CHAPTER

12

The antiaging role of mitophagy

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Abbreviations

AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
ATP	adenosine triphosphate
BNIP3	BCL2/adenovirus E1B 19kDa protein-interacting protein 3
BNIP3L/NIX	BNIP3-like protein
BRCA1	breast cancer type 1 susceptibility protein
CK2	casein kinase 2
CR	calorie restriction
CRMs	calorie restriction mimetics
DAF-16	abnormal dauer formation 16
DAMPs	damage-associated molecular patterns
dct-1	DAF-16/FOXO Controlled, germline Tumor affecting-1
Drp1	Dynamin-related protein 1
FDB	flexor digitorum brevis
FGF2	fibroblast growth factor 2
FOXO	forkhead box O
FUNDC1	FUN14 domain containing 1
FZO-1	FZO (Fzo mitochondrial fusion protein) related
GABARAP	GABAA receptor-associated protein
GAK	cyclin G-associated kinase
IF	intermittent fasting
IMM	inner mitochondrial membrane
IVDD	intervertebral disk degeneration
LC3	Microtubule-associated proteins 1A/1B light chain 3
LIR	LC3 interacting region

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MFF	mitochondrial fission factor
MFN1/2	mitofusin 1/ mitofusin 2
mtDNA	mitochondrial DNA
mTOR	mammalian target of rapamycin
mTORC1	mTOR complex 1
NAD^+	nicotinamide adenine dinucleotide
NBR1	neighbor of BRCA1 gene 1
NIP3	(also known as BNIP3)
NMN	nicotinamide mononucleotide
NR	nicotinamide ribose
NRF2	nuclear factor erythroid 2 like 2
OA	osteoarthritis
OMM	outer mitochondrial membrane
OPA1	optic atrophy 1
pdr-1	Parkinson's disease related-1
PGC-1alpha	peroxisome proliferator-activated receptor gamma coactivator 1α
PINK1	Phosphatase and Tensin homolog (PTEN)-induced kinase 1
PRKCD	protein kinase c delta
PTEN	Phosphatase and Tensin homolog
PUF-8	PUF (Pumilio/FBF) domain-containing protein 8
PUM2	Pumilio RNA binding family member 2
RHEB	RAS homolog enriched in brain
ROS	reactive oxygen species
SA-β-gal	senescence-associated beta-galactosidase
SKN-1	SKiNhead-1
SQSTM1	Sequestosome 1
SRC	sarcoma proto-oncogene, non receptor tyrosine kinase
TANK	TRAF family member associated NFKB activator
TAX1BP1	Tax1-binding protein 1
TBK1	TANK-binding kinase 1
ULK1	unc-51 like autophagy activating kinase 1
UPRmt	mitochondrial unfolded protein response
YY1	Yin yang 1

Introduction

Aging is defined as the inevitable and irreversible process that leads to a decline in biological function and, ultimately, to death. The hallmarks of aging have been identified by many studies and include mitochondrial dysfunction, cellular senescence, telomere attrition, genomic instability, epigenetic alterations, loss of proteostasis, stem cell exhaustion, changes in intercellular communication and deregulation in nutrient sensing [1]. Aging is the primary risk factor for many diseases, such as neurodegeneration, cardiovascular disorders, obesity, and cancer. As life expectancy increases, so does the risk of age-related diseases. Thus, current research is concentrated on the field of healthy aging, exploring possible ways to extend healthspan alongside lifespan and maintain a higher quality of life at older ages. Since mitochondrial dysfunction is one of the hallmarks of aging, many researchers have been focusing on the crucial role of proper mitochondrial function and homeostasis and its importance in organismal fitness and the prevention of age-related diseases [1,2].

Mitochondria are energy producing organelles that constantly undergo fusion and fission, resulting in the formation of dynamic and morphologically distinct networks [3]. These two

processes regulate not only the shape of the organelles, but also their ability to perform various functions, such as cellular respiration, Ca^{2+} signaling, preservation of mitochondrial DNA (mtDNA), and regulation of apoptosis [3]. Dysfunctional or superfluous mitochondria are removed through a selective type of autophagy, defined as mitophagy. By controlling mitochondrial turnover under basal conditions and adjusting the population of mitochondria according to the metabolic demands of the cell, mitophagy performs a largely housekeeping role. However, under stress conditions, mitophagy is induced for the selective removal of damaged or dysfunctional organelles, which helps maintain a healthy and functional mitochondrial population. Although there are certain differences in the molecular pathways of mitophagy across different species, the key mitophagy modulators are functionally conserved (Table 12.1) [4]. Nevertheless, mitophagy alone cannot maintain mitochondrial homeostasis. It is rather the proper coordination between mitophagy and mitochondrial biogenesis that is crucial for cellular health and proper function [5]. Conclusively, it has been established by many studies, in various model organisms, that impaired mitophagy and accumulation of dysfunctional mitochondria occur during aging and can contribute to accelerated aging, progeria, or neurodegeneration [1].

Despite this massive research, how exactly mitochondrial dysfunction, decline in mitophagy, and other mitochondrial quality control mechanisms contribute to aging and the pathophysiology of age-related disorders remains unknown. Interestingly, accumulating evidence suggests that induction of autophagy, and specifically selective autophagy, such as mitophagy, can delay the aging process and alleviate symptoms in certain age-associated diseases, such as neurodegeneration [6]. Collectively, although it is still unclear to what extent mitochondrial function and mitophagy affect aging, accumulating findings suggest that targeting molecular pathways of mitophagy may be a potential therapeutic intervention to maintain healthy aging and prevent the organismal health decline that can lead to disease.

TABLE 12.1 Key mediators of mitophagy pathways across species.

Organisms					
Homo sapiens	***Mus musculus***	***Drosophila melanogaster***	***Caenorhabditis elegans***	***Danio rerio***	***Saccharomyces cerevisiae***
PINK1	PINK1	Pink1	PINK1	Pink1	–
PARK2	PARK2	Park	PDR-1	Park2	–
–	–	–	–	–	ATG32
GABARAP/LC3	GABARAP/LC3	Atg8a	LGG-1/LGG-2		ATG8
BNIP3, NIX/ BNIP3L	BNIP3, NIX/ BNIP3L	–	DCT-1	Dct-1	–
FUNDC-1	FUNDC-1	–	FUNDC-1	Fundc-1	CG5676
PRKCD	PRKCD	–	TPA-1	Prkcda/ Prkcdb	PKC1
GAK	GAK	Auxilin	GAKH-1	–	–

Here, we briefly review the mechanisms of mitophagy in different organisms under basal and stress conditions. Then, we discuss how mitophagy is altered during aging and how this contributes to accelerated aging, progeroid disorders, and age-related diseases. Finally, we discuss the different pathways and compounds that can induce mitophagy to promote healthy aging.

