins degradation, apoptosis might inactivate the fusogenic capacity of mitofusins, perhaps directly mediated by activated BAX/BAK oligomers (Karbowski et al., 2006). Consistently, when in its apoptotic conformation, BAX was proposed to inhibit Mfn2 activity (Hoppins et al., 2011). Moreover, BAK was reported to promote mitochondrial fragmentation during apoptosis by dissociating from Mfn2 and associating with Mfn1 (Brooks et al., 2007). Reciprocally, in healthy cells, BAX and BAK were proposed to promote mitochondrial fusion (Karbowski et al., 2006; Hoppins et al., 2011). Mechanistically, BAX and BAK seem to be required for Mfn2 assembly in fusion-prone complexes (Karbowski et al., 2006). Indeed, Mfn2 foci observed in wild type cells were impaired in the absence of these pro-apoptotic proteins, resulting in a more even mitochondrial distribution of Mfn2. Finally, BAX/BAK-loss induces mitochondrial fragmentation in similar extent to *Mfn2* knockout. Interestingly, mitofusins have also been linked with other types of cell death such as necroptosis, pyroptosis and ferroptosis (Xie et al., 2018; Bock and Tait, 2020). Necroptosis induction promoted ubiquitination and degradation of Mfn1, without affecting Mfn2 (Baker et al., 2014). Instead, upregulation of Mfn2 levels correlated with reduced pyroptosis in the liver, reverted by Oroxylin A, which allowed resisting to lipid deposition and ROS overproduction (Kai et al., 2020). In contrast, Mfn2 fusion activity was stimulated by the same BAX mutants that induced necrosis (Whelan et al., 2012; Karch et al., 2013) and hepatic knockdown of Mfn2 reduced ferroptosis, provoked by arsenite (Wei et al., 2020). In sum, the dual interplay between mitofusins and cell death points to multiple cross-talk effects that require future clarification.

MITOPHAGY: A PRO-SURVIVAL OR A PRO-APOPTOTIC MECHANISM?

Mitophagy, autophagy and apoptosis–as major quality control mechanisms–are intimately related and often reported to affect each other (Maiuri et al., 2007; Kubli and Gustafsson, 2012; Anding and Baehrecke, 2015; Bloemberg and Quadrilatero, 2019). It is overall accepted that mitophagy prevents cell death, by clearing damaged and toxic mitochondria, thus constituting a pro-survival mechanism. However, pro-apoptotic and anti-tumor consequences of mitophagy were also reported (**Figure 2**).

Anti-apoptotic Effects of Mitophagy

The majority of the findings showing a direct regulation of these two processes support the idea that mitophagy occurs as a pro-survival mechanism. Prevention of mitophagy, by Parkin and/or PINK1 inhibition, or by cyclosporine A or 3-MA treatment, induced CytC release and caspase activity, stimulating apoptosis (Tian et al., 2019; Kang et al., 2020; Li C. et al., 2020; Lin et al., 2020). Consistently, mitophagy induction via Urolithin A or FCCP treatment prevented apoptosis (Tian et al., 2019; Lin et al., 2020). Importantly, the inverse correlation between apoptosis and mitophagy was also observed under physiological conditions involving hypoxia. During follicle development, the follicle stimulating hormone is responsible for ensuring survival of porcine granulosa cells, despite their hypoxic environment (Li C. et al., 2020). In fact, the follicle stimulating hormone induced mitophagy, thereby protecting from hypoxia-induced apoptosis. The anti-apoptotic effects of mitophagy are also relevant in the context of liver, where an important role is attributed to mitofusins, as detailed in the next two chapters. For example, in murine hepatocytes, the impairment of mitophagy by downregulation of Parkin or PINK1, or by the knockout of both, was shown to significantly increase the hepatic apoptotic rate (Chen et al., 2019; Wang H. et al., 2019; Zheng et al., 2020). Similarly, in hepatocellular carcinoma HepG2 cells, treatment with Mdivi-1, a mitophagy inhibitor, led to induction of apoptosis (Kang et al., 2019). Inversely, interference with

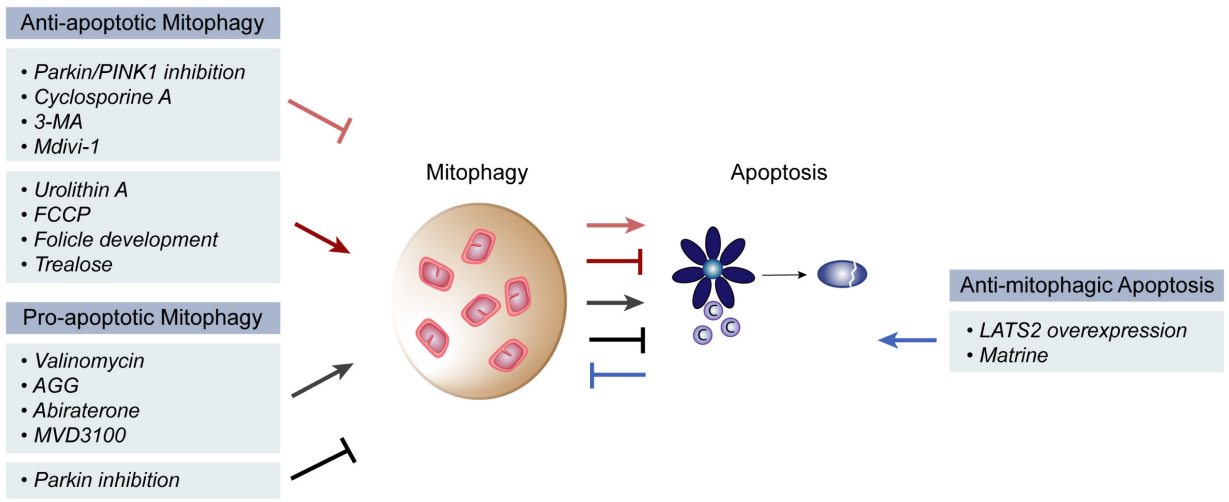

FIGURE 2 | Pro- and anti- apoptotic roles of mitophagy. Mitophagy is reported to both induce and inhibit apoptosis in different physiological and pathological contexts. Inhibition of mitophagy via Parkin/PINK1 inhibition, Cyclosporine A, 3-MA or Mdivi-1 treatment has the ability to induce mitochondrial apoptosis, while induction of mitophagy that occurs during follicle development or upon urolithin A, FCCP or trealose treatment, has the opposite effect. On the other hand, pro-apoptotic roles of mitophagy were described upon mitophagy induction via valinomycin, AGG, abiraterone or MVD3100 treatment. Further, mitophagy suppression by parkin inhibition also inhibited apoptosis. Finally, an anti-mitophagic role of apoptosis could be observed upon overexpression of the large tumor suppressor gene 2 (LATS2) gene or by matrine treatment.

apoptosis also impacted on mitophagy. Apoptosis induction via matrine treatment, or by expression of the large tumor suppressor gene 2 (*LATS2*) suppressed mitophagy (Wei et al., 2018; Tian et al., 2019). Cell death was prevented by mitophagy activation, through HIF-1α-PINK1-Parkin, resolving hypoxic stress. General autophagy was also linked to apoptosis. Induction of autophagy with trealose delayed MOMP, while downregulation of autophagy components activated apoptosis, by increasing the levels of the p53-dependent apoptosis mediator (PUMA) (Thorburn et al., 2014). Mechanistically, autophagy inhibition caused accumulation of the transcription factor FOXO3a, increasing *PUMA* mRNA levels, thus sensitizing cells to apoptosis (Fitzwalter et al., 2018).

Pro-apoptotic Effects of Mitophagy

A pro-apoptotic mechanism of mitophagy was also reported. Induction of mitophagy in cancer cells, by treatment with valinomycin, *Abrus* agglutinin (AGG), abiraterone or MDV3100 (anti-cancer drugs), led to an increase in apoptosis, facilitating cancer recovery (Panda et al., 2018; Ding et al., 2019; Han et al., 2019). The Bcl-2 family member BCL-B and the levels of phosphorylated Parkin appeared to be central in this response (Ding et al., 2019). Consistently, Parkin silencing decreased the death rate of hepatocellular carcinoma cells (Prieto-Domínguez et al., 2016). Further, a concomitant increase in mitophagy and apoptosis was observed upon *PUMA* overexpression or by treatment with the ribosome inhibitor AGG, which depended on BAX (Yee et al., 2010; Panda et al., 2018). Consistently, inhibition of mitophagy led to decreased caspase activity and apoptosis (Panda et al., 2018). Thus, synergistic induction of mitophagy and apoptosis, observed in glioblastomas cells or in lymph node prostate carcinoma cells (Panda et al., 2018; Han et al., 2019), appears to be relevant in suppressing cancer proliferation. However, given that anti-cancer drugs affect mitochondrial function, a causal pro-apoptotic role of mitophagy induction is still controversial.

Interestingly, beyond compensatory or synergistic effects, the ubiquitylation state of VDAC was shown to differentially affect either mitophagy or apoptosis. While polyubiquitylated VDAC1 is able to induce mitophagy, monoubiquitylated VDAC1 seems to exert a protective effect over apoptosis (Ham et al., 2020). However, the conditions by which VDAC gets mono or polyubiquitylated and the respective E3 ligases involved are still to be identified.

Interaction Between Mitophagic and Apoptotic Components

The relationship between mitophagy and apoptosis is supported by evidence of physical interactions between several players of each process. First, the anti-apoptotic BCL-2 proteins Bcl-xL and MCL1 were shown to prevent mitophagy by physically interacting with Parkin, preventing its stable recruitment to mitochondria (Hollville et al., 2014; Yu et al., 2020). Second, the Bcl-2 pro-apoptotic family member BNIP3 is also a mitophagy receptor that physically interacts with LC3, via its LIR domain, promoting mitophagy. Interestingly, upon starvation, Beclin-1 participates in autophagosome formation, by associating with the hVps34/Class III PI3K complex, which localizes autophagy proteins to the autophagosome membrane. However, in nutrient rich conditions, the anti-apoptotic protein Bcl-2 interacts physically with the autophagic protein Beclin-1, preventing autophagy (Pattingre et al., 2005). By binding to Beclin-1, Bcl-2 impedes the formation of the hVps34/Class III PI3K complex, blocking autophagy. In contrast to this role of anti-apoptosis components in preventing autophagy via Beclin-1 sequestration,

Beclin-1 was shown to induce apoptosis, by localizing to mitochondria (Wirawan et al., 2010). In fact, Beclin-1 is cleaved by caspases during apoptosis induction (Cho et al., 2009; Luo and Rubinsztein, 2010; Wirawan et al., 2010) and a Beclin-1 cleaved form localizes to mitochondria, sensitizing cells to apoptosis by enhancing CytC and HtrA2/Omi release (Wirawan et al., 2010). Similarly, after being cleaved by calpain, the autophagic protein Atg5 associated to the anti-apoptotic protein $Bclx_1$, enhancing apoptosis (Yousefi et al., 2006).

