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Mitophagy in human health, ageing and disease

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Abstract

Maintaining optimal mitochondrial function is a feature of health. Mitophagy removes and recycles damaged mitochondria and regulates the biogenesis of new, fully functional ones preserving healthy mitochondrial functions and activities. Preclinical and clinical studies have shown that impaired mitophagy negatively affects cellular health and contributes to age-related chronic diseases. Strategies to boost mitophagy have been successfully tested in model organisms, and, recently, some have been translated into clinics. In this Review, we describe the basic mechanisms of mitophagy and how mitophagy can be assessed in human blood, the immune system and tissues, including muscle, brain and liver. We outline mitophagy's role in specific diseases and describe mitophagy-activating approaches successfully tested in humans, including exercise and nutritional and pharmacological interventions. We describe how mitophagy is connected to other features of ageing through general mechanisms such as inflammation and oxidative stress and forecast how strengthening research on mitophagy and mitophagy interventions may strongly support human health.

Mitophagy is a specific form of autophagy (from the Greek, *phagos*: eating, consuming) aimed at tagging, removing and recycling damaged and depolarized mitochondria[1]. Mitophagy also clears superfluous organelles, such as sperm mitochondria, after fertilisation. Mitophagy works in coordination with mitochondrial dynamics and biogenesis to replenish the mitochondrial pool with new, healthy organelles[2]. This continuous quality-

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Author contributions

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Competing interests

J.F. and D.D'A. are employees of Amazentis. J.A. is a founder and/or consultant of MitoBridge-Astellas, MetroBiotech, Amazentis, Vandria, OrsoBio and NOV Metapharma. A.P. and L.F. declare no conflict of interest.

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control process is critical to maintaining an optimal level of mitochondrial functions, such as oxidative phosphorylation (OxPhos) and ATP synthesis, Ca^{2+} regulation, reactive oxygen species (ROS) production and mitochondrial DNA (mtDNA) maintenance, which are essential for cellular health.

Impaired mitophagy causes the accumulation of dysfunctional mitochondria and contributes to the pathogenesis of many chronic diseases. Mitophagy is considered both a hallmark of ageing[3] and a hallmark of health[4]. Hence, a comprehensive understanding of the mechanisms that regulate mitophagy is clinically relevant, and interventions boosting mitophagy can potentially increase healthspan by delaying the onset of chronic diseases.

In early life, programmed mitophagy is enacted to support developmental processes[5,6]. Later on, a basal level of mitophagy allows the continuous recycling of damaged mitochondria over the life of a cell[7,8]. Finally, mitophagy is acutely enhanced when stressors challenge mitochondrial integrity and function[9].

The signalling cascades that activate and modulate mitophagy have been extensively studied in model organisms and have been reviewed elsewhere[1,10], although some details of mitophagy regulation and interaction with other biological processes still need to be fully understood. The first part of our Review will describe different biological mechanisms involved in the mitophagy pathway. We will then focus on the growing number of studies investigating mitophagy's role in human pathophysiology, with sections dedicated to different organs and disease conditions. Sections will cover the role of mitophagy in skeletal and heart muscle function, brain ageing and neurodegeneration, immune health, metabolic function and cancer (summarized in Table 1). This is followed by an overview of the main mechanisms of mitophagy dysfunction and how, by interacting with other biological processes, they affect health and disease. Finally, we outline the emerging evidence that boosting mitophagy supports health and improves the clinical course of diseases associated with mitochondrial dysfunction.

An introduction to mitophagy

Among different mitophagy classification standards, one frequently used approach is based on dependence on the PINK1–parkin signalling axis; accordingly, mitophagy can be grouped as PINK1–parkin dependent and PINK1–parkin independent[1] (Fig. 1).