The molecular pathways of mitophagy

Mitophagy can occur through distinct signaling pathways, depending on the type of stimuli and the cellular context: basal conditions, developmental changes, and stress-related circumstances [7]. The process is probably initiated by the dynamin-related protein 1 (Drp1)-mediated fission of dysfunctional mitochondria, assisted by the degradation of the membrane anchored guanosine triphosphate hydrolases (GTPases): mitofusin (MFN) 1 and 2 [8]. MFN1 and MFN2 are normally responsible for mitochondrial fusion, along with the inner mitochondrial membrane (IMM) protein optic atrophy 1 (OPA1) [8].

The phosphatase and tensin homolog-induced putative kinase 1/Parkin pathway

The most well investigated pathway of mitophagy is mediated by Phosphatase and Tensin homolog (PTEN)-induced putative kinase protein 1 (PINK1) and Parkin. PINK1 is a kinase composed of three domains: an N-terminal domain, necessary for its localization to the outer mitochondrial membrane (OMM), a kinase domain and a cytosolic C-terminal domain [9]. PINK1 is found both in the cytosol and integrated in the membrane of the mitochondria [9]. PINK1 is constantly and rapidly degraded, but upon mitochondrial depolarization, it accumulates on the OMM of dysfunctional mitochondria [10]. Subsequently, PINK1 recruits and phosphorylates the cytosolic E3 ubiquitin ligase Parkin [10]. This leads to a continuous Parkin-mediated ubiquitination and PINK1-mediated phosphorylation of OMM proteins [11]. These polyubiquitin chains will be recognized by autophagy receptors such as p62, optineurin, a neighbor of the BRCA1 gene 1 (NBR1), and Tax-1 binding protein 1 (TAX1BP1). Autophagy receptors then interact with the microtubule-associated proteins 1A/1B light chain 3 (LC3) and the GABAA receptor-associated protein (GABARAP) on the membrane of autophagosomes for transport of cargo and delivery to the lysosomes for degradation [2,12]. The PINK1/Parkin pathway is implicated in various mitochondrial processes, such as fission, fusion, and trafficking to efficiently remove dysfunctional mitochondria.

Receptor-mediated mitophagy

Mitophagy constantly occurs in an organism at a basal level and is upregulated under stress conditions. Basal mitophagy is probably a homeostatic mechanism to maintain mitochondrial mass [11] and is mainly PINK1/Parkin-independent, as many studies in mice and *Drosophila* [11,13] have strongly suggested. *Drosophila* null mutants for PINK1 or Parkin show a high number of formed mitolysosomes in neuromuscular tissues [11], while PINK1 knock out mice display the same levels of mitophagy as wild type animals [13]. In mammals, the PINK1/Parkin-independent pathway of mitophagy is mediated by the interaction of LC3 with different receptors, such as FUN14 domain containing 1 (FUNDC1) and homologous members of the BCL2/adenovirus E1B 19 kDa protein-interacting protein (BNIP)

family: BNIP3 and BNIP3L/NIX, through their LC3 interacting region (LIR) motifs [2]. In yeast, the autophagy-related 32 (Atg32) mitophagy receptor, which shows common features with NIX, recruits and interacts with Atg8 and/or Atg11 through their LIR motifs to initiate mitophagy under stress conditions, such as nitrogen starvation [14,15]. This binding substitutes the ubiquitination step that is required for the PINK1/Parkin pathway. Specifically, Atg32 is phosphorylated by the serine and/or threonine casein kinase-2 (CK2), which promotes the interaction of Atg32 with Atg8 and Atg11, for mitophagy to occur [16].

Under basal conditions, BH3-only protein-induced mitophagy is mainly taking place. For example, NIX-mediated mitophagy is required for the maturation of the mammalian erythroid cells [17]. NIX gets upregulated and disturbs the membrane potential of red blood cells during their terminal differentiation process [17]. Defective NIX-mediated mitophagy in red blood cells results in increased activation of caspases, display of phosphatidylserine, and clearance of erythroid cells by macrophages [17]. BNIP3-mediated mitophagy is initiated by inhibition of cytochrome c release from the mitochondria and phosphorylation of the serine resides in its LIR motif to interact with LC3, to allow autophagosomal engulfment of the mitochondria [18]. NIX/BNIP3L is a receptor with a WXXL like motif facing the cytosol, which can interact with the autophagy proteins LC3/GABARAP to partially initiate mitophagy [15]. A recent study also identified two novel mediators of the PINK1/Parkin-independent mitophagy that occurs under basal conditions [19]. Specifically, the cyclin G-associated kinase (GAK) and the protein kinase c delta (PRKCD) are shown to induce mitophagy both in vitro and in vivo, since inhibition of the GAK orthologue, *gakh-1*, in *Caenorhabditis elegans* and the PRKCD paralogue in zebrafish significantly reduces the basal levels of mitophagy [19].

On the other hand, hypoxia-induced mitophagy is mediated either by FUNDC1 or BNIP3/NIX. Under normal conditions, the activity of the OMM FUNDC1 is blocked by phosphorylation at Tyr18 in its LIR motif by Src tyrosine kinase [4]. However, under hypoxia, FUNDC1 is dephosphorylated to colocalize and bind with LC3 for mitophagy to be activated [4]. In contrast, studies in *C. elegans* and zebrafish demonstrate that *dct-1* (DAF-16/FOXO Controlled, germline Tumor affecting 1) and *bnip3*, respectively, are required for hypoxia-induced mitophagy [7,20]. Additionally, treatment of HeLa cells with the hypoxia mimic $CoCl_2$ causes ubiquitination of OMM proteins and subsequent mitochondrial fragmentation that leads to BNIP3/NIX-mediated mitophagy [21]. Interestingly, although the damaged mitochondria were highly ubiquitinated in this study, stabilization of both BNIP3 and NIX and autophagosomal engulfment of the mitochondria did not rely on ubiquitin binding autophagy adapters [21]. This further confirms the finding that the binding between a mitochondrial receptor and LC3 during receptor-mediated mitophagy does not require ubiquitination.

Alterations in mitophagy during aging and age-related diseases

Alterations in mitophagy during aging

Deterioration of mitochondrial function is one of the main hallmarks of eukaryotic aging [22]. This phenomenon can be primarily attributed to the accumulation of damage from reactive oxygen species (ROS) that escape neutralization via the various antioxidant

mechanisms of the cell (such as scavenger enzymes) and is exacerbated as the respiratory chain complexes themselves start to malfunction. This decline is accompanied by drastic alterations in mitochondrial morphology, as the organelles increase in size while decreasing in number. The normal structure of the mitochondrial cristae is also disrupted [23]. The production of energy in the form of adenosine triphosphate (ATP) is disrupted, and various other metabolic and signaling processes that are associated with these organelles become deregulated [24,25]. In addition, mtDNA is damaged, and mutations in mtDNA accumulate [24]. This gradual deterioration becomes especially strong in postmitotic cells, such as neurons and cardiomyocytes [25,26]. All of these deviations from healthy mitochondrial function can obviously be quite disruptive for a cell on their own; however, their significance is even greater when one considers that they can affect the emergence of other aging-related phenotypes, triggering a cascade of decline [24]. Indeed, there is evidence that mitochondrial dysfunction can accelerate telomere shortening, induce changes in DNA methylation and chromatin structure, and even trigger an inflammatory response at the intercellular level [24].