INTERPLAY BETWEEN MITOCHONDRIAL FUSION, MITOPHAGY AND APOPTOSIS

Mitofusins–mainly MFN2 but recently also MFN1–have been broadly suggested as decision-making players in the interplay between mitophagy and apoptosis. Several pathological contexts affecting distinct physiological systems, such as heart, liver and brain, have implied a role of mitofusins. Generally, mitofusins are downregulated in disease states, being pathophysiology improved by restoring mitofusin's levels, thus highlighting a broad therapeutic potential of these proteins (**Figure 3**). Regarding Mfn1, peripheral nerve injury and consequent muscular denervation upregulated the miRNA 142a-5p, which was bioinformatically predicted to target *Mfn1* (Yang et al., 2020). Consistently, denervated muscles presented decreased levels of Mfn1. Moreover, a miRNA-142a-5p mimic led to downregulation of *Mfn1* and to increased mitophagy and apoptosis of skeletal muscle cells, which were rescued by a miRNA-142a-5p-inhibitor. Experiments performed *in vivo*, in mice sciatic nerve transection models, confirmed these observations, pointing to an important role of Mfn1 in protecting from mitophagy and apoptosis (Yang et al., 2020).

Decreased levels of MFN2 inhibited mitophagy and increased apoptosis. While rather exacerbating damage caused by stress in healthy cells, compromising mitophagy and MFN2 lead to better disease prognoses in cancer. For example, in gastric cancer, increased levels of Yes-associated protein (YAP) contribute to cellular proliferation and metastasis (Yan H. et al., 2018). Knockdown of *YAP* inhibited Sirtuin 1 (SIRT1) activity, consequently decreasing *MFN2* expression and mitophagy. This increased apoptosis and oxidative stress, preventing migration of the cancer cells. Upregulation of mitophagy in YAP-deficient cells, with FCCP or by reactivation of SIRT1, reversed apoptosis induction. This substantiated the importance of MFN2 and mitophagy inhibition in gastric cancer treatment (Yan H. et al., 2018). Similarly, upon treatment of esophageal squamous cell carcinoma with the cytokine IL-24, drug-resistance responses were associated with increased mitophagy (Zhang J. et al., 2019). Drug-resistance also correlated with decreased levels of macrophage stimulating factor 1 (MST1). Overexpression of *MST1* inhibited ERK activity, decreasing MFN2 levels and consequently preventing mitophagy. This was accompanied by better anti-cancer efficacy of IL-24. Importantly, independent silencing of *MFN2* or chemical inhibition of ERK led to the same mitophagic outcome (Zhang J. et al., 2019). In sum, the pro-survival role of MFN2 and mitophagy in cancer cells suggest it as a potential target for inhibition in oncogenic treatments.

The role ERK-MFN2 in mitophagy was also described in cerebral ischemia-reperfusion (IR) injury (Zhang and Yu, 2018). Here, decreased mitophagy and increased apoptosis are associated with brain damage. The protein Nr4a1 was upregulated upon IR injury, which induced brain damage by increasing apoptosis and by inhibiting mitophagy. Mitophagic rescue and apoptotic inhibition, caused by *Nr4a1* deletion, were lost in absence of *Mfn2*, pointing to a dependence of Nr4a1on Mfn2 for IR injury. IR injury also repressed ERK and

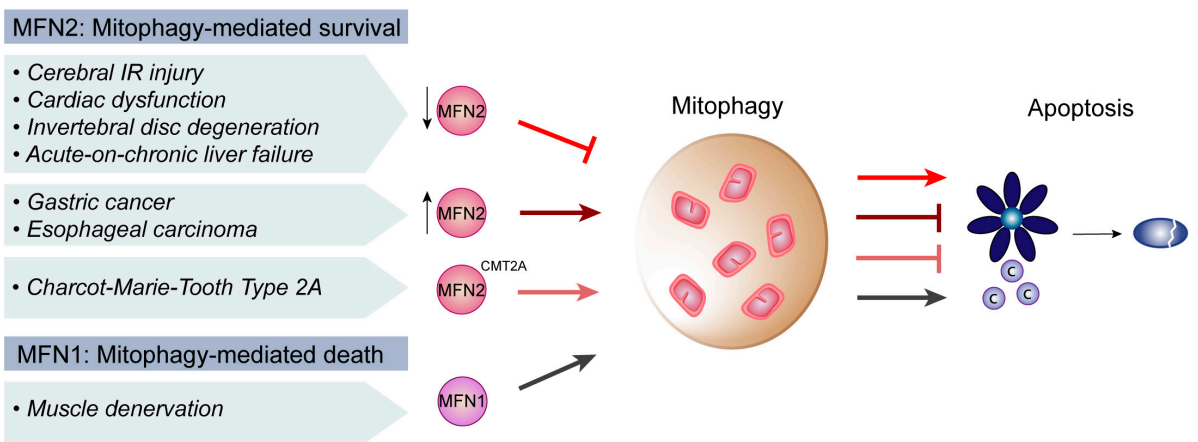

FIGURE 3 | Regulation of mitophagy and apoptosis by mitofusins in disease. In cerebral ischemia-reperfusion (IR) injury and coronary heart disease, there is a presumable decrease of Mitofusin 2 (MFN2) levels, which repressed mitophagy and induced apoptosis. Similarly, in the context of intervertebral disc degeneration, acute-on-chronic liver failure and cardiac injury, decreased MFN2 levels were concomitant with decreased mitophagy and increased apoptosis. Consistently, digestive track cancer presented increased levels of MFN2, increased mitophagy and decreased apoptosis. Moreover, MFN2 mutations causative of the neuropathy Charcot-Marie-Tooth Type 2A were also reported to induce mitophagy and block apoptosis. In contrast, upon muscle denervation, low levels of Mitofusin 1 (MFN1) lead to an induction of mitophagy which promoted apoptosis, supporting an anti-apoptotic role of mitofusins.

CREB phosphorylation, again reverted by Nr4a1 loss, implying the MAPK-ERK-CREB signaling pathway in this response. Furthermore, in Nr4a1-deficient cells, blockage of the MAPK-ERK-CREB signaling pathway decreased the levels of Mfn2 and no longer allowed apoptotic inhibition. In sum, this study points to an important role of Mfn2 and of the MAPK-ERK-CREB pathway in the brain, by controlling mitophagy and apoptosis upon IR injury (Zhang and Yu, 2018). The importance of MFN2 in the interplay between mitophagy and apoptosis was confirmed in the context of coronary heart disease, however instead involving the AMPK-CREB pathway (Li P. et al., 2020). Inflammation in human umbilical vein endothelial cells, caused by oxidized low-density lipoprotein (ox-LDL), induced the phosphatase and tensin homolog (PTEN), decreased mitophagy and increased apoptotic cell death. PTEN downregulation reversed these phenotypes, being the rescue dependent on the presence of MFN2. Furthermore, ox-LDL treatment disrupted AMPK activity and decreased CREB phosphorylation, being both phenotypes rescued by PTEN deletion. Finally, AMPK inhibition led to concomitant reduction of *MFN2* expression, pointing to a role of the AMPK-CREB pathway and of MFN2-induced mitophagy in preventing apoptosis and heart injury (Li P. et al., 2020). Also in heart, Mfn2 was implicated in the regulation of cardiomyocyte cell death mediated by the kinase Lats2 (Tian et al., 2019). *Lats2* overexpression resulted in decreased levels of Mfn2, reduced mitophagy and increased cardiomyocyte apoptosis. Consistent with the usual association of cardiomyocyte death with hypoxia, the levels of peroxiredoxin 3 (Prx3), a reactive oxygen species scavenger, were decreased upon *Lats2* overexpression. In *Lats2* overexpression conditions, Prx3 re-expression rescued Mfn2 levels and mitophagy, pointing to an important role of Prx3 (Tian et al., 2019). Thus, it would be interesting to test if reactivation of Prx3-Mfn2-mitophagy reverses Lats2-induced cardiomyocyte death. In sum, stress conditions seem to cause a decrease in mitophagy, caused by Mfn2 depletion through the downregulation of associated signaling pathways.

Mitofusin 2 downregulation, causing decreased mitophagy or autophagy, was also observed in cardiac injury mimicked by angiotensin II (Xiong et al., 2019), in intervertebral disc degeneration (IVDD) (Chen et al., 2020) and in acute-on-chronic liver failure (ACLF) (Xue et al., 2019a,b). Common to these reports, low levels of MFN2/Mfn2 and concomitant decrease in autophagy/mitophagy were accompanied by apoptosis induction. Importantly, MFN2 re-expression rescued autophagy/mitophagy levels and reversed apoptosis. Reinforcing the great dependence of these two cellular processes on each other, Chen et al. (2020) further showed that such protective effects of MFN2 over apoptosis are dependent on the autophagic flux during IVDD (Chen et al., 2020). In sum, these reports correlate disease to decreased MFN2, to decreased mitophagy and to induction of apoptosis. Inversely, in CMT2A neuropathy models, where MFN2 itself is present in a mutated form, mitophagy was induced and apoptosis was reduced (Rizzo et al., 2016). Indeed, using motor neurons differentiated from CMT2A patient fibroblasts-derived iPSCs as a cellular model of the disease, the authors observed increased autophagic clearance of mitochondria and decreased levels of BAX, caspase 8 and caspase 3 cleavage as well as increased levels of the anti-apoptotic BCL-2 protein (Rizzo et al., 2016). In conclusion, mitofusins, especially MFN2, regulate cell death by mediating mitophagy (**Figure 3**).