The phosphatase and tensin homologue (PTEN)-induced kinase (PINK)1–parkin-dependent pathway is the best characterized and considered the canonical pathway for mitophagy[1]. PINK1 is localized on the outer mitochondrial membrane and in basal conditions is continuously cleaved and inactivated. Upon mitochondrial stress or programmed mitophagy, reduction in mitochondrial membrane potential prevents cleavage and degradation of PINK1, thereby allowing its accumulation on the mitochondrial surface. Accumulated PINK1 recruits and phosphorylates the E3 ubiquitin (Ub) ligase parkin, unblocking its enzymatic activity and leading to the ubiquitination of outer membrane mitochondrial proteins. In turn, PINK1 phosphorylates newly formed Ub moieties generating phospho (p)-Ub chains exposed to the cytosol. p-Ub chains act as a docking site to recruit adaptor

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proteins, such as optineurin and calcium-binding and coiled-coil domain 2 (CALCOCO2, also known as NDP52) (ref. 11). The autophagic adaptor protein p62 is also recruited to mitochondria and is essential for final clearance. Through the microtubule-associated protein light chain 3 (LC3), these proteins allow buildup of the mitophagosome, a bilayer lipidic membrane that encloses the mitochondrion and fuses with lysosomes, where mitochondria are degraded by enzymes active in an acidic environment (Fig. 1a).

PINK1–parkin-independent mitophagy pathways involve other players, such as BCL2-interacting protein 3 (BNIP3), NIP-3-like protein X (NIX) and FUN14 domain-containing protein 1 (FUNDC1), which can recruit LC3 independently of PINK1–parkin to promote mitophagosome formation[10]. BNIP3 interacts with and supports the stabilisation of PINK1 (refs. 11,12). Furthermore, both BNIP3 and NIX can modulate parkin recruitment[13], indicating a potential interplay between PINK1–parkin-dependent and -independent pathways (Fig. 1b). A variety of other mitophagy receptors participate in mitophagy execution including BCL2L13, FKPB8, PHB2 and cardiolipins as well as the mitophagy–autophagy receptors AMBRA1, TAXIBP1 and NBR1 (refs. 1,14).

Recent literature discussed noncanonical mechanisms for removing and recycling mitochondria in addition to canonical PINK1–parkin-dependent and -independent pathways. Lysosomal-driven degradation of mitochondrial-derived vesicles (MDVs) has been identified, which can eliminate damaged but not depolarized mitochondria as an early response to oxidative stress[15,16]. While the exact mechanisms of MDV generation are unclear, their biogenesis requires PINK1 and parkin priming and herniation of mitochondrial membranes likely favoured by oxidized cardiolipins[17,18] (Fig. 1c). In addition, mitochondria can be extruded from cells through exospheres, extracellular vesicles containing mitochondria, formed by cytoplasmic membrane blebbing. These extracellular vesicles can be taken up by distal cells and release their mitochondrial cargo, which is then degraded by canonical mitophagy processes. This mechanism contributes to the transfer and degradation of damaged mitochondria from cardiomyocytes[19,20] or brown adipose tissue[21] to macrophages and from retinal ganglion cell axonal mitochondria to astrocytes[22] (Fig. 1d). A similar mechanism was shown in *Caenorhabditis elegans*[23] to clean neuronal cells as a response to compromised proteostasis (Fig. 1d). In the setting of hypoxia, the generation of enlarged and dysfunctional organelles (megamitochondria) has been identified to promote mitochondrion–lysosome contacts followed by lysosome engulfment by the mitochondrion and mitochondrial self-digestion[24] (Fig. 1e).

Mitophagy is tightly connected with mitochondrial behaviours such as mitochondrial dynamics and biogenesis. Mitochondrial dynamics involve continuous division (fission) and fusion of organelles that both maximize efficiency and adaptability of OxPhos and allow the elimination of damaged mitochondrial components and/or dilution of organelle damage, resulting in a dynamic allostatic equilibrium. Efficient mitophagy requires mitochondrial fission to portion damaged mitochondria into smaller entities that are easier to be engulfed and degraded by autophagosomes. To this aim, the recruitment of the mitochondrial dynamic protein dynamin-related protein 1 (DRP1) is required for mitochondrial fragmentation and consequent parkin-, FUNDC1- and BNIP3-mediated mitophagy execution[13,25]. DRP1 activity is also upregulated by PINK1, thus preventing its phosphorylation through protein