Beyond this decline in the function of mitochondria themselves, research has indicated that there is also a direct correlation between aging and the malfunction of mechanisms that are responsible for the maintenance of healthy mitochondria, such as mitophagy. This means that the accumulation of damage and defects becomes increasingly easier and faster [27]. Notably, there is a lot of evidence suggesting that mitophagy is disrupted in various tissues and organisms [28]. For instance, Sun et al. [29] demonstrated that mitophagy levels can be reduced by up to 70% in the Dentate Gyrus regions of old mice. Work by others has linked age-related sarcopenia in mice and humans to the impairment of mitophagy in tissue samples [30–32] and specifically in muscle satellite stem cells [33]. Research in the hearts of mice has revealed a decrease in mitochondrial degradation with age [34] and an accumulation of dysfunctional mitochondria due to the disruption of mitochondrial fission and mitophagic turnover in aging animals [35,36]. Mitophagy also appears to be impaired in samples from old human patients with heart failure [37]. Mitochondrial function and clearance via mitophagy are also negatively impacted during aging in mouse cerebral vasculature, which can possibly also disrupt the blood-brain barrier [38]. Furthermore, mitophagy is reduced in aging macrophages, with potential consequences in excessive induction of inflammation [39]. Work in our lab has shown a gradual accumulation of mitochondria during aging in *C. elegans*, which was also linked to an age-related decrease in mitophagy [5]. It is worth mentioning that in addition to direct inhibition of mitophagic pathways, mitophagic clearance can also be impeded due to alterations in mitochondrial dynamics, and specifically the inhibition of mitochondrial fission that allows damage to be isolated and permits effective turnover [40].

Alterations in mitophagy of age-related diseases

Beyond its involvement in healthy aging, mitophagy is also associated with a multitude of age-related diseases, be it progeroid disorders that lead to premature and accelerated aging or diseases whose emergence and severity of symptoms are tightly correlated with the age of the patient, such as neurodegeneration (Fig. 12.1), cancer, cardiovascular diseases, and degenerative joint diseases.

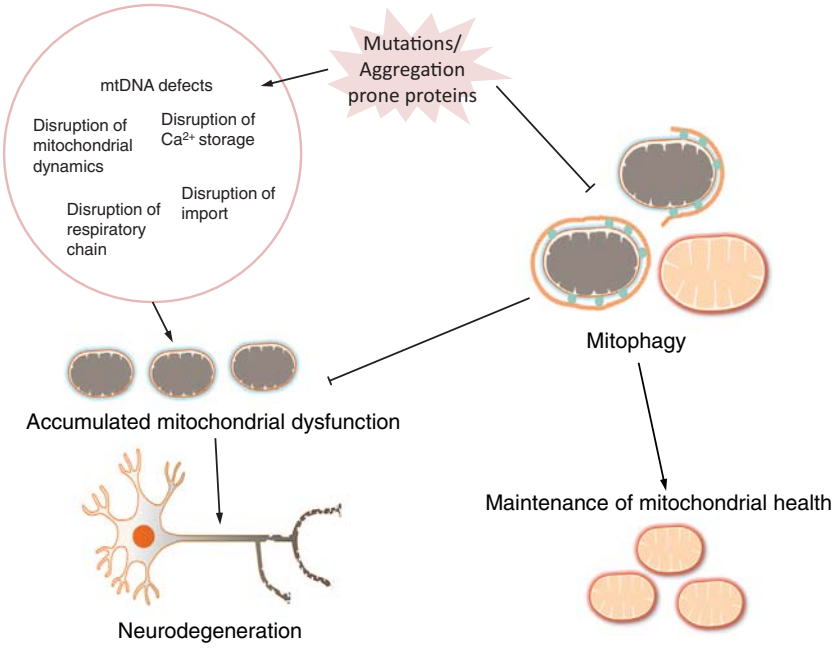

FIGURE 12.1 Role of mitochondrial alterations and mitophagy in a generalized model of neurodegenerative diseases. Mutations that directly target mitochondrial components or produce aggregation prone proteins that can directly interact with mitochondrial components may lead to dysfunctional mitochondria. Mutations that inhibit mitophagy prevent the removal of these dysfunctional organelles, allowing them to accumulate and eventually disrupt neuronal function enough to cause degeneration.

Werner syndrome, a progeroid disease characterized by severe dysregulation of energy metabolism, impaired mitophagy due to nicotinamide adenine dinucleotide (NAD^+) insufficiency was demonstrated to be a significant constituent of the phenotype and a potential therapeutic target [41]. Similar observations have been made in the cases of other progeroid disorders such as ataxia telangiectasia [42], xeroderma pigmentosum group A [43,44], and Cockayne syndrome group B [45].

Alzheimer's disease (AD) is the most common neurodegenerative disease in humans. It is characterized by the loss of cholinergic neurons in the brain, particularly in the hippocampus, leading to memory defects and eventually major cognitive decline [46]. There is ample evidence linking AD with mitochondrial dysfunction, both in patients and in animal models [40,47]. Experiments give contradictory evidence on whether mitophagy is induced or inhibited in postmortem samples from AD patients and in models of the disease [48,49]. However, it seems that external induction of mitophagy in animal models can ameliorate some of the pathology [49].

Parkinson's disease (PD) is the second most common neurological disease and involves the loss of dopaminergic neurons in the substantia nigra of patients, leading to dramatic motor defects such as tremors, bradykinesia, and change of gait [50]. The disease is tightly connected with mitochondrial dysfunction, as patients and animal models commonly exhibit defects in the respiratory chain, mtDNA alterations (deletions and decreases in

copy number), or altered Ca^{2+} sequestration [51]. Mitochondrial genes are directly connected to the emergence of familial forms of PD with the mitophagy initiators PINK1 and Parkin being the most prominent culprits [40]. Interestingly, induction of mitophagy through overexpression of these genes can have a protective effect and reduce PD-associated symptoms [52].