MFN2 AND MITOPHAGY IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

A high-incidence disease where a strong interplay between mitophagy and apoptosis can be found is NAFLD, which affects around 25% of the world's population and is the most common hepatic disease in the western countries (Kumar et al., 2020). It is a step-wise liver disease characterized by a spectrum of heterogeneous clinical manifestations. It is initiated by the excessive accumulation of fat in the liver, named steatosis. Although patients can exhibit steatosis without further complications, in many cases steatosis evolves to hepatic inflammation, fibrotic restructuring, cirrhosis, hepatocellular carcinoma and ultimately liver failure (Parthasarathy et al., 2020; **Figure 4**). Other disorders can constitute risk factors for NAFLD development, such as type 2 diabetes mellitus, obesity, metabolic syndrome, cardiovascular disease, and chronic kidney disease (VanWagner and Rinella, 2017; Li A. A. et al., 2020). Interestingly, a bidirectional link was observed for some of these, since NAFLD is a risk factor for the development of cardiovascular disease, atherosclerosis and chronic kidney disease (VanWagner and Rinella, 2017).

NAFLD and Mitochondria

There are numerous scientific reports showing an induction of apoptosis in the progression of NAFLD (Feldstein et al., 2003; Ferreira et al., 2011; Li C. P. et al., 2014; Alkhouri et al., 2015; Gonçalves et al., 2015; Kanda et al., 2018). Enhanced apoptosis in NAFLD hepatocytes correlates with activation of caspases (Feldstein et al., 2003; Ferreira et al., 2011) and both FAS -an apoptosis signal transduction factor- and its ligand -FAS-L- were found upregulated in NAFLD (Feldstein et al., 2003; Li C. P. et al., 2014; Alkhouri et al., 2015). NAFLD and mitochondria are also intimately related, being mitochondrial dysfunction and structural changes hallmarks of this hepatic disease. Upon high lipid intake, when adipocyte storage is no longer sufficient, hepatocytes uptake, store and metabolize lipids as well (Singh et al., 2009). In the liver, free fatty acids can then either undergo β-oxidation within mitochondria or be esterified into triglycerides (**Figure 3**). In early stages of NAFLD, mitochondria upregulate their activity, resolving lipid overload. However, continuous excess of lipid intake can impair mitochondrial function (Flores-Toro et al., 2016; Lee et al., 2019). Both NAFLD patient biopsies and animal models frequently present functional impairment of β-oxidation and of the respiratory chain and altered mitochondrial morphology (Paradies et al., 2014). Hence, a failure in mitochondrial quality control by mitophagy, and the subsequent accumulation of dysfunctional mitochondria, can contribute to the pathological accumulation of fatty acids in the liver (**Figure 3**). Consistently, reduced levels of PINK1/Parkin and thus reduced mitophagy was found in a NAFLD mouse model, upon high-fat diet (Gonçalves et al., 2015). Moreover,

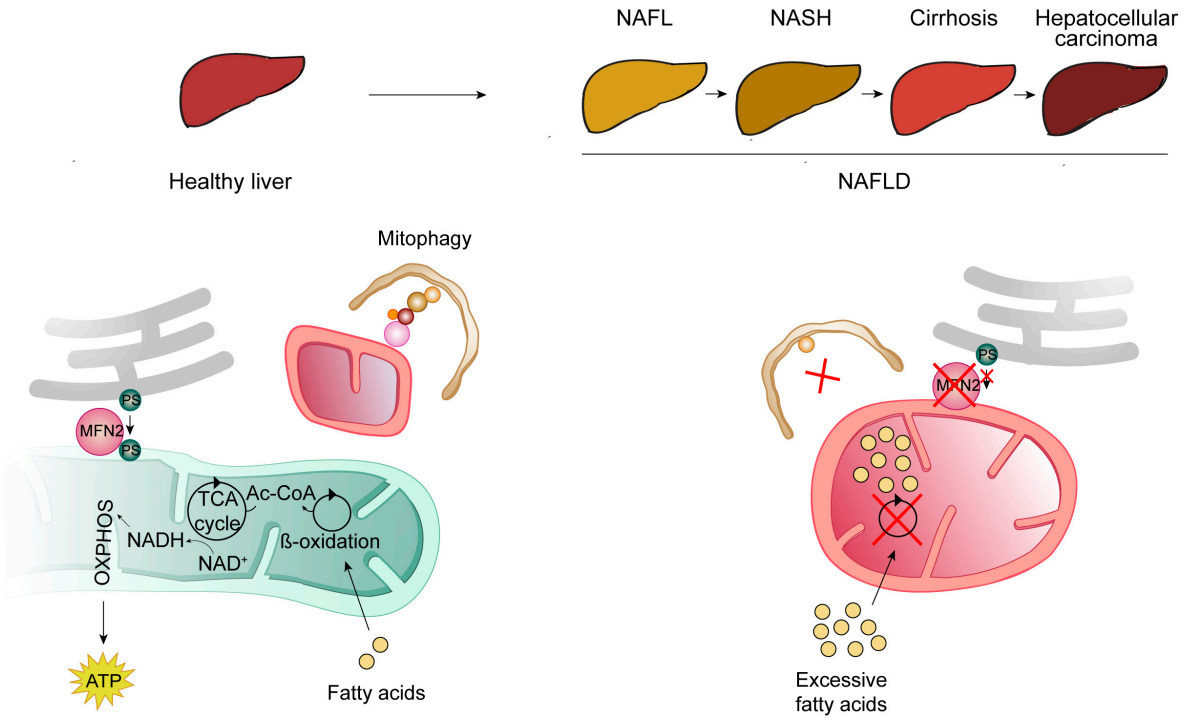

FIGURE 4 | Mitochondrial contribution for the development of non-alcoholic fatty liver disease (NAFLD). NAFLD consists of several heterogeneous clinical manifestations, usually organized in a development-stepwise-spectrum. The first step (NAFL) consists of the pathological accumulation of fat in the hepatocytes, due to excessive lipid intake and insufficient adipose storage. NAFL can evolve to further steatosis, accompanied by inflammation and scarring [(non-alcoholic steatohepatitis (NASH)], followed by cirrhosis and, ultimately, hepatocellular carcinoma. Mitochondrial dysfunction, also caused by low levels of MFN2, contributes to the development of NAFLD. In a healthy liver (left panel), free fatty acids are metabolized by β-oxidation into Acetyl-CoA (Ac-CoA), which can then enter the citric acid (TCA) cycle, from which NADH is produced and further used by the oxidative phosphorylation chain (OXPHOS) to produce energy in the form of ATP. These healthy mitochondria are kept in check by mitophagy (for details see **Figure 1**). Also in a healthy situation (left panel), MFN2 was proposed to serve as a tether between mitochondria and ER and thereby to also bind to and promote the transfer of phosphatidylserine (PS) from the ER to mitochondria. In a situation of excessive lipid intake and NAFLD development (right panel), mitophagy is impaired, leading to the accumulation of dysfunctional mitochondria, unable to metabolize fatty acids, which accumulate in hepatic mitochondria and lead to steatosis. Furthermore, the absence of MFN2 impairs PS transfer to mitochondria, affecting further lipid biosynthesis, consequently leading to ER stress in hepatocytes.

PINK1/Parkin-dependent induction of mitophagy, by the administration of quercetin–a plant flavonoid–rescued hepatic steatosis, both in *in vitro* and *in vivo* models of NAFLD (Liu et al., 2018). Similar beneficial results were obtained when using low doses of sorafenib, an anti-tumor drug used in hepatocellular carcinoma treatment (Jian et al., 2020). Sorafenib prevented progression of non-alcoholic steatohepatitis (NASH, the primary stage of NAFLD), in mice and monkeys, by inducing mitochondrial uncoupling, associated with activation of mitophagy. Importantly, endurance training reversed the status of several high fat-diet markers, leading to a decrease in Ca^{2+}-dependent mitochondrial swelling, in both BAX and caspase 8 and 9 activities and also a rescue of PINK1/Parkin levels and of the mitochondrial biogenesis markers TFAM and PGC-1α (Gonçalves et al., 2015). Thus, exercise can help mitigate over-nutrition damage in mitochondrial functions and reverse apoptosis induction, preventing the development of NAFLD (Gonçalves et al., 2015).

Mechanistically, it is not well understood how mitophagy is impaired in NAFLD. However, recent studies under high-fat diet brought some insights onto why lack of mitophagy and enhanced apoptosis disrupt liver functionality. Notably, these NAFLD mouse models revealed increased levels of nuclear receptor subfamily 4 group A member 1 (Nr4a1), which repressed Bnip3 (Zhou et al., 2017). BNIP3 protein levels are regulated by nutrient availability, being reduced in fasting conditions. Moreover, although first annotated as a cell-death regulatory factor, several studies reported roles of Bnip3 in mitochondrial dysfunction and in mitophagy, being recognized as a mitophagy receptor, which can also assist in cell survival (Gao et al., 2020). Absence of Bnip3 as well as high-fat diet lead to mitophagy inhibition along with increased cell death (Zhou et al., 2017). *BNIP3* deleted mice exhibit lipid accumulation and steatosis, *in vitro* and *in vivo*, consistent with increased hepatocyte lipogenesis and decreased β-oxidation of fatty acids (Glick et al., 2012). Furthermore, *BNIP3* null murine hepatocytes present an increase in mitochondrial mass, associated with a decline in mitochondrial function, consistent with defective mitophagy (Glick et al., 2012). In NAFLD mouse models, loss of Nr4a1 relieved Bnip3 repression and re-activated mitophagy. Importantly, it also mitigated NAFLD-phenotypes, namely mice body and liver weight, hepatocyte vacuolation, hepatic lipid

accumulation, steatosis and hepatic fibrosis (Zhou et al., 2017). Melatonine, used as anti-oxidant, anti-inflammatory and anti-obesogenic drug, also attenuated NAFLD-associated phenotypes induced by high-fat diet, suggesting it could possibly be used as a supplement for the treatment of NAFLD (Zhou et al., 2017).