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kinase A (PKA)[26] and leading to fission and improved mitophagy[27,28]. Conversely, activated parkin was shown to abolish fusion through ubiquitination and degradation of the fusion mediators mitofusin (MFN)1 and MFN2 (refs. 29,30). Finally, mitophagy activity acts in coordination with mitochondrial biogenesis[31]. The energy sensor AMP-activated protein kinase (AMPK) phosphorylates peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), which enhances both generation of newly synthesized organelles[32,33] and mitophagy, by promoting the transcription of nuclear respiratory factor 1 (NRF1) and NRF2. Indeed, the PGC-1α–NRF1 axis promotes FUNDC1-mediated mitophagy; and sequestosome 1 (SQSTM1) or p62 is a transcriptional target of NRF2 (ref. 34). AMPK also regulates mitophagy through UNC-51-like autophagy-activating kinase 1 (ULK1)-mediated autophagosome formation[35] and direct phosphorylation of mitochondrial fission factor[36], which promotes PINK1–parkin-mediated mitophagy[37]. Conversely, parkin appears to activate mitochondrial biogenesis by degrading its substrate PARIS, which is a PGC-1α inhibitor[38] and by enhancing transcription factor A, mitochondrial (TFAM)-mediated transcription activity[39].

Mitophagy and the muscle

Skeletal muscle

Skeletal muscle is key for mobility and metabolic homeostasis. It holds remarkable plasticity, engaging different metabolic reactions depending on muscle fibre type and physiological needs. Energy requirements of the muscle vary substantially between rest and active contraction, with an increase of about 100-fold[40]. During muscle contraction, energy in the form of ATP is required for actin–myosin cross-bridge cycling. Still, the available ATP is only sufficient for 2 s of continuous contraction. While energy accumulation in the phosphocreatine system maintains sufficient energy availability for a few more seconds, fresh energy production is soon required to maintain function[41]. Both anaerobic and aerobic reactions are engaged to contribute to ATP synthesis and meet such massive muscle energy requirements. The contribution of anaerobic metabolism becomes substantial above a certain threshold of exercise intensity[40]. However, muscles draw energy mostly from aerobic, mitochondrial oxidative metabolism during prolonged submaximal exercise. Thus, mitophagy is of the outmost importance in maintaining high-quality mitochondria for energy production and muscle contraction. Mitochondria and mitophagy also assist in muscle stem cell differentiation and muscle regeneration[42,43].

Inducing mitophagy in experimental models has been shown to promote muscle function and mobility using genetic modelling[37,44,45], dietary interventions and pharmacological approaches[46–50]. Mitophagy activation has also been proven to replenish the regenerative capacity of aged satellite cells in mice. This indicates that the age-related decrease in satellite cell regenerative capacity can be partly ascribed to reduced mitochondrial signalling control[43]. A growing number of clinical studies investigated mitophagy in human skeletal muscle as a target to counteract age-related decline in muscle mass and function to prevent loss of independence in older age (Fig. 2a and Table 1).

In human skeletal muscle, ageing significantly reduces mitochondrial function[51], and there is evidence that such decline affects muscle strength and walking performance[52,53].

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However, decline in mitochondrial function is not always associated with a proportional reduction in mitochondrial mass, suggesting accumulation of dysfunctional mitochondria that are not efficiently removed because of impaired mitophagy[54–56]. Persistent reduced mitochondrial signalling control due to mitophagy impairment may also stall mitochondrial biogenesis[57,58].

Dysfunctional mitophagy upon muscle ageing was suggested by most but not all studies. Skeletal muscle of physically inactive frail older women exhibits reduced expression of the mitophagy-related genes *PRKN* (encoding the cytosolic ubiquitin-E3-ligase protein parkin), *ATG7* (autophagy-related 7), *BECN1* and *BNIP3* (ref. 59). Reduced levels of parkin were also observed in muscle biopsies from physically active older men compared to young men[54]. Another research group showed that p62 and BNIP3 protein content was increased and negatively correlated with muscle volume[60]. A separate study failed to demonstrate different levels of parkin, BNIP3 and p62 in sedentary older adults compared to young controls[55]. However, energy shortage due to prolonged impaired OxPhos function limits the capacity of cells to execute this energetically expensive process. The fact that damaged mitochondria still accumulate with ageing clearly indicates that mitophagy is impaired and suggests that mitophagy boosters counteract, at least in part, the harmful effect of muscle ageing and/or delay its impact on mobility.

Dysfunctional mitophagy also plays a pathogenetic role in skeletal muscle disease conditions. Patients with Duchenne muscular dystrophy have defective muscle mitophagy[47]. Abnormal mitophagy has also been documented in the muscle of patients with degenerative sporadic inclusion body myositis[61,62]. There is clinical evidence of reduced mitophagy in diaphragm myopathy with severe heart failure (HF), with patients showing reduced mitochondrial number and function and lower levels of autophagy–mitophagy proteins LC3 and p62 (ref. 63). Finally, muscle proteomic analyses in people with peripheral artery disease suggest that mitophagy is impaired in peripheral artery disease, leading to an unbalanced accumulation of mitochondrial-versus nuclear-expressed respiratory complex subunits, with a consequent reduction in mitochondrial function[64].