Huntington's disease (HD) is a hereditary neurodegenerative disease caused by a mutation in the gene of huntingtin (HTT), resulting in abnormal extension of the polyglutamine track in the N-terminal region of the htt gene. This renders the gene product pathogenic and aggregation prone. As a result, polyglutamine-expanded huntingtin accumulates inside cells, forming toxic oligomeric species and aggregates, and eventually causes cell death, particularly in neurons [53]. Several publications have connected HD with the deregulation of autophagy and particularly autophagic cargo recognition/loading. Autophagy impairment especially affects lipid droplets and mitochondria with the latter exhibiting a higher incidence of depolarization [53]. Evidence suggests that huntingtin acts as scaffold for selective autophagy through its interaction with the critical factors p62/SQSTM1 and Unc-51 like autophagy activating kinase (ULK1) [54]. This seems to be the same for mitophagy as well, as recent work demonstrates that mutant HTT can disrupt the initiation of the mitophagic process and the recruitment of mitophagy receptors to damaged organelles [55].

Mitophagy-related genes have been shown to exhibit abnormal regulation in cancer patients, although their role can range greatly from tumor promoting to tumor suppressing depending on the context and type of cancer [56]. A common phenomenon in cancer cells is the Warburg effect, a shift of ATP production from oxidative phosphorylation to glycolysis even under aerobic conditions. There is evidence that mitophagy can either promote or inhibit this effect, depending on the circumstances [56,57]. Mitophagy has also been associated with the maintenance of cancer stem cell stemness through its effects on mitochondria-associated signaling [58,59]. Finally, mitophagy appears to be important in preventing the release of mitochondrial components into the extracellular space where they can act as damage-associated molecular patterns (DAMPs or alarmins), triggering inflammation, which can have a significant tumor promoting effect [60].

Intervertebral disk degeneration (IVDD) and osteoarthritis (OA) are prevalent age-related degenerative joint diseases. They involve the degradation of cartilaginous tissue [61]. Mitochondrial dysfunction and mitophagy deregulation in the cells that produce and regulate the components of the cartilaginous extracellular matrix contribute to this pathology and represent potential targets for therapeutic intervention [62,63].

The antiaging role of mitophagy

During the past few years, the role of mitophagy in aging has been studied in depth, and it has been established that mitophagy is a prolongevity process (Fig. 12.2). In *C. elegans*, DCT-1 is the major protein that regulates mitophagy independently of the stimuli of induction. This was verified by the fact that deletion of *dct-1* in wild type worms impairs mitophagy and results in the same phenotype as that of old worms that are characterized by mitochondrial accumulation [5]. Moreover, coordination between mitophagy and mitochondrial biogenesis can antagonize aging, as DCT-1 is regulated by the transcription factors

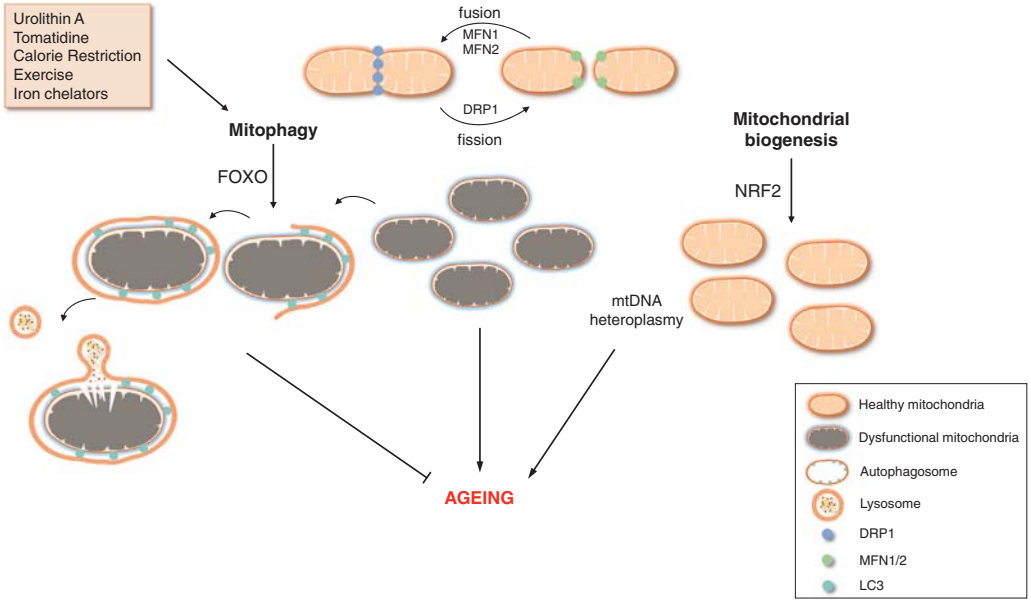

FIGURE 12.2 Mitochondrial homeostasis is required for healthy aging. The balance between mitochondrial fission and fusion is important for lifespan regulation and for coordination between mitochondrial biogenesis and mitophagy, while the accumulation of dysfunctional mitochondria is associated with aging and age-related diseases. mtDNA heteroplasmy, which exists in healthy aging, can also contribute to aging when induced. Several compounds, such as urolithin A, tomatidine, and iron chelators, and several lifestyle choices, such as regular exercise and calorie restriction, can promote healthspan and lifespan through induction of mitophagy.

SKN-1 (SKiNhead-1) and DAF-16, the respective homologs of the mammalian nuclear factor erythroid 2 like 2 (NRF2) and forkhead box O (FOXO) [5]. In contrast, mitochondrial fission is mainly thought as a pro aging process, but it is a prerequisite for mitophagy. Indeed, deletion of the fission gene *drp-1* reduces lifespan [64]. Unexpectedly, simultaneous deletion of fission and fusion genes in *C. elegans*, *drp-1* and *fzo-1*, extends lifespan, highlighting the importance of homeostasis of the mitochondrial network in the regulation of lifespan [64]. Finally, an RNA-binding protein, Pum2, and its orthologue in *C. elegans*, PUF-8, have been shown to accelerate aging, by directly affecting mitophagy and mitochondrial dynamics. Pum2/PUF-8 binds to the RNA of the OMM protein, mitochondrial fission factor (MFF/MFF-1), and inhibits its translation. Knockout of Pum2 in aged mice enhances BNIP3-mediated mitophagy and improves myoblast respiration [65]. Moreover, *puf-8* RNAi reverts the aging phenotype of old worms and upregulates the mitophagy genes *dct-1* and *pink-1* in an MFF-1-dependent manner [65]. Finally, Parkin overexpression in cardiomyocytes of aged mice resulted in a reduction of dysfunctional mitochondria and also of aging biomarkers, such as SA-β-gal activity [36].

Mitochondrial DNA damage is one of the hallmarks of aging, and accumulation of partial deletions of mtDNA (ΔmtDNAs) is observed in age-related diseases, such as neurodegeneration. Animal models that express proofreading deficient mitochondrial DNA polymerase exhibit reduced lifespan and accelerated emergence of aging phenotypes [66].