High-fat stress, both *in vivo* and *in vitro*, also led to upregulation of the growth suppressor Mst1 (Zhou T. et al., 2019). *Mst1* deletion rescued the metabolic NAFLD signature phenotypes, namely body weight, blood glucose levels, triglycerides levels, total cholesterol and levels of lipid metabolism enzymes (Zhou T. et al., 2019). Equally, *Mst1* deletion suppressed abnormal liver structure, liver weight, hepatocytes size and liver fibrosis. The effects of hepatic injury reversion attained by *Mst1* deletion were further attributed to a reduction in oxidative stress and inflammation response. Consistently, *Mst1* deletion ameliorated hepatic steatosis (Jeong et al., 2018). Finally, downregulation of *Mst1* also rescued mitochondrial potential and prevented mPTP opening, CytC release and caspase activation (Zhou T. et al., 2019). Moreover, rescue of apoptosis was dependent on Parkin (Zhou T. et al., 2019), pointing to a strong correlation between apoptosis and mitophagy in the development of NAFLD. Besides NAFLD, MST1/Mst1 also repressed mitophagy during cardiac ischemia-reperfusion injury and colorectal cancer (Li et al., 2018; Yu et al., 2019). Thus, although still to be mechanistically defined, Mst1 appears to generally cause inhibition of mitophagy. In conclusion, Mst1 and Nr4a1 inhibition restored mitophagy and reduced cell death, alleviating high-fat stress, of clinical relevance in the context of NAFLD.

Despite most studies reporting an inhibition of mitophagy at the basis of NAFLD development, mitophagy induction along with hepatocytes' apoptosis was also observed (Pang et al., 2018). Upon oleic acid treatment, shown to induce NASH in HepG2 cells (Cui et al., 2010), reticulophagy is induced, preventing the death of hepatocytes. However, continuous lipid intake induced mitophagy, accompanied by an increase in hepatocytes' apoptosis, which could be prevented by PINK1 downregulation (Pang et al., 2018). Nevertheless, why excessive lipid intake caused mitophagy induction, rather than the mostly described mitophagy failure and mitochondrial dysfunction, is still unclear.

NAFLD and MFN2

Morphological alterations of mitochondria, characterized by very enlarged organelles, have been broadly observed upon liver dysfunctions, constituting a hallmark of NAFLD and alcoholic liver injury (Caldwell et al., 1999; Wakabayashi, 2002; Noureddin et al., 2013; Lotowska et al., 2014; Neuman et al., 2014; Kleiner and Makhlouf, 2016; Yamada et al., 2018). Importantly, two recent studies provided causal implications of mitochondrial shape and dynamics proteins in NAFLD (Yamada et al., 2018; Hernández-Alvarez et al., 2019). First, blocking either fusion or fission was harmful in hepatocytes, suggesting that extreme mitochondrial lengths–fragmented or hypertubular–affected liver functionality (Yamada et al., 2018). Consistently, mitochondrial stasis, created by simultaneously blocking mitochondrial division (Drp1 knockout) and fusion (Opa1 knockout) re-established mitochondrial size and mitigated pathological markers. Moreover, re-establishment of mitochondrial size rescued mitophagy and liver damage (Yamada et al., 2018). Second, MFN2 was proposed to directly assist in phosphatidylserine (PS) transfer from the ER into mitochondria, thus protecting against NAFLD (Hernández-Alvarez et al., 2019; **Figure 3**). In this study, both liver biopsies from NASH patients and mouse models of steatosis showed reduced Mfn2 levels. Consistently, murine liver-specific ablation of *Mfn2* led to abnormal lipid metabolism, chronic hepatic inflammation, apoptosis, fibrosis and liver cancer (Hernández-Alvarez et al., 2019). Mechanistically, *Mfn2* ablation in the liver was accompanied by a decrease in the levels of phosphatidylserine synthase 1 and 2. Moreover, Mfn2 was able to selectively bind PS, indicating a direct role of MFN2 in phospholipid regulation (Hernández-Alvarez et al., 2019). The

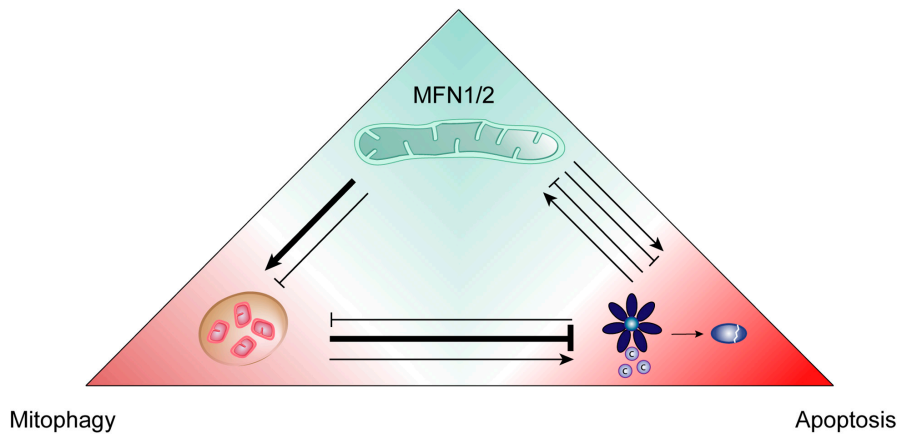

FIGURE 5 | Positive and negative interactions between mitofusins, mitophagy and apoptosis. The quality control processes of mitophagy and apoptosis can positively and negatively regulate each other and, in turn, this interplay can be itself influenced by mitofusins (MFN1/2). Mitophagy mostly behaves as an anti-apoptotic mechanism, promoting cellular survival. However, it can also contribute to cell death by apoptosis. Mitofusins add another layer of regulation since they are known to promote both mitophagy and apoptosis. Nevertheless, these proteins can also be found to negatively impact mitophagy induction.

authors therefore suggested that MFN2 hepatic deficiency leads to inefficient PS transfer from ER to mitochondria. Then, the subsequent inability to synthetize other phospholipids, such as phosphatidylethanolamine, causes ER stress (Hernández-Alvarez et al., 2019). Indeed, ER stress was shown to induce NAFLD-related phenotypes (DeZwaan-McCabe et al., 2013). Finally, re-expression of Mfn2 was able to restore normal liver metabolism, suggesting therapeutic potential (Hernández-Alvarez et al., 2019). In conclusion, MFN2 downregulation, mitophagy defects and pathological accumulation of lipids are determinant in the development of NAFLD disease.

CONCLUDING REMARKS

The crosstalk between the major surveillance mechanisms of mitophagy and apoptosis amplifies the cellular capacity to ensure cellular homeostasis. Recent studies placed mitofusins at the cross-roads of these two quality control processes (**Figure 5**). The identification of mitofusins' manifold central functions, as described here, provides the basis for future mechanistic understanding of this interdependence. Future studies will certainly shed light on the molecular details involving mitofusins in mitophagy, apoptosis and their interplay. As such, mitofusins hold promise for developing targeted therapeutic approaches.

AUTHOR CONTRIBUTIONS

Both authors conceived the study and the figures. MJ wrote the manuscript and drew the figures. ME-H coordinated the study. Both authors contributed to the article and approved the submitted version.

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REVIEW
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Mitophagy Receptors in Tumor Biology

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Mitochondria are multifunctional organelles that regulate cancer biology by synthesizing macromolecules, producing energy, and regulating cell death. The understanding of mitochondrial morphology, function, biogenesis, fission and fusion kinetics, and degradation is important for the development of new anticancer strategies. Mitophagy is a type of selective autophagy that can degrade damaged mitochondria under various environmental stresses, especially oxidative damage and hypoxia. The key regulator of mitophagy is the autophagy receptor, which recognizes damaged mitochondria and allows them to enter autophagosomes by binding to MAP1LC3 or GABARAP, and then undergo lysosomal-dependent degradation. Many components of mitochondria, including mitochondrial membrane proteins (e.g., PINK1, BNIP3L, BNIP3, FUNDC1, NIPSNAP1, NIPSNAP2, BCL2L13, PHB2, and FKBP8) and lipids (e.g., cardiolipin and ceramides), act as mitophagy receptors in a context-dependent manner. Dysfunctional mitophagy not only inhibits, but also promotes, tumorigenesis. Similarly, mitophagy plays a dual role in chemotherapy, radiotherapy, and immunotherapy. In this review, we summarize the latest advances in the mechanisms of mitophagy and highlight the pathological role of mitophagy receptors in tumorigenesis and treatment.

Keywords: mitophagy, cancer, cell death, autophagy, mitochondria

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INTRODUCTION

Autophagy, which was first observed under an electron microscope by Belgian scientist Christian de Duve in the 1950s, is a cellular phenomenon of "self-eating" by lysosomes (Yang and Klionsky, 2010). At present, based on the transport mode of cytosolic cargoes to lysosomes, autophagy is mainly divided into three types: macroautophagy (hereafter referred to as autophagy), microautophagy, and chaperone-mediated autophagy (Dikic and Elazar, 2018). As an important degradation mechanism, the process of autophagy involves the formation of lipid-related autophagosomes by wrapping various cargoes (e.g., damaged organelles, unused proteins, and invading pathogens), and then fusing them with lysosomes to form autophagosomes and degrading their contents (Klionsky and Emr, 2000; Xie et al., 2020b). At the molecular level, autophagy-related (ATG) genes and proteins play a vital role in regulating the dynamic formation of autophagic membrane structures, mainly through protein-protein interactions (Kang et al., 2011; Dikic and Elazar, 2018; **Figure 1**). These ATG protein interactions are further modulated by various factors, especially kinase-mediated protein posttranslational modification (McEwan and Dikic, 2011; Xie et al., 2015). Generally, the activation of autophagy is an important defense mechanism that promotes cell survival and recovery under harmful stresses, such as starvation and hypoxia