Cardiac muscle

Cardiac muscle continuous contraction under high load demands constant and abundant energy availability. Such energy derives mainly from mitochondrial fatty acid oxidation, making mitochondria key for heart contractility. Mitochondria occupy 23–30% of the myocardium cellular volume[65], and preclinical models indicated that both PINK1–parkin-dependent[66] and -independent mitophagy[67] are required to preserve heart mitochondrial homeostasis. Maintaining adequate levels of mitophagy is an essential mechanism for cardioprotection during ageing[68]. Heart transcriptomics in patients with both dilated and ischaemic cardiomyopathy showed changes that are indicative of altered mitochondrial signalling as a common signature of end-stage HF. Co-repression of the protein ubiquitination pathway in these patients suggests altered autophagy–mitophagy processes[69]. Supporting this interpretation, significantly lower levels of PINK1 protein have been found in the heart of patients with end-stage HF than in controls[70], and levels of the mitophagy protein parkin were significantly lower in patients with idiopathic dilated cardiomyopathy[68].

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Finally, an isoform shift of AMPK from AMPKα2 to AMPKα1 has been observed in failing heart samples from patients with HF[71]. As AMPKα2, but not AMPKα1, can phosphorylate and activate PINK1 (ref. 71), the isoform shift might contribute to reduced ability to activate mitophagy during HF (Fig. 2a and Table 1).

Mitophagy in brain ageing and neurodegeneration

Brain integrity and function strongly depend on mitochondrial OxPhos. While the brain comprises only 2% of body weight, it consumes about 20% of its oxygen supply[72,73]. Because of such high energy demand, brain mitochondria are under considerable stress, and mitophagy-mediated repair and recycling are neuroprotective against brain ageing and neurodegenerative diseases[14] (Fig. 2a).

In healthy older adults, p-Ub proteins have been shown to accumulate in the hippocampus, often within vacuoles, while only a few p-Ub granules were detected in younger individuals[74]. It has been suggested that such accumulation of p-Ub, a well-established marker of PINK1–parkin-related mitophagy, reflects increased mitochondrial damage and/or decreased mitophagy flux[74].

Defective mitophagy also contributes to the development of mild cognitive impairment (MCI), Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration[75]. Patients with both MCI and AD showed reduced circulating levels of autophagy-related gene (ATG)5 and parkin proteins compared to healthy controls[76]. Reduced protein levels of the mitophagy regulators ULK1 and TANK-binding kinase 1 (TBK1) have been reported in both hippocampal tissues and induced pluripotent stem cell-derived neurons from patients with AD compared to controls[77]. Similarly, levels of the mitophagy receptor PINK1 were decreased in the hippocampus of patients with AD versus healthy individuals in two independent works[77,78]. In other studies, p-Ub has been found to accumulate in the brains of patients with AD, and, depending on the brain region, it colocalizes with mitochondria, granulovacuolar degeneration bodies and/or lysosomes[79–82]. Therefore, mitophagy decline is thought to be accelerated in the AD brain. However, depending on the brain region, such a decline may be caused by reduced expression of mitophagy components, stalled trafficking or reduced lysosomal activity and mitophagy flux (Fig. 2b). Preclinical studies with mitophagy boosters showed efficacy in activating mitophagy and reverting AD phenotypes[77,83,84]. Clinical trials are now required to confirm that such interventions effectively counteract the development of AD in humans.

There is strong evidence that loss of function of mitophagy components is implicated in the pathogenesis of PD. The parkin gene (*PRKN*) was named as such after discovering that its loss-of-function mutation was associated with autosomal PD[85]. Several mutations in this gene have been linked to autosomal recessive juvenile PD[86], and other PD-related genes have indirectly affected mitochondrial quality control. For instance, the deglycase DJ-1, encoded by the *PARK7* gene, holds a key protective role against oxidative stress and preserves mitochondrial homeostasis[87], and mutations in DJ-1 are causally related to autosomal recessive PD[88]. Studies in PD animal models showed that impaired mitophagy is causally linked to increased inflammatory responses and loss of dopaminergic neurons[89].