Generation of heteroplasmic *Drosophila* that contained a mixture of wild type and mutant mtDNA revealed that the selection against mitochondria that contained the mutant mtDNA was female germline specific and involved the mitophagy proteins Atg1 and BNIP3 [67]. Additionally, a heteroplasmy of mtDNA mutations exists between different tissues, and this is probably maintained through a specific mechanism, rather than a random process. A recent study in *C. elegans* demonstrated that PINK1 and the Parkin homolog, PDR-1, are indispensable to maintain different levels of heteroplasmy of a deleterious mitochondrial genome mutation (ΔmtDNA) across various tissues [68]. ΔmtDNA tends to accumulate more in cells that have a lower response to mitophagy, which results in body wall muscle cells having higher ΔmtDNA abundance than other tissues. Knockdown of *pdr-1* and *pink-1* seems to equalize ΔmtDNA heteroplasmy across the whole organism [68].

Compounds that act as mitophagy modulators

Several natural compounds are shown to extend lifespan by the induction of mitophagy. Treatment of *C. elegans* and mice with the food metabolite, urolithin A, significantly extended lifespan and improved muscle function through the activation of autophagy and mitophagy genes [69]. Interestingly, urolithin A had a different effect on the mitochondrial content of young versus old worms, upregulating mitophagy and biogenesis, respectively [69]. This result supports the notion that the coordination between mitophagy and mitochondrial biogenesis is dynamic and is altered depending on the current needs of the cell. Moreover, a clinical trial revealed that oral administration of urolithin A in humans upregulated fatty acid oxidation and transcription of mitochondrial genes, improving overall muscle mitochondrial health [70]. Another compound that has been demonstrated to extend lifespan is the aglycone of alpha-tomatine that is found in unripe tomatoes, tomatidine. Tomatidine treatment in *C. elegans* improved pharyngeal pumping and swimming, which decrease with age, while inhibited sarcopenia [71]. Tomatidine maintained cellular function by enhancing PINK-1/DCT-1-mediated mitophagy both in vitro and in vivo, probably through stress response pathways, such as the NRF2/SKN-1 pathway and activation of the mitochondrial unfolded protein response (UPRmt) [71].

Another natural compound, which normally exists in all eukaryotic cells and is known to exhibit longevity effects across many species, such as yeast, *C. elegans*, flies, and mice, is spermidine [72]. These studies have proposed that spermidine promotes longevity through autophagy induction, since inhibition of autophagy completely suppresses the lifespan promoting effects of supplementation with spermidine [72]. Moreover, experiments on AD nematode models clearly showed that spermidine extended lifespan and alleviated AD-associated phenotypes, such as memory impairment and locomotion, through the induction of PINK-1 and PDR-1, the main mitophagy mediators [73]. Thus, spermidine can also be used for healthspan and lifespan extension through mitophagy induction.

NAD^+ is a crucial cofactor involved in most cellular pathways, such as cellular senescence and metabolism, that gradually decreases with aging and age-related diseases, ranging from diabetes to cancer [74]. Accordingly, supplementation with the NAD^+ precursors nicotinamide mononucleotide (NMN) or nicotinamide ribose (NR) can have beneficial effects on both healthspan and lifespan [74]. For example, NR supplementation alleviated both memory and learning impairment in *C. elegans* models of AD, through

PINK-1/PDR-1-mediated mitophagy induction [49]. The notion that NAD^+ promotes healthspan and lifespan through mitophagy was verified by a study that showed that downregulation of PINK-1, DCT-1 or PDR-1, the major mitophagy mediators in *C. elegans*, completely abolished its beneficial effects [42]. Finally, experiments in mice have demonstrated that NR supplementation extends both healthspan and lifespan by inducing the UPRmt and the synthesis of prohibitins [75]. Conclusively, dietary supplementation with either spermidine or NAD precursors can display an antiaging role.

Lastly, rapamycin is a well known autophagy inducing chemical compound, which can inhibit the mammalian target of rapamycin (mTOR) pathway and extend lifespan. However, rapamycin has a hormetic effect, as it can demonstrate the opposite effect in higher doses, so it should be administered only in low doses [76]. Indeed, a recent study showed that low doses of rapamycin in WRL-68 cells for 24 hours can induce dephosphorylation of mTOR in Ser 2448 and lead to its inactivation. Moreover, rapamycin can promote mitochondrial biogenesis, through the elevation of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1a) and Yin yang 1 (YY1) [76]. Furthermore, rapamycin induced PINK1/Parkin-mediated mitophagy and upregulated BECN1, which encodes the autophagic protein Beclin-1. This, in coordination with enhanced mitochondrial biogenesis, increased the mitochondrial density of the cells, leading to an antiaging effect [76].

Calorie restriction and exercise promote longevity

Many studies claim that exercise, calorie restriction (CR), and intermittent fasting (IF), namely fasting for 12–16 hours and eating only the rest of the hours during the day, extend both healthspan and lifespan. Starvation or CR is known to promote autophagy, which is negatively and positively regulated by the function of the mTOR and AMP-activated protein kinase (AMPK), respectively [27]. Studies show that glucose deprivation activates Ulk1 through AMPK-dependent phosphorylation at Ser 317 and Ser 777, while inhibiting mTOR complex 1 (mTORC1) activity [77]. However, mTORC1 can disrupt the interaction between AMPK and Ulk1 by directly phosphorylating Ulk1 at Ser 757 to block initiation of autophagy when not required by the cellular nutritional status [77]. In addition, during hypoxia, mTOR can be indirectly inactivated by receptor-mediated mitophagy, when BNIP3 directly interacts with its upstream activator, Ras homolog enriched in brain (Rheb) [78]. Ulk1 can also negatively regulate autophagy by phosphorylating AMPK to block its activity, resulting in a negative feedback loop [79]. Moreover, AMPK induced mitophagy in mouse flexor digitorum brevis (FDB) skeletal muscles during the recovery period after six hours of exercise. Immediately after exercise training, AMPK phosphorylated Ulk1 at Ser 555, whereas Drp1 was hyperphosphorylated in an AMPK-independent manner, probably to initiate mitochondrial fission required for mitophagy [80].