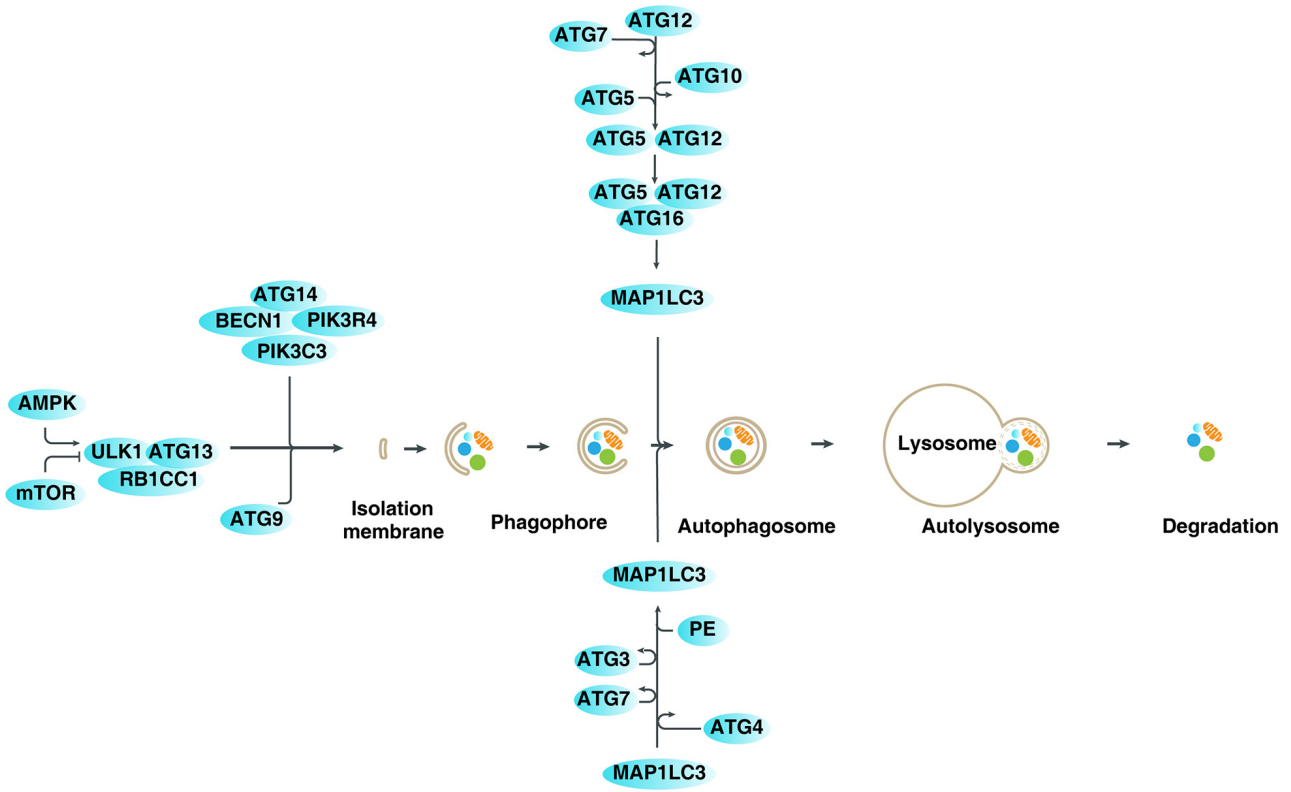

FIGURE 1 | Mechanisms and regulation of autophagy in mammalian cells. Autophagy is a dynamic process involving the formation of several specific membrane structures, such as phagophores, autophagosomes, and autolysosomes. ATG proteins, in association with various regulators, are involved in regulating the dynamic process of membrane structure formation, leading to the degradation of various cargoes in lysosomes.

(Kroemer et al., 2010). The autophagic degradation products can be reused for protein synthesis and energy production, although the underlying mechanism of this process is unclear. In contrast, an excessive activation of autophagy may lead to cell death, which is called autophagy-dependent cell death (Bialik et al., 2018; Galluzzi et al., 2018; Tang et al., 2019). In particular, recent studies indicate that ferroptosis is a type of autophagy-dependent cell death (Hou et al., 2016; Bai et al., 2019; Li et al., 2020; Xie et al., 2020a), highlighting the importance of autophagy in the degradation of proteins involved in iron and lipid metabolism (Zhou et al., 2020; Liu et al., 2020). It is also worth noting that the term "autophagic cell death" is used to describe the phenotype of increased autophagy during the induction of cell death, regardless of the effect of autophagic response on cell fate (Kroemer and Levine, 2008). Therefore, autophagy plays a dual role in cell survival and cell death, which is related to human disease, especially cancer and neurodegenerative diseases (Levine and Kroemer, 2019).

Depending on whether specific autophagic receptors (also known as autophagic adaptor proteins) are needed to degrade specific substrates, autophagy can be non-selective or selective (Zaffagnini and Martens, 2016). In recent years, a large number of types of selective autophagy have been found to regulate cell homeostasis, such as mitophagy (Harper et al., 2018), pexophagy (Cho et al., 2018), lipophagy (Kounakis et al., 2019), ferritinophagy (Mancias et al., 2014), and clockophagy (Yang et al., 2019). Among them, mitophagy is the most-studied selective autophagy, which eliminates damaged or aging mitochondria by recognizing different components of mitochondrial structure via various autophagy receptors (Lemasters, 2005). Dysregulated mitophagy is closely related to many physiological and pathological processes, such as aging, neurodegenerative diseases, and cancer (Palikaras et al., 2018). In this review, we first introduce the structure and function of mitochondria, and then focus on the molecular mechanisms of mitophagy. Finally, we describe the pathologic role of mitophagy regulators in tumor development and therapy, and will discuss new directions for cancer treatment.

Structure and Function of Mitochondria

Mitochondria are double-membrane organelles present in most eukaryotic cells, and their size, number, and appearance are different on different cells (Herst et al., 2017; Pfanner et al., 2019). Like chloroplasts in plants and algae, mitochondria may have evolved from primitive bacteria (Gray, 2012). The main chemical components of mitochondria include water, protein, and lipids. In addition, mitochondria have a small amount of small molecules, such as coenzymes and nucleic acids. Proteins, including soluble and insoluble proteins, account for 65 to 70% of the dry weight of mitochondria. Soluble proteins are

mainly the enzymes located in the mitochondrial matrix and the periphery of the membrane, whereas insoluble proteins constitute the main body of the membrane, part of which is composed of mosaic proteins or enzymes (Pfanner et al., 2019). Lipids in mitochondria are mainly distributed in two layers of membranes, accounting for 20 to 30% of the dry weight. Phospholipids in mitochondria account for more than 75% of total lipids. The amount of phospholipids in the mitochondrial membrane of different tissues of the same organism is relatively stable. Abundant cardiolipin and less cholesterol are the obvious differences between the structure of mitochondria and other cell membranes (Montero et al., 2010).

From the outside to the inside, the mitochondria can be divided into four functional areas: the outer mitochondrial membrane (OMM), the intermembrane space (the space between the outer and inner membranes), the inner mitochondrial membrane (IMM), and the matrix (space within the inner membrane) (Pfanner et al., 2019). The OMM is smoother and acts as the boundary membrane of organelles, while the IMM folds inward to form mitochondrial cristae (e.g., lamellar cristae, tubular cristae, and vesicular cristae), which complicate biochemical reactions. Mitochondria are the main sites for oxidative phosphorylation and synthesis of adenosine triphosphate (ATP) in cells, and provide chemical energy for cellular activities as the "powerhouse of the cell." In addition to supplying energy for cells, mitochondria are also involved in various processes, such as cell differentiation, signal transduction, cell growth, the cell cycle, and cell death (Herst et al., 2017; Bock and Tait, 2020). Dysfunctional mitochondria are unable to execute oxidative phosphorylation and consequently accumulate reactive oxygen species (ROS) in the cells. Mitochondrial oxidative stress is associated with a variety of pathologies, especially age-related diseases (e.g., cancer). In order to avoid mitochondrial dysfunction, some conservative mechanisms have evolved to control the quality of mitochondria. Among them, mitophagy plays a central role in preventing mitochondrial damage by promoting mitochondrial turnover. Understanding the signal transduction and molecular modification of mitophagy is important for improving the homeostasis of mitochondria (Palikaras et al., 2018).

Molecular Mechanisms of Mitophagy

Mitochondrial depolarization refers to the process in which the membrane potential of the mitochondria changes from negative to positive in the direction of depolarization (Zorova et al., 2018). During the electron transport process, as electrons flow down the chain of the redox complex located in the IMM, protons flow into the space between the IMM and the OMM. Therefore, the intermembrane space becomes positive, and the IMM becomes electrochemically polarized. The backflow of protons is related to the production of ATP. In this state, the mitochondria are polarized. When proton flow is independent of ATP production, mitochondria are considered to be depolarized (Zorova et al., 2018). Fission-induced mitochondrial depolarization is an important factor that triggers mitophagy to reduce oxidative stress (Twig and Shirihai, 2011). The molecular mechanisms involved in mitophagy are complex, and recognition of depolarized mitochondria requires a variety of cargo receptors and regulators. In general, mitophagy can be mediated through ubiquitin (Ub)-dependent and Ub-independent receptor pathways, as described below (Harper et al., 2018; **Figure 2**).