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However, *Prkn*-knockout mice fail to develop PD-related phenotypes, suggesting that the pathophysiology of PD may be more complex and cautioning about the translation of preclinical data[90]. Parkin-associated biomarkers were shown to be altered in patients with PD. Reduced plasma levels of p-Ub have been reported in patients with loss-of-function PINK1 PD compared to age-matched healthy controls[91]. Additionally, a study in human skin fibroblasts showed broad insensitivity to mitophagy activation in samples from patients with PD compared to healthy controls. This loss of function is mediated by impaired mitochondrial recruitment of parkin and of the multifunctional kinase and mitophagy activator LRKK[92].

Gene variants related to mitochondrial function and mitophagy have been associated with other neurodegenerative disorders. Polymorphisms in the *OPTN* gene, encoding the mitophagy adaptor optineurin, are associated with ALS[93,94]. *SQSTM1* (ref. 90) and *TBK1* (ref. 95) are two other genes involved in mitophagy regulation and with ALS disease-associated mutations[96,97].

Given the role of mitophagy in neurodegeneration, further clinical studies are warranted to investigate how mitophagy dysfunction occurs in healthy older adults, that is, by genetic predisposition, environmental causes or combinations. Developing new human mitophagy biomarkers could help design strategies to support mitochondrial function and mitophagy for preventing and slowing down neurological disorders.

Mitophagy and the immune system

The immune system protects organisms against viruses, bacteria, microorganisms and biological contaminants, continuously surveils for damaged cells and tissues and actively participates in tissue repair. Mitophagy is critical in maintaining optimal mitochondrial functions and an efficient shift between oxidative metabolism to glycolysis for specific immune cells to be engaged and contribute to cell-specific immune regulatory functions[98]. Mitophagy also regulates immunity by restraining the production and circulation of mitochondrial components that can trigger immune cell responses (see Mechanisms of impaired mitophagy and impacted biological processes for more details; Fig. 2a and Table 1).

Genetic and pharmacological manipulation of ex vivo human immune cells and preclinical models indicated that mitophagy plays a critical role in preserving homeostasis and supporting the survival of T cells, microglia[99] and macrophages[5].

In humans, several lines of research connect impaired mitophagy in immune cells and the development of immunosenescence, a process involving age-related morphological and functional changes in cells of innate and adaptive immunity, leading to a blunted response to acute challenges paralleled by a state of chronically activated, mild inflammatory stimulation, called ‘inflammaging’ (refs. 100,101).

Primary $CD8^+$ T cells of older people show low levels of lysosomal (LysoID) and autophagosomal LC3 markers and a reduced overlap of LC3 and the acidic compartment of $CD8^+$ T cells, indicating reduced basal macroautophagy[102]. Autolysosome accumulation

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has been reported in $CD8^+$ T lymphocytes undergoing in vitro replicative senescence and induced sensitisation to cell death[103]. Recent evidence reported stalled mitophagy in $CD4^+$ lymphocytes of older adults as indicated by transmission electron microscopy images showing accrual of dysfunctional mitochondria in autophagic compartments of these cells[104].

Accordingly, the autophagic flux of activated isolated $CD4^+$ T cells decreased with age and was preserved in progenies of centenarians, a model of successful ageing[105]. Conversely, ex vivo peripheral blood mononuclear cells (PBMCs) from older people cultured in artificial medium hold higher basal autophagic flux[106,107] than younger PBMCs and show lower responsiveness to the autophagy inducer rapamycin[106], suggesting a potential differential sensitivity of basal and stimulated autophagy to ageing. These findings indicate that developing a standardized measure for assessing mitophagy is of the utmost importance and suggest that preserving mitochondrial turnover may protect against age-associated accumulation of damaged mitochondria in immune cells, inflammaging and blunted immune defence in older adults.

Mitophagy defects have also been found in other blood cell diseases. For instance, retention of damaged mitochondria in red blood cells due to inadequate mitophagy-dependent mitochondrial removal has been described in people with sickle cell disease and is associated with subphenotypes that could be related to disease complications[108].