Indeed, a recent study verified that induction of mitophagy in skeletal muscle cells acutely activates AMPK-dependent phosphorylation of another kinase, Tank-binding kinase 1 (TBK1), and that the AMPK signaling is required for mitochondrial fission and PINK1/Parkin-mediated mitophagy [81]. In the zebrafish skeletal muscle, fasting causes an increase in the mitophagy index, enlargement of mitolysosomes, and the new production of small mitolysosomes, probably generated by fission [7]. Additionally, mitophagy seemed to occur via a piecemeal mechanism by utilizing $LC3^+$ organelles, probably for the adaptation and maintenance of the mitochondrial network [7]. Weir et al. [64]

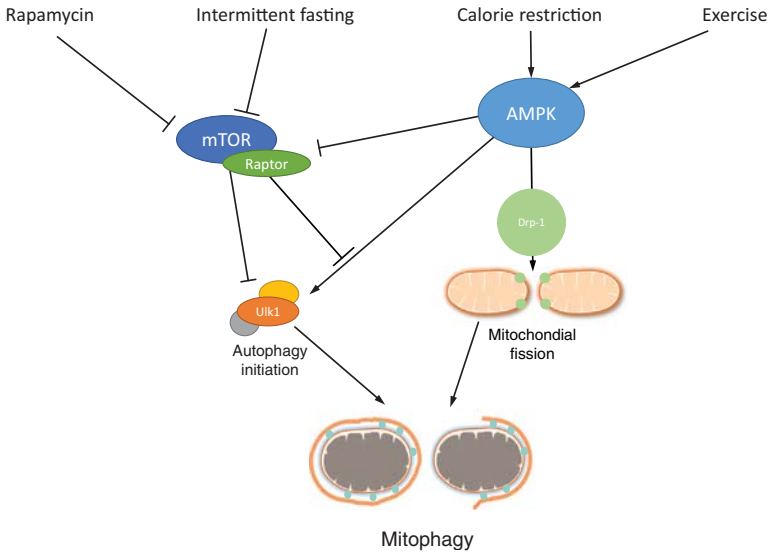

FIGURE 12.3 Regulation of mitophagy by the AMPK–mTOR signaling axis. Manipulations such as intermittent fasting, calorie restriction, and exercise can induce mitophagy through the induction of AMPK signaling and the inhibition of mTOR.

demonstrated that both fusion and fission are required for the beneficial effects of AMPK- and CR-mediated longevity. Research has also focused on the identification of compounds that can be classified as calorie restriction mimetics (CRMs). A CRM example is aspirin, whose active metabolite, salicylate, reduced protein acetylation by inhibiting the enzymatic activity of the EP300 acetyltransferase and stimulating both autophagy and mitophagy [82]. Finally, iron chelators could also be beneficial for lifespan induction, as they can mimic hypoxia. Iron deprivation triggers frataxin silencing and extends lifespan in *C. elegans* by inducing PDR-1 and DCT-1-mediated mitophagy, which seems to be indispensable for the extension of lifespan [20].

Recently, besides CR, IF has attracted a lot of attention [83]. IF is found to activate all the signaling pathways that respond to stress, including enhanced autophagy and DNA repair, so IF is suggested not only for healthspan promotion but also for the treatment for age-related diseases [84]. In addition, IF inactivates the mTOR signaling, like CR does, promotes mitochondrial biogenesis, and upregulates cyclic AMP response element binding protein and neurotrophic factors, such as the brain derived neurotrophic factor and fibroblast growth factor 2 (FGF2) [84]. Thus, CR and IF are interventions that can promote healthspan and lifespan by inducing both general autophagy and selective autophagic pathways like mitophagy, resulting in cellular clean up and removal of toxic components (Fig. 12.3).

Conclusions

Overall, mitochondria are a cornerstone of eukaryotic life and death through their involvement in ATP production, regulation of apoptosis and necrosis, Ca^{2+} storage, and their role as a central hub of many if not most metabolic pathways [8]. It is therefore hardly surprising that their function and dysfunction can have such a prominent

involvement in aging and the multitude of diseases that are associated with it. This means that research in mitochondria and mitophagy can always have surprising implications far beyond the intended scope and provide critical insights for a variety of conditions. It also means that research for the development of existing and the discovery of new pharmaceutical and genetic methods of manipulating mitophagy can grant us with incredibly versatile and potentially lifesaving tools.

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Author contributions

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References

[1] Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, et al. Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol 2019;15(10):565–81.
[2] Palikaras K, Lionaki E, Tavernarakis N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat Cell Biol 2018;20(9):1013–22.
[3] Pham AH, McCaffery JM, Chan DC. Mouse lines with photo-activatable mitochondria to study mitochondrial dynamics. Genesis 2012;50(11):833–43.
[4] Liu L, Feng D, Chen G, Chen M, Zheng Q, Song P, et al. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. Nat Cell Biol 2012;14(2):177–85.
[5] Palikaras K, Lionaki E, Tavernarakis N. Coordination of mitophagy and mitochondrial biogenesis during ageing in C. elegans. Nature 2015;521(7553):525–8.
[6] Madeo F, Tavernarakis N, Kroemer G. Can autophagy promote longevity? Nat Cell Biol 2010;12(9):842–6.
[7] Wrighton PJ, Shwartz A, Heo JM, Quenzer ED, LaBella KA, Harper JW, et al. Quantitative intravital imaging in zebrafish reveals in vivo dynamics of physiological-stress-induced mitophagy. J Cell Sci 2021;134(4).
[8] Yoo SM, Jung YK. A molecular approach to mitophagy and mitochondrial dynamics. Mol Cell 2018;41(1):18–26.
[9] Zhou C, Huang Y, Shao Y, May J, Prou D, Perier C, et al. The kinase domain of mitochondrial PINK1 faces the cytoplasm. Proc Natl Acad Sci U S A 2008;105(33):12022–7.
[10] Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol 2010;189(2):211–21.