Ub-Dependent Receptors

PTEN-induced kinase 1 (PINK1) is a serine/threonine protein kinase that localizes to mitochondria (Valente et al., 2004). Parkin RBR E3 ubiquitin protein ligase (PRKN/PARKIN/PARK2) is a component of the multiprotein E3 ubiquitin ligase complex, which can catalyze the covalent attachment of the ubiquitin part to the substrate protein (Shimura et al., 2000). Mutations in PINK1 and PRKN are implicated in Parkinson's disease, an aging-related disease associated with mitochondrial abnormalities and motor nerve damage (Pickrell and Youle, 2015). Importantly, the activation of the PINK1-PRKN pathway is the first and most studied regulatory mechanism of mitophagy. When mitochondria are damaged or depolarized, PINK1 stabilizes on the OMM, where it recruits and activates PRKN (Vives-Bauza et al., 2010). After being transported from the cytoplasm to the mitochondria, PRKN ubiquitinates many OMM proteins [e.g., translocase of outer mitochondrial membrane 20 (TOMM20/TOM20), mitofusin 1 (MFN1), and mitofusin 2 (MFN2)], resulting in the recruitment of autophagy receptors such as sequestosome 1 (SQSTM1/p62) and optineurin (OPTN) (Geisler et al., 2010; Wong and Holzbaur, 2014). The ubiquitinated proteins with autophagy receptors are then bound to the autophagosome-associated proteins [microtubule-associated protein 1 light chain 3 (MAP1LC3/LC3/Atg8) or GABA type A receptor-associated protein (GABARAP)] via their LC3 interacting domain (LIR) to direct the isolation membrane/phagophore of growing autophagosomes to surround damaged mitochondria (Wild et al., 2014). Finally, engulfed mitochondria are degraded and eliminated in autolysosomes (Geisler et al., 2010; Wong and Holzbaur, 2014). The activity and function of PINK1 or PRKN in mitophagy is further regulated by various binding partners and changes in mitochondrial dynamics (e.g., mitochondrial fission). Of note, some mammalian cells (Eiyama and Okamoto, 2015) do not express PINK1 or PRKN, indicating that PINK1- and PRKN-mediated mitophagy may have tissue and cell-specific effects in preventing mitochondrial dysfunction.

Ub-Independent Receptors

BCL2 Interacting Protein 3-Like

The BCL2 apoptosis regulator (BCL2) family includes pro-apoptotic proteins [e.g., BCL2-associated X, apoptosis regulator (BAX), BCL2 antagonist/killer 1 (BAK1/BAK), and BH3 interacting domain death agonist (BID)] and anti-apoptotic proteins [e.g., BCL2 and BCL2-like 1 (BCL2L1/BCLXL)]. Members of the anti-apoptotic BCL2 protein family are overexpressed in many malignant tumors and become targets for tumor treatment (Adams and Cory, 2007). In addition to regulating mitochondrial apoptosis by controlling mitochondrial outer membrane permeabilization, the OMM protein BCL2 interacting protein 3-like (BNIP3L/NIX, a pro-apoptotic BCL2

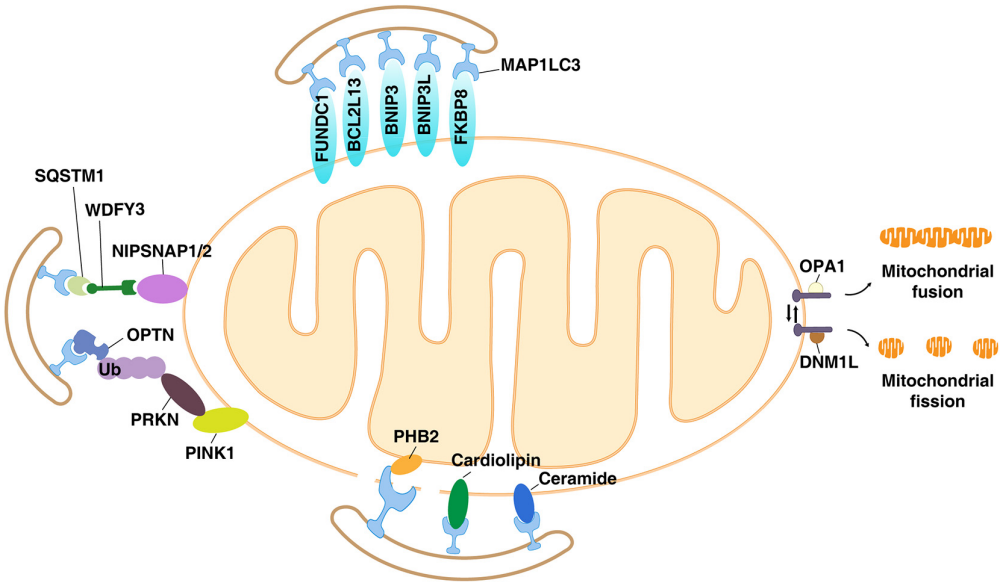

FIGURE 2 | The Ub-dependent and Ub-independent receptor pathways of mitophagy. Many components of the mitochondria, including mitochondrial membrane proteins (e.g., PINK1, BNIP3L, BNIP3, FUNDC1, NIPSNAP1, NIPSNAP2, BCL2L13, PHB2, and FKBP8) and lipids (e.g., cardiolipin and ceramides), act as mitophagy receptors in a context-dependent manner.

family member) is involved in mediating mitophagy (Schweers et al., 2007; Sandoval et al., 2008). Unlike binding BCL2 during apoptosis (Zhang and Ney, 2009), BNIP3L directly binds to MAP1LC3 or GABARAP during mitophagy (Schwarten et al., 2009). Moreover, BNIP3L-mediated mitophagy may not be associated with the ubiquitination of BNIP3L, indicating that it is an Ub-independent receptor for mitophagy (Ney, 2015).

Functionally, the activation of BNIP3L-dependent mitophagy is essential for the programmed mitochondrial elimination during erythroid maturation, and BNIP3L-depleted mice show anemia (Schweers et al., 2007; Sandoval et al., 2008). Furthermore, BNIP3L instead of PRKN is responsible for mitophagy induction in HeLa cells (a cell line derived from patients with cervical cancer) (Ding et al., 2010). In addition, transcriptional factor hypoxia-inducible factor 1 subunit alpha (HIF1A/HIF1α)-mediated BNIP3L upregulation is required for hypoxia-induced mitophagy (Sowter et al., 2001), indicating a potential role of BNIP3L-mediated mitophagy in hypoxic tumor microenvironments (TMEs). Structurally, BNIP3L positioning on OMM requires the transmembrane domain, and BNIP3L dimerization is responsible for MAP1LC3 recruitment (Marinkovic et al., 2020). BNIP3L further binds to MAP1LC3 at the amino terminus of BNIP3L through the LIR motif (Schwarten et al., 2009). These structural studies provide information for the development of BNIP3L-targeted drugs.

BCL2 Interacting Protein 3

BCL2 interacting protein 3 (BNIP3) is a BH3-only protein and acts as a pro-apoptotic BCL2 family member (Vande Velde et al., 2000). It interacts with the anti-apoptotic BCL2, thereby overcoming the inhibitory effect of BCL2 on apoptosis (Zhang and Ney, 2009). BNIP3 at the OMM regulates the opening of the pores in the mitochondrial double membrane to mediate the transport of lysosomal proteins from the cytoplasm to the mitochondrial matrix, thereby leading to the degradation of damaged proteins in the mitochondria in response to oxidative damage (Zhang and Ney, 2009). BNIP3 also has a LIR domain through which it interacts with MAP1LC3 and mediates mitochondrial degradation through mitophagy (Novak et al., 2010; Park et al., 2013). Like its homolog BNIP3L, BNIP3 forms a dimer during mitophagy and its expression is regulated by HIF1A during hypoxia (Sowter et al., 2001; O'Sullivan et al., 2015). BNIP3 is also highly expressed in the hypoxic environment of solid tumors. Although both BNIP3 and BNIP3L mediate hypoxia-induced mitophagy (Sowter et al., 2001), the functional complementarity and differences of these two proteins in cancer-related mitophagy remain largely unclear. In addition, BNIP3 may affect the fission or fusion of mitochondria by binding to OPA1 mitochondrial dynamin-like GTPase (OPA1) or dynamin 1-like (DNM1L/DRP1), thereby promoting mitophagy (Lee et al., 2011). These findings highlight the role of mitochondrial dynamics in regulating mitophagy.

FUN14 Domain Containing 1

In addition to BNIP3L and BNIP3, FUN14 domain containing 1 (FUNDC1) was also found to be expressed in OMM as an autophagy receptor for mitophagy during hypoxia (Liu et al., 2012). The activity of FUNDC1 in hypoxia-induced mitophagy is regulated by phosphorylation and dephosphorylation events. Unc-51–like autophagy-activating kinase 1 (ULK1/ATG1) is the only kinase of the ATG family and a component of the ULK1-ATG13 RB1-inducible coiled-coil 1 (RB1CC1) complex, which initiates the formation of autophagosomes in mammalian cells (Hosokawa et al., 2009; Jung et al., 2009). ULK1 has

many phosphorylation sites, and these phosphorylation sites have different functions in regulating autophagy (Xie et al., 2015). Phosphorylation of ULK1 at serine17 mediates ULK1 translocation to mitochondria and subsequently binds to FUNDC1 during hypoxia (Wu et al., 2014). In contrast, the dephosphorylation of FUNDC1 by PGAM family member 5, mitochondrial serine/threonine protein phosphatase (PGAM5) under hypoxia increases its binding to MAP1LC3 through LIR, and recruits the isolation membrane that binds to MAP1LC3, further forming autophagosomes to engulf damaged mitochondria in mammalian cells (Liu et al., 2012; Chen et al., 2014). Certain proteins [e.g., mitochondrial E3 ligase membrane-associated ring CH-type finger 5 (MARCHF5/MARCH5) and cytosolic molecular chaperone heat shock protein family A (hsp70) member 8 (HSPA8/HSC70)] bind to FUNDC1, which further regulate the protein stability of FUNDC1 to fine-tune mitophagy during hypoxia (Chen et al., 2017; Li et al., 2019b). Interestingly, the PRKN-mediated ubiquitination may promote the transport of MARCHF5 from mitochondria to peroxisomes, resulting in a decrease in mitophagy (Koyano et al., 2019). FUNDC1 also acts as a platform for regulating mitochondrial dynamics (e.g., fission and fusion) and mitophagy by interacting with DNM1L and OPA1 (Chen et al., 2016). In particular, the dissociation of FUNDC1 from DNML1 to form a complex with OPA1 inhibits mitochondrial fission and mitophagy (Chen et al., 2016). These findings further support the idea that mitochondrial dynamics and quality control are inseparably intertwined.