Mitophagy and metabolic disorders

High ROS production, low levels of ATP and dysmorphic mitochondria have been documented in preclinical models of type 2 diabetes (T2D) and liver disease[109,110]. Altered mitochondrial turnover is also increasingly recognized in the context of human metabolic disorders. However, the relationship between defective mitophagy and metabolic diseases is complex and potentially bidirectional. On one side, mitophagy defects could be an upstream cause of reduced energy availability and excessive oxidative stress, triggering metabolic abnormalities. On the other hand, metabolic diseases may cause a scarcity of energy that makes homeostatic mitophagy activity less effective (Fig. 2b(iv))[111].

Mitophagy in non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is excessive intra-hepatic lipid accumulation unrelated to alcohol use[112]. This can evolve into non-alcoholic steatohepatitis (NASH), which is characterized by liver inflammation predisposing to cirrhosis and, ultimately, hepatic cellular carcinoma. The relationship between these diseases and mitophagy function has been explored mostly in preclinical models with few investigations in humans.

Ablation of ATGs, lysosomal biogenesis transcription factor EB (TFEB) and the mitophagy mediator PINK1 has been shown to induce lipid accumulation and trigger liver steatosis in preclinical models[113,114]. Activation of autophagy by pharmacological interventions or liver-targeted *TFEB* gene transfer reduced liver fibrosis and ameliorated liver disease in mice[115,116]. These findings indicate that targeting mitophagy may be a crucial step to counteract metabolic disorders. Reduced activity of the key metabolic and

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mitophagy regulator AMPK has also been reported in preclinical models of NAFLD[117]. A mitochondrial-localized pool of AMPK has also been shown to regulate mitochondrial bioenergetics, promoting mitochondrial quality via mitophagy[118]. Whether mitochondrial AMPK signalling is reduced in the context of NAFLD is still unknown but could explain, at least in part, the altered mitochondrial turnover and bioenergetic failure observed.

In humans, no data are available on mitophagy biomarkers and NAFLD. However, data on mitochondrial quality suggest a contribution of mitophagy to disease progression. Increased hepatocyte mitochondrial oxidative capacity has been shown as a compensatory mechanism to buffer excessive lipid availability and avoid lipid accumulation in humans with NAFLD. However, this metabolic flexibility is lost in NASH[119], as the overall mitochondrial capacity is impaired, despite an increase in mitochondrial mass. These mitochondria accumulating in the human liver were shown to be inefficient at metabolizing lipids and produce higher levels of ROS and toxic lipid metabolites[119]. Therefore, targeting mitophagy could be a strategy for balancing metabolic homeostasis, maintaining high-quality and low ROS-producing organelles and protecting or halting NAFLD and NASH[120].

Mitophagy in insulin resistance and T2D

A decline in mitophagy flux has been reported in preclinical models of T2D[98], both directly in the liver and the pancreas and also in muscle, kidneys and adipose tissue as a result of systemic metabolic imbalance[121].

Studies in preclinical models indicate that hyperglycaemia in insulin resistance and hyperinsulinaemia blunt PINK1–parkin-dependent mitophagy in the liver[122]. PINK1–parkin-dependent mitophagy has also been reported to protect pancreatic beta cells and improve glucose intolerance in diabetic mice, and most of this protective effect seems to derive from protection of beta cells from inflammatory injury[123]. There is evidence that entire and/or portioned mitochondria are transferred by noncanonical mitophagy in response to an altered metabolic milieu and/or flux[21,124].

In humans, reduced expression of *PINK1* have been reported in muscle and adipose tissue of patients with T2D compared with healthy particpants[125]. PINK1 levels were decreased also in PBMCs isolated from patients with both diabetic nephropathy (DN) and diabetes without kidney disease. This decline was associated with significant impairment of both maximal respiration and reserve capacity in patients with DN compared to diabetic control patients but not healthy controls[126]. Finally, mitochondrial function assessed in the muscle by magnetic resonance imaging spectroscopy has been associated with severity and length of insulin resistance in non-diabetic persons[127].

Mitophagy and cancer

The role of mitophagy in cancer is complex, and reduced mitophagy may support cancer development by inducing accumulation of damaged mitochondria and oxidative stress favoring genomic instability[128]. Indeed, high-frequency loss of *PRKN* heterozygosis has been observed in breast and ovarian tumours and is associated with reduced *PRKN* expression[129] and poor outcome[130]. Loss of *PRKN* copy number was detected also in a

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fraction of patients with colon carcinoma, and, in preclinical models, parkin loss of function accelerated cancer development, suggesting a tumour-suppressive role of parkin[131].