[11] Lee JJ, Sanchez-Martinez A, Martinez Zarate A, Beninca C, Mayor U, Clague MJ, et al. Basal mitophagy is widespread in Drosophila but minimally affected by loss of Pink1 or parkin. J Cell Biol 2018;217(5):1613–22.
[12] Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature 2015;524(7565):309–14.
[13] McWilliams TG, Prescott AR, Montava-Garriga L, Ball G, Singh F, Barini E, et al. Basal mitophagy occurs independently of PINK1 in mouse tissues of high metabolic demand. Cell Metab 2018;27(2):439–49 e5.
[14] Kanki T, Wang K, Cao Y, Baba M, Klionsky DJ. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. Dev Cell 2009;17(1):98–109.
[15] Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, et al. Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep 2010;11(1):45–51.
[16] Kanki T, Kurihara Y, Jin X, Goda T, Ono Y, Aihara M, et al. Casein kinase 2 is essential for mitophagy. EMBO Reports 2013;14:788–94. Available from: https://doi.org/10.1038/embor.2013.114.
[17] Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, et al. Essential role for Nix in autophagic maturation of erythroid cells. Nature 2008;454(7201):232–5.
[18] Zhu Y, Massen S, Terenzio M, Lang V, Chen-Lindner S, Eils R, et al. Modulation of serines 17 and 24 in the LC3-interacting region of Bnip3 determines pro-survival mitophagy versus apoptosis. J Biol Chem 2013;288(2):1099–113.
[19] Munson MJ, Mathai BJ, Ng MYW, Trachsel-Moncho L, de la Ballina LR, Schultz SW, et al. GAK and PRKCD are positive regulators of PRKN-independent mitophagy. Nat Commun 2021;12(1):6101.
[20] Schiavi A, Maglioni S, Palikaras K, Shaik A, Strappazzon F, Brinkmann V, et al. Iron-starvation-induced mitophagy mediates lifespan extension upon mitochondrial stress in C. elegans. Curr Biol 2015;25(14):1810–22.
[21] Sulkshane P, Ram J, Thakur A, Reis N, Kleifeld O, Glickman MH. Ubiquitination and receptor-mediated mitophagy converge to eliminate oxidation-damaged mitochondria during hypoxia. Redox Biol 2021;45:102047.
[22] Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153(6):1194–217.
[23] Chen G, Kroemer G, Kepp O. Mitophagy: an emerging role in aging and age-associated diseases. Front Cell Dev Biol 2020;8:200.
[24] van der Rijt S, Molenaars M, McIntyre RL, Janssens GE, Houtkooper RH. Integrating the hallmarks of aging throughout the tree of life: a focus on mitochondrial dysfunction. Front Cell Dev Biol 2020;8:594416.
[25] Mendoza A, Karch J. Keeping the beat against time: mitochondrial fitness in the aging heart. Front Aging 2022;3:951417.
[26] Markaki M, Tavernarakis N. Mitochondrial turnover and homeostasis in ageing and neurodegeneration. FEBS Lett 2020;594(15):2370–9.
[27] Markaki M, Palikaras K, Tavernarakis N. Novel insights into the anti-aging role of mitophagy. Int Rev Cell Mol Biol 2018;340:169–208.
[28] De Gaetano A, Gibellini L, Zanini G, Nasi M, Cossarizza A, Pinti M. Mitophagy and oxidative stress: the role of aging. Antioxid (Basel) 2021;10(5).
[29] Sun N, Yun J, Liu J, Malide D, Liu C, Rovira II, et al. Measuring in vivo mitophagy. Mol Cell 2015;60(4):685–96.
[30] Drummond MJ, Addison O, Brunker L, Hopkins PN, McClain DA, LaStayo PC, et al. Downregulation of E3 ubiquitin ligases and mitophagy-related genes in skeletal muscle of physically inactive, frail older women: a cross-sectional comparison. J Gerontol A Biol Sci Med Sci 2014;69(8):1040–8.
[31] Gouspillou G, Godin R, Piquereau J, Picard M, Mofarrahi M, Mathew J, et al. Protective role of Parkin in skeletal muscle contractile and mitochondrial function. J Physiol 2018;596(13):2565–79.
[32] Sebastian D, Sorianello E, Segales J, Irazoki A, Ruiz-Bonilla V, Sala D, et al. Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway. EMBO J 2016;35(15):1677–93.
[33] Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortet L, Rodriguez-Ubreva J, Rebollo E, et al. Autophagy maintains stemness by preventing senescence. Nature 2016;529(7584):37–42.
[34] Stotland A, Gottlieb RA. Alpha-MHC MitoTimer mouse: in vivo mitochondrial turnover model reveals remarkable mitochondrial heterogeneity in the heart. J Mol Cell Cardiol 2016;90:53–8.
[35] Liang W, Moyzis AG, Lampert MA, Diao RY, Najor RH, Gustafsson AB. Aging is associated with a decline in Atg9b-mediated autophagosome formation and appearance of enlarged mitochondria in the heart. Aging Cell 2020;19(8):e13187.

[36] Hoshino A, Mita Y, Okawa Y, Ariyoshi M, Iwai-Kanai E, Ueyama T, et al. Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. Nat Commun 2013;4:2308.
[37] Billia F, Hauck L, Konecny F, Rao V, Shen J, Mak TW. PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. Proc Natl Acad Sci U S A 2011;108(23):9572–7.
[38] Tyrrell DJ, Blin MG, Song J, Wood SC, Goldstein DR. Aging impairs mitochondrial function and mitophagy and elevates interleukin 6 within the cerebral vasculature. J Am Heart Assoc 2020;9(23):e017820.
[39] Zhong W, Rao Z, Xu J, Sun Y, Hu H, Wang P, et al. Defective mitophagy in aged macrophages promotes mitochondrial DNA cytosolic leakage to activate STING signaling during liver sterile inflammation. Aging Cell 2022;21(6):e13622.
[40] Rodolfo C, Campello S, Cecconi F. Mitophagy in neurodegenerative diseases. Neurochem Int 2018; 117:156–66.
[41] Fang EF, Hou Y, Lautrup S, Jensen MB, Yang B, SenGupta T, et al. NAD(+) augmentation restores mitophagy and limits accelerated aging in Werner syndrome. Nat Commun 2019;10(1):5284.
[42] Fang EF, Kassahun H, Croteau DL, Scheibye-Knudsen M, Marosi K, Lu H, et al. NAD(+) replenishment improves lifespan and healthspan in Ataxia Telangiectasia models via mitophagy and DNA repair. Cell Metab 2016;24(4):566–81.
[43] Fang EF, Scheibye-Knudsen M, Brace LE, Kassahun H, SenGupta T, Nilsen H, et al. Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/SIRT1 reduction. Cell 2014;157(4):882–96.
[44] Valentin-Vega YA, Maclean KH, Tait-Mulder J, Milasta S, Steeves M, Dorsey FC, et al. Mitochondrial dysfunction in ataxia-telangiectasia. Blood 2012;119(6):1490–500.
[45] Scheibye-Knudsen M, Mitchell SJ, Fang EF, Iyama T, Ward T, Wang J, et al. A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. Cell Metab 2014;20(5):840–55.
[46] Kerr JS, Adriaanse BA, Greig NH, Mattson MP, Cader MZ, Bohr VA, et al. Mitophagy and Alzheimer's disease: cellular and molecular mechanisms. Trends Neurosci 2017;40(3):151–66.
[47] Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. J Neurosci 2006;26(35):9057–68.
[48] Hu Y, Li XC, Wang ZH, Luo Y, Zhang X, Liu XP, et al. Tau accumulation impairs mitophagy via increasing mitochondrial membrane potential and reducing mitochondrial Parkin. Oncotarget 2016;7 (14):17356–68.
[49] Fang EF, Hou Y, Palikaras K, Adriaanse BA, Kerr JS, Yang B, et al. Mitophagy inhibits amyloid-beta and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. Nat Neurosci 2019;22(3):401–12.
[50] Killackey SA, Philpott DJ, Girardin SE. Mitophagy pathways in health and disease. J Cell Biol 2020;219(11).
[51] Chen C, Turnbull DM, Reeve AK. Mitochondrial dysfunction in Parkinson's disease—cause or consequence? Biol (Basel) 2019;8(2).
[52] Kamp F, Exner N, Lutz AK, Wender N, Hegermann J, Brunner B, et al. Inhibition of mitochondrial fusion by alpha-synuclein is rescued by PINK1, Parkin and DJ-1. EMBO J 2010;29(20):3571–89.
[53] Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, et al. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci 2010;13(5):567–76.
[54] Rui YN, Xu Z, Patel B, Chen Z, Chen D, Tito A, et al. Huntingtin functions as a scaffold for selective macroautophagy. Nat Cell Biol 2015;17(3):262–75.
[55] Franco-Iborra S, Plaza-Zabala A, Montpeyo M, Sebastian D, Vila M, Martinez-Vicente M. Mutant HTT (huntingtin) impairs mitophagy in a cellular model of Huntington disease. Autophagy 2021;17(3):672–89.
[56] Vara-Perez M, Felipe-Abrio B, Agostinis P. Mitophagy in cancer: a tale of adaptation. Cells 2019;8(5).
[57] Agnihotri S, Golbourn B, Huang X, Remke M, Younger S, Cairns RA, et al. PINK1 is a negative regulator of growth and the Warburg effect in glioblastoma. Cancer Res 2016;76(16):4708–19.
[58] Liu K, Lee J, Kim JY, Wang L, Tian Y, Chan ST, et al. Mitophagy controls the activities of tumor suppressor p53 to regulate hepatic cancer stem cells. Mol Cell 2017;68(2):281–92 e5.
[59] Whelan KA, Chandramouleeswaran PM, Tanaka K, Natsuizaka M, Guha M, Srinivasan S, et al. Autophagy supports generation of cells with high CD44 expression via modulation of oxidative stress and Parkin-mediated mitochondrial clearance. Oncogene 2017;36(34):4843–58.
[60] Grazioli S, Pugin J. Mitochondrial damage-associated molecular patterns: from inflammatory signaling to human diseases. Front Immunol 2018;9:832.