The 4-Nitrophenylphosphatase Domain and Non-neuronal SNAP25-Like Protein Homolog 1

Both 4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (NIPSNAP1) and NIPSNAP2 play a major role in vesicular transport (Seroussi et al., 1998). Under normal conditions, they are located in the IMM and act as modulators of calcium channels (Brittain et al., 2012). However, they also localize to the OMM during mitochondrial depolarization to recruit autophagy receptors, MAP1LC3 homologs, and other proteins, and effectively serve as an "eat me" signal for triggering PRKN-dependent mitophagy (Princely Abudu et al., 2019). For example, the recruitment of autophagy receptors, such as calcium binding and coiled-coil domain 2 (CALCOCO2/NDP52), SQSTM1, NBR1 autophagy cargo receptor (NBR1), tax1-binding protein 1 (TAX1BP1), and WD repeat and FYVE domain containing 3 (WDFY3/ALFY), to depolarized mitochondria is mediated by NIPSNAP1 and NIPSNAP2 during mitophagy (Princely Abudu et al., 2019). Accordingly, NIPSNAP1 and NIPSNAP2, which require OMM localization, interact with MAP1LC3 or GABARAP as preferred interaction partners (Princely Abudu et al., 2019). Although zebrafish lacking Nipsnap1 show a decrease in mitochondria in the brain, which is coupled with the production of ROS, the loss of dopaminergic neurons, and a strong decrease in movement (Princely Abudu et al., 2019), the impact of NIPSNAP1 or NIPSNAP2-mediated mitophagy in neurodegenerative disease in mice or humans remains unknown.

BCL2-Like 13

BCL2-like 13 (BCL2L13/Bcl-rambo) is an OMM protein, a member of the pro-apoptotic BCL2 family with four conserved BH domains (Murakawa et al., 2015). The overexpression of BCL2L13 induces caspase-3–dependent apoptosis, which can be blocked by co-expression of inhibitor of apoptotic proteins (IAPs) (Kataoka et al., 2001). However, unlike other BCL2 members, BCL2L13 does not require the BH domains to induce apoptosis, but instead relies on mitochondrial localization by the transmembrane domain (Kataoka et al., 2001). In addition to promoting apoptosis, BCL2L13 also acts as a homolog of Atg32 in mammalian cells, mediating mitochondrial fragmentation and subsequent mitophagy (Murakawa et al., 2015). The OMM protein Atg32 is an autophagy receptor for mitophagy in yeast, and interacts with Atg8 and Atg11 (Liu et al., 2012). BCL2L13 interacts with MAP1LC3 through a conserved LIR sequence, leading to autophagosome engulfment of damaged mitochondria (Otsu et al., 2015). BCL2L13-mediated mitochondria also require fission mechanisms to drive mitochondrial fragmentation (Murakawa et al., 2015). The BCL2L13 gene is involved in a wide range of cancers, but whether BCL2L13-mediated mitophagy affects tumor development is still poorly understood.

Prohibitin 2

Prohibitin 2 (PHB2) is a conserved protein found in the mitochondria and the nucleus of eukaryotic cells, and plays a role in development, lifespan regulation, and various cellular processes (including mitochondrial dynamics) (Wei et al., 2017; Zhou et al., 2018). Notably, PHB2 was initially identified as a specific repressor of estrogen receptor in the nucleus by competitively inhibiting the binding between nuclear receptor coactivator 1 (NCOA1/SRC-1) and estrogen receptors (Montano et al., 1999; Kasashima et al., 2006). PHB2 can combine with PHB1 to form a large ring complex on the mitochondrial membrane and act as a molecular chaperone to stabilize mitochondrial proteins, thereby supporting mitochondrial morphogenesis and preventing cell death (Tatsuta et al., 2005). Moreover, mitochondrial PHB2 acts as an autophagy receptor for the clearance of damaged mitochondria in mammalian cells and *C. elegans* (Wei et al., 2017). In many cases, the IMM protein requires the rupture of the OMM to recruit the mitophagy molecular machinery (including mitophagy receptors and MAP1LC3) (Wei et al., 2017). However, in some cases, this dynamic positional change of the IMM protein is not necessary for mitophagy. Alternatively, PHB2 may act as a direct autophagy receptor in the IMM and binds to MAP1LC3 through the classical LIR motif, thereby degrading mitochondria (Wei et al., 2017). However, PHB2 promotes PINK1-PRKN–mediated mitophagy in a MAP1LC3-independent manner via the presenilin-associated rhomboid-like (PARL)-PGAM5 axis (Yan et al., 2020). Thus, both OMM receptors and IMM receptors participate in mitophagy-mediated mitochondrial removal.

FKBP Prolyl Isomerase 8

FKBP prolyl isomerase 8 (FKBP8/FKBP38) is a member of the immunophilin family, which has a conserved peptidyl prolyl *cis*/*trans*-isomerase domain. FKBP8 not only plays a role in

immune regulation, but also participates in protein quality control (e.g., protein folding and trafficking) (Okamoto et al., 2006; Janssens et al., 2014; Xu et al., 2019). When combined with calmodulin and calcium, FKBP8 becomes active (Edlich et al., 2005). FKBP8 is anchored by the transmembrane domain and is mainly distributed in mitochondria (Shirane-Kitsuji and Nakayama, 2014). Mitochondrial FKBP8 acts as a molecular chaperone of BCL2 or heat shock proteins to inhibit apoptosis (Chen et al., 2008; Misaka et al., 2018). In addition to its anti-apoptotic function in response to various mitochondrial stresses, FKBP8 is also an autophagy receptor for damaged mitochondria (Bhujabal et al., 2017). FKBP8 has a typical LIR motif, and can strongly recruit MAP1LC3 to damaged mitochondria in HeLa cells during mitophagy (Bhujabal et al., 2017). Consequently, the overexpression of FKBP8 promotes mitochondrial fission, leading to mitophagy (Bhujabal et al., 2017). Unlike other autophagy receptors that usually degrade with cargo, FKBP8 escapes autophagosome degradation during mitophagy and instead relocates to the endoplasmic reticulum to bind BCL2 (Bhujabal et al., 2017). Thus, FKBP8 partially protects mitochondria from damage through mitophagy activation.

Mitochondrial Membrane Lipids

Cardiolipin is a diphosphatidylglycerol lipid, first found in animal hearts. It is an important component of the IMM, accounting for 20% of its total lipid composition (Paradies et al., 2014). In addition to mitochondria, cardiolipin can also be found in the membranes of most bacteria (Carranza et al., 2017). Cardiolipin homeostasis plays a key role in regulating mitochondrial function, and is involved in metabolism, cell death, and mitochondrial quality control (Dudek, 2017). For example, cardiolipin is necessary for the enzymatic activity of the respiratory chain complex and acts as a proton trap for oxidative phosphorylation (Dudek, 2017). The distribution of cardiolipin on the OMM not only triggers apoptosis, but also induces mitophagy to clear damaged mitochondria by interacting with MAP1LC3 (Chu et al., 2013), indicating that cardiolipin is an important eat me signal that regulates cell death and survival after mitochondrial injury.

Other lipids that contribute to mitophagy come from ceramides, which are composed of sphingosine and fatty acids. For example, C_{18}-ceramide synthesized by ceramide synthase 1 (CERS1) induces mitophagy and tumor suppression in head and neck squamous cell carcinoma cells (Sentelle et al., 2012) and acute myeloid leukemia cells (Dany et al., 2016) *in vitro* and *in vivo*. Mechanistically, ceramide can bind to MAP1LC3 on the mitochondrial membrane to trigger mitophagy after DNM1L-mediated mitochondrial fission (Dany and Ogretmen, 2015). These findings provide another strategy for removing damaged mitochondria through the phospholipid components of the mitochondrial membrane.

Mitophagy in Cancer

The role of autophagy in tumor biology is complex, which depends not only on the type of tumor, but also on the stage and context of the tumor (Levy et al., 2017). In general, autophagy plays a role in blocking the initiation of tumorigenesis because it inhibits genome instability and inflammation. In contrast, in established tumors, cancer cells may use autophagy to meet their metabolic requirements and enhance the resistance to cell death, leading to increased growth and invasiveness. Similarly, dysfunctional mitophagy is a characteristic phenomenon of cancer. Most mitophagy receptors or regulators are involved in cancer; however, whether they act as tumor promoters or tumor suppressors seems to be highly dependent on tumor type and TME (**Table 1**), which is described below (Panigrahi et al., 2019; Praharaj et al., 2019; Vara-Perez et al., 2019; Ferro et al., 2020).

Mitophagy Inhibits Tumorigenesis

The PINK1-PRKN pathway is considered to be the main pathway of mitophagy in cancer cells (Bernardini et al., 2017). A loss of PINK1 or PRKN function impairs mitochondrial quality control, which further leads to the accumulation of ROS, thereby affecting cell function. The mutation or depletion of PINK1 or PRKN is often detected in a variety of tumors, such as lung cancer, glioma, and colon cancer (Bernardini et al., 2017). For example, the PRKN gene and human colorectal cancer are obviously associated with adenomatous polyps, and the expression of PRKN can inhibit the proliferation of colon cancer cells (Poulogiannis et al., 2010). The hybridization of PRKN knockout mice with colorectal adenomatous polyposis mice significantly accelerates the development of intestinal adenomas in newborn mice, and the diversity of polyps is also significantly increased, indicating that PRKN is a tumor suppressor gene in colon cancer (Poulogiannis et al., 2010). In addition, in a KRAS-driven tumor model, the depletion of PINK1 or PRKN promotes pancreatic tumorigenesis in mice (Li et al., 2018). Mechanistically, PINK1- and PRKN-mediated autophagy degradation of mitochondrial iron importers [e.g., solute carrier family 25 member 37 (SLC25A37) and solute carrier family 25 member 28 (SLC25A28)] suppresses pancreatic tumors by attenuating mitochondrial iron accumulation, inflammasome activation, high-mobility group box 1 (HMGB1) release, and subsequent immune checkpoint expression (Li et al., 2018). Therefore, the pharmacological or genetic inhibition of mitochondrial iron-dependent signaling prolongs the survival of animals and reverses the phenotype of mitophagy deficient-mediated pancreatic tumors *in vivo* (Li et al., 2018). These findings suggest that PINK1-PRKN pathway-mediated mitophagy links iron metabolism to tumor immunity during tumor formation (Kang et al., 2019). Unlike extracellular HMGB1 that promotes tumor growth, intracellular HMGB1 can regulate autophagy and mitophagy to inhibit the development of pancreatic cancer (Tang et al., 2010, 2011; Kang et al., 2017; Kang and Tang, 2018).