Some works reported increased levels of mitophagy components. A study analysing human breast cancer biopsies reported an increase in mRNA expression of the genes *PRKN*, *BNIP3*, *BECN1* and *MAP1LC3A* when comparing tumour cells with peritumoural tissues[132]. Also, 31.9% of patients with early endometrial adenocarcinomas from a small study cohort had increased *BNIP3* levels, which correlated with higher hypoxia-inducible factor 1α (HIF-1α) levels and poorer survival[133]. It has yet to be determined whether this increase is a compensatory mechanism to protect against cancer cell development or may be linked to tumour growth by promoting mitochondrial homeostasis[128].

This bimodal direction of mitophagy biomarkers in cancer likely depends on tumour type, stage, microenvironment and genetic alterations. For instance, in a humanized mouse model of pancreatic ductal adenocarcinoma with mutation in the oncogene *KRAS*, genetic ablation of macroautophagy components *Atg5* or *Atg7* halted the progression of neoplastic cells. However, in the same model with mutations in the genes encoding KRAS and p53, macroautophagy ablation led to enhanced tumour formation[134]. Noncanonical mitophagy should also be further studied in oncology, given that processes like MDV formation have been suggested to compensate for defective canonical mitophagy and promote cancer cell survival[135].

Regulation of mitophagy may also play a role in cancer drug sensitivity, especially when using anti-mitochondrial drugs. A phase II clinical study showed that the tricarboxylic acid cycle inhibitor devimistat was more effective in older adults with potentially reduced mitophagy. Inhibition of mitochondrial fission or autophagy components in cell-based models sensitizes cancer to devimistat. To explain these findings, it was hypothesized that autophagy and/or mitophagy inhibition prevents the removal of devimistat-induced toxic ROS byproducts and causes cell death[136]. Similar results were shown preclinically in mice with SH-SY5Y xenografted tumours, in which inhibition of PINK1–parkin-mediated mitophagy enhanced the effect of the anticancer drug magnolol[137].

Overall, a more comprehensive understanding of the relationships between human cancer features and mitophagy changes is warranted for determining the role of mitophagy in cancer progression and clarifying the clinical relevance of targeting mitophagy in oncology.

Mechanisms of impaired mitophagy and impacted biological processes

The sections above outlined changes in mitophagy and associated biomarkers in different tissues during human ageing and diseases. Clinical and preclinical studies delineated distinct mechanisms underlying mitophagy alterations (Fig. 2b). In certain conditions, mitophagy defects derive from downregulation of components of the mitophagy machinery, either caused by mutations or by the influence of upstream regulators (Fig. 2b(i)). This may lead to insufficient levels of mitophagy effectors, with consequent deficient removal and increased accumulation of damaged mitochondria. Conversely, other studies reported increased levels of mitophagy components with ageing and diseases (Fig. 2b(ii)). It can be hypothesized

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that this is a compensatory response to a status of excessive mitochondrial damage. The frequent accumulation of mitophagy receptors, damaged mitochondria or mitophagosome vesicles suggests that the process stalled before lysosomes can degrade mitochondria. This is in line with mutations and functional deficits in lysosomal activity observed in several human diseases (Fig. 2b(iii)). A likely overarching mechanism may be an overall cellular energy deficiency (Fig. 2b(iv)). Lack of energy may blunt the efficient synthesis and folding of mitophagy components and impair the correct assembly and function of autophagosomes and lysosomes. Consequently, dysfunctional mitophagy is associated with detrimental effects on cellular homeostasis and is interconnected with other features of poor health, ageing and disease, such as inflammation and oxidative stress (Fig. 2c).