[61] Sun K, Jing X, Guo J, Yao X, Guo F. Mitophagy in degenerative joint diseases. Autophagy 2021;17(9):2082–92.
[62] Zhang Z, Xu T, Chen J, Shao Z, Wang K, Yan Y, et al. Parkin-mediated mitophagy as a potential therapeutic target for intervertebral disc degeneration. Cell Death Dis 2018;9(10):980.
[63] Ansari MY, Khan NM, Ahmad I, Haqqi TM. Parkin clearance of dysfunctional mitochondria regulates ROS levels and increases survival of human chondrocytes. Osteoarthr Cartil 2018;26(8):1087–97.
[64] Weir HJ, Yao P, Huynh FK, Escoubas CC, Goncalves RL, Burkewitz K, et al. Dietary restriction and AMPK increase lifespan via mitochondrial network and peroxisome remodeling. Cell Metab 2017;26(6):884–96 e5.
[65] D'Amico D, Mottis A, Potenza F, Sorrentino V, Li H, Romani M, et al. The RNA-binding protein PUM2 impairs mitochondrial dynamics and mitophagy during aging. Mol Cell 2019;73(4):775–87 e10.
[66] Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 2004;429(6990):417–23.
[67] Lieber T, Jeedigunta SP, Palozzi JM, Lehmann R, Hurd TR. Mitochondrial fragmentation drives selective removal of deleterious mtDNA in the germline. Nature 2019;570(7761):380–4.
[68] Ahier A, Dai CY, Kirmes I, Cummins N, Hung GCC, Gotz J, et al. PINK1 and parkin shape the organism-wide distribution of a deleterious mitochondrial genome. Cell Rep 2021;35(9):109203.
[69] Ryu D, Mouchiroud L, Andreux PA, Katsyuba E, Moullan N, Nicolet-Dit-Felix AA, et al. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. Nat Med 2016;22(8):879–88.
[70] Andreux PA, Blanco-Bose W, Ryu D, Burdet F, Ibberson M, Aebischer P, et al. The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial and cellular health in humans. Nat Metab 2019;1(6):595–603.
[71] Fang EF, Waltz TB, Kassahun H, Lu Q, Kerr JS, Morevati M, et al. Tomatidine enhances lifespan and healthspan in C. elegans through mitophagy induction via the SKN-1/Nrf2 pathway. Sci Rep 2017;7:46208.
[72] Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, et al. Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 2009;11(11):1305–14.
[73] Yang X, Zhang M, Dai Y, Sun Y, Aman Y, Xu Y, et al. Spermidine inhibits neurodegeneration and delays aging via the PINK1-PDR1-dependent mitophagy pathway in C. elegans. Aging (Albany NY) 2020;12(17):16852–66.
[74] Guo J, Chiang WC. Mitophagy in aging and longevity. IUBMB Life 2022;74(4):296–316.
[75] Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, et al. NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. Science 2016;352(6292):1436–43.
[76] Mahalakshmi R, Priyanga J, Bhakta-Guha D, Guha G. Hormetic effect of low doses of rapamycin triggers anti-aging cascades in WRL-68 cells by modulating an mTOR-mitochondria cross-talk. Mol Biol Rep 2022;49(1):463–76.
[77] Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011;13(2):132–41.
[78] Li Y, Wang Y, Kim E, Beemiller P, Wang CY, Swanson J, et al. Bnip3 mediates the hypoxia-induced inhibition on mammalian target of rapamycin by interacting with Rheb. J Biol Chem 2007;282(49):35803–13.
[79] Loffler AS, Alers S, Dieterle AM, Keppeler H, Franz-Wachtel M, Kundu M, et al. Ulk1-mediated phosphorylation of AMPK constitutes a negative regulatory feedback loop. Autophagy 2011;7(7):696–706.
[80] Laker RC, Drake JC, Wilson RJ, Lira VA, Lewellen BM, Ryall KA, et al. Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. Nat Commun 2017;8(1):548.
[81] Seabright AP, Fine NHF, Barlow JP, Lord SO, Musa I, Gray A, et al. AMPK activation induces mitophagy and promotes mitochondrial fission while activating TBK1 in a PINK1-Parkin independent manner. FASEB J 2020;34(5):6284–301.
[82] Pietrocola F, Castoldi F, Markaki M, Lachkar S, Chen G, Enot DP, et al. Aspirin recapitulates features of caloric restriction. Cell Rep 2018;22(9):2395–407.
[83] Mehrabani S, Bagherniya M, Askari G, Read MI, Sahebkar A. The effect of fasting or calorie restriction on mitophagy induction: a literature review. J Cachexia Sarcopenia Muscle 2020;11(6):1447–58.
[84] Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. Ageing Res Rev 2017;39:46–58.