As discussed above, the activation of HIF1A increases the expression of BNIP3 and subsequent mitophagy. In turn, the expression of BNIP3 may affect HIF1A stability. This HIF1A-BNIP3–mediated mitophagy pathway is also implicated in controlling tumorgenesis in some cancers, such as triple-negative breast cancer (TNBC) (Chourasia et al., 2015). In fact, during the metastasis of TNBC, HIF1A-dependent BNIP3 expression is often suppressed or absent (Chourasia et al., 2015). The combination of BNIP3 deletion and high HIF1A expression

TABLE 1 | Role of mitophagy regulators in tumorigenesis.

Mitophagy regulator	Tumor type	Function in cancer	Mechanisms	References
BNIP3	Breast tumor	Tumor suppressor	Inhibits glycolysis and angiogenesis	Chourasia et al., 2015
BNIP3	Pancreatic cancer	Tumor suppressor	Promotes hypoxia-induced cell death	Okami et al., 2004
BNIP3	Colorectal cancer	Tumor suppressor	Promotes hypoxia-induced cell death	Murai et al., 2005; Bacon et al., 2007
BNIP3	Gastric cancer	Tumor suppressor	Promotes hypoxia-induced cell death	Murai et al., 2005
BNIP3L	Pancreatic ductal adenocarcinoma	Tumor promoter	Increases glucose metabolism and antioxidant capacity	Humpton et al., 2019
Ceramide	Head and neck squamous cell carcinoma, acute myeloid leukemia cells	Tumor suppressor	Promotes cell death	Sentelle et al., 2012; Dany et al., 2016
FUNDC1	Hepatocellular carcinoma	Tumor suppressor	Inhibits inflammation	Li et al., 2019a
FUNDC1	Laryngeal cancer	Tumor promoter	Promotes cell proliferation and survival	Hui et al., 2019
FUNDC1	Cervical cancer	Tumor suppressor	Promotes apoptosis	Hou et al., 2017
PHB2	Cervical/non-small cell lung/colorectal cancer cells	Tumor suppressor	Promotes activation of PINK1-PRKN pathway	Yan et al., 2020
PHB2	Non-small cell lung carcinoma	Tumor promoter	Promotes cell proliferation and migration	Zhang et al., 2020
PINK1	Pancreatic ductal adenocarcinoma	Tumor suppressor	Inhibits inflammation and antitumor immunity	Li et al., 2018
PRKN	Pancreatic ductal adenocarcinoma	Tumor suppressor	Inhibits inflammation and antitumor immunity	Li et al., 2018; Yin et al., 2018
PRKN	Colon cancer	Tumor suppressor	Inhibits cell proliferation	Poulogiannis et al., 2010

predicts poor metastasis-free survival for TNBC (Chourasia et al., 2015). The increased aggressiveness of breast tumors in BNIP3-depleted mice is related to a decrease in mitophagy and the increased stability of HIF1A, indicating that BNIP3 can inhibit HIF1A and mitochondrial dysfunction (Chourasia et al., 2015). In addition, BNIP3 has a tumor suppressor effect in pancreatic cancer (Okami et al., 2004), colorectal cancer (Murai et al., 2005; Bacon et al., 2007), and gastric cancer (Murai et al., 2005), which is related to hypermethylation of the BNIP3 promoter. Whether the epigenetic silencing of BNIP3 can help reduce mitophagy and subsequent tumorigenesis remains unanswered.

FUNDC1 is another player in hypoxia-mediated mitophagy through its dephosphorylation (Liu et al., 2012). In cervical cancer, the expression of FUNDC1 was higher in tumors than in adjacent normal tissues (Hou et al., 2017). This high FUNDC1 expression is negatively correlated with tumor progression and patient prognosis, indicating a potential role of FUNDC1 in the suppression of tumor growth of cervical cancer (Hou et al., 2017). In addition, FUNDC1-mediated mitophagy protects laryngeal cancer cells against oxidative stress (Hui et al., 2019), which correlates with tumorigenic potential. Conditional knockout of FUNDC1 in the liver also initiates liver cancer by activating inflammation (Li et al., 2019a).

Mitophagy Promotes Tumor Progression

In some cases, the activation of a specific mitophagy pathway may promote tumor growth and development. Although both BNIP3 and BNIP3L are similar modulators of mitophagy and apoptosis, BNIP3L, unlike BNIP3 which inhibits tumorgenesis, plays an opposite role in promoting tumorigenesis. For example, the loss of BNIP3L in the KPC (*LSL-Kras*^{G12D}*; Tp53*^{R172H}*; Pdx1-Cre*) model of pancreatic ductal adenocarcinoma (PDAC) delays tumor occurrence, which is associated with reduced mitophagy and attenuated progression from the pancreatic intraepithelial neoplasia stage to PDAC (Humpton et al., 2019). These findings raise an unsolved question about the role of BNIP3L-dependent mitophagy in mutated KRAS and mutated TP53-driven tumorigenesis. One possibility is that different types of mitophagy may produce different TMEs, which further affects inflammation response and tumor immunity. It is also a challenge to distinguish the mitophagy-dependent and -independent role of BNIP3L in tumor biology.

Mitophagy and Tumor Therapies

The main reason for treatment failure in cancer is the resistance of cancer cells to drugs, which leads to tumor recurrence and metastasis. Dysfunctional autophagy and mitophagy lead to drug resistance through multiple mechanisms, including inhibiting cell death, especially apoptosis (Levy et al., 2017). Cancer stem cells (CSCs) are self-renewing cell types that contribute to tumor onset, expansion, drug resistance, recurrence, and metastasis after treatment (Reya et al., 2001; Ward and Dirks, 2007). Mitochondria are an important source of ROS within most cells, including cancer cells. Elevated ROS production is a powerful inducer of apoptosis during chemotherapy. Mitophagy-mediated mitochondria degradation limits the production of ROS, thereby exerting a cytoprotective effect during chemotherapy and helping CSCs resist apoptosis (Ianniciello et al., 2018; Levy et al., 2020). Reversing mitophagy-mediated protective mechanisms may be one of the ways to reverse CSC chemotherapy resistance (Wang et al., 2020). For example, leukemia stem cells (LSCs) are resistant to traditional chemotherapy drugs because LSCs can attain a lower rate of energy metabolism and ROS production through fission-dependent mitophagy (Pei et al., 2018). LSCs increase the expression of fission, mitochondrial 1 (FIS1) through the adenosine 5′-monophosphate-activated protein kinase (AMPK)

pathway (Pei et al., 2018). Blocking FIS1 gene expression blocks the mitophagy pathway by inhibiting glycogen synthase kinase 3 (GSK3) activity (Pei et al., 2018). The use of GSK3 inhibitors to target the AMPK-FIS1-GSK3-mediated mitophagy pathway may become a radical cure for acute myeloid leukemia (Pei et al., 2018). Doxorubicin (brand name: adriamycin) is used to treat different types of cancers. The inhibition of mitophagy enhances the anticancer activity of doxorubicin in colorectal cancer cells (Yan et al., 2017). Higher mitophagic levels are also found in CSCs in cisplatin-resistant oral squamous cell carcinoma and oxaliplatin-resistant human colorectal cancer (Naik et al., 2018; Takeda et al., 2019), supporting a widely pro-survival role of mitophagy in various chemo-resistant cancer cells.

In radiotherapy, increased mitophagy can also promote survival, which is mediated by the PINK1-PRKN pathway. Therefore, the inhibition of PINK1-PRKN–mediated mitophagy restores the radiosensitivity of tumor cells (Zheng et al., 2015). Temozolomide-perillyl alcohol (TMZ-POH) conjugate induces lysosomal dysfunction and subsequent impaired mitochondrial flux in non-small cell lung cancer cells and makes them sensitive to radiation, thereby showing TMZ-POH as a potential radiosensitizer (Chang et al., 2018). Ionizing radiation can trigger a series of cellular DNA damage responses, and the dynamic interaction between these responses and mitophagy remains to be revealed.

Compared with chemotherapy and radiotherapy, immunotherapy (e.g., using cytokines, antibodies, or immune checkpoint inhibitors) has shown emerging and great potential in inhibiting tumor growth. Accordingly, more research has focused on the dual roles of mitophagy in immunotherapy. On the one hand, inhibition of the mitophagic axis enhances tumor necrosis factor-based immunotherapy to control the survival and progression of cervical and gastric cancer cells (Yan et al., 2018; Zhao et al., 2019). On the other hand, enhanced mitophagy may induce immunogenic cell death, thereby inhibiting tumor growth through the activation of cytotoxic T lymphocytes in liver cancer cells (Yu et al., 2020). These findings further support that mitophagy may be an effective target for modified tumor immunotherapy.

CONCLUSION AND PERSPECTIVES

Mitochondria are multifunctional organelles that play an important role in cancer through the synthesis of macromolecules, energy production, and cell death regulation. Understanding the regulation of mitochondrial morphology, function, biogenesis, fission and fusion dynamics, and degradation is important for the development of new anticancer strategies. Dysfunctional mitophagy is a feature of the TME in many cancers and plays multiple roles in regulating tumor metabolism. On the one hand, mitophagy prevents the accumulation of damaged mitochondria, thereby maintaining energy production for tumor growth. On the other hand, mitophagy may suppress tumors by limiting the production of ROS, which is a well-known factor in causing gene mutation and chromosomal instability. Therefore, it is not surprising that mitophagy is a regulator of tumor biology, acting as either a suppressor or a facilitator of tumorigenesis.

The identification of various mitophagy-related autophagic receptors (including mitochondrial OMM, IMM, or lipid components) has accelerated our knowledge of the complexity of mitophagy in tumor biology. Therefore, understanding the molecular mechanism and function of mitophagy during different types of mitochondrial stress and damage may be critical for developing the next generation of cancer treatment methods. It is also important to develop convenient and reliable methods or biomarkers to assess the activity of mitophagy in humans. In addition, distinguishing the function of mitophagy between normal cells and cancer cells may be important for improving the targeting of tumor therapy and reducing its toxicity.

AUTHOR CONTRIBUTIONS

YX and DT conceived of the topic for this review. All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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