While playing a relevant role as signalling molecules for physiological purposes[138], high levels of ROS emerging from age-related altered mitophagy and reduced antioxidant buffering can inflict oxidative damage on macromolecules and organelle components (that is, lipids (lipid peroxidation), proteins (proteotoxic stress) and nucleic acids) and play a relevant role in pathological processes implicated in ageing and associated diseases[138]. Damaged mitochondria can also release in the cytosol or at the extracellular level a set of molecules identified as mitochondrial damage-associated molecular patterns (mtDAMPs)[139], including oxidized mtDNA, mitochondrial RNA (mtRNA) and cardiolipins[140] (Fig. 3). Cytosolic mtDNA is sensed as ‘non-self’ by pattern recognition receptors of innate immunity due to its bacterial ancestry, lack of histones and hypomethylated CpG motifs[141]. It can activate inflammatory pathways, such as the NLR family, pyrin domain-containing 3 (NLRP3) inflammasome, the DNA-sensing cyclic GMP–AMP synthase (cGAS)–stimulator of interferon response cGAMP interactor 1 (STING) pathway, the checkpoint for DNA transcription and Toll-like receptor (TLR) systems. While NLRP3 inflammasome activation culminates in caspase-1 activation and pro-inflammatory signalling through interleukin (IL)-1 and IL-18, activation of TLRs and the cGAS–STING pathway induces cellular senescence and type I interferon signalling[142]. mtRNA can also be recognized as ‘non-self’ by the mtRNA-sensing mitochondrial antiviral signalling protein (MAVS)–retinoic acid-inducible protein 1 (RIG1) complex and trigger the interferon γ response. Finally, cardiolipins are known to be herniated from the inner mitochondrial membrane[143], leading to cytochrome c-mediated apoptosis[144], but can also act as mtDAMPs when released and eventually oxidized in the cytosol, bind to TLR4 and activate pro-inflammatory signals through nuclear factor (NF)-κB[145] (Fig. 3). Preclinical studies support a causal link between mitophagy and mtDAMP-mediated inflammation. Cell-based studies indicate that increased mitophagy can attenuate mtDNA oxidation and release and blunt TLR9 pro-inflammatory signalling[146]. In mice, PINK1 and parkin activity was shown to mitigate STING-induced inflammation[147], and mitophagy activation increased microglial phagocytic efficiency and mitigated neuroinflammation via inhibition of NLRP3 signalling[77].

Evidence of a link between mitophagy and mtDAMP-induced inflammation emerged from recent studies also in humans. Mitophagy impairment and mtDNA leakage were shown to activate STING signalling in aged macrophages of older individuals with liver injury[148]. Moreover, low protein expression of PINK1 and high levels of free mtDNA have been found in people with interstitial lung diseases, including idiopathic pulmonary fibrosis, hypersensitivity pneumonitis and autoimmune-related interstitial lung disease[146].

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Furthermore, cell-free mtDNA and associated sterile inflammation have been proposed as major contributors to the pro-atrophy signalling pathways leading to age-related muscle wasting[149].

A link between defective mitochondrial turnover, inflammation and impaired human immunity also comes from colocalization of mitochondria with high ROS emission and calcium dysregulation in the autophagy compartments of aged human $CD4^+$ T cells[104]. Altered mitophagy along with changes in the repertoire of inflammatory cells and mtDNA deletion load have been described in muscle fibres of people with sporadic inclusion body myositis and has a role in its progression[61]. These alterations were identified in most respiratory-deficient muscle fibres for which a strong correlation between T lymphocyte infiltration and the percentage of fibres holding mtDNA mutations was observed[62]. Respiratory-deficient muscle fibres were also more prone to atrophy than respiratory-competent controls, perhaps as a consequence of the bulk inflammatory response mounted by T lymphocytes and muscle-resident macrophages[62].

Insights on mechanisms of mitophagy dysfunction and cross-talk with other hallmarks of ageing across organs could derive from the study of models of premature ageing. Mitophagy loss was first described in Cockayne syndrome and, together with accumulation of mtDNA[150], was further identified in diseases such as ataxia–telangiectasia[151], xeroderma pigmentosum[152,153] and Werner syndrome[154]. Interestingly, mitophagy activation has been shown to partially counteract the development of ataxia–telangiectasia and Werner syndrome disease in preclinical models[155,156].

In summary, different mechanisms of mitophagy dysfunction converge on causing inefficient OxPhos, increased ROS production and accumulation of oxidative damage. In addition, mtDAMPs release can trigger pro-inflammatory signals that may further amplify cellular damage. Conversely, optimal mitophagy allows cyclic removal of mitochondria, prevents cellular damage signals and maintains optimal respiratory efficiency. The latter is due to the preservation of a pool of mitochondria with better intrinsic mitochondrial capacity and to the increase in mitochondrial content by coordinated activation of mitochondrial biogenesis (Fig. 2c).

Interventions to boost mitophagy in humans

Exercise

The effect of exercise on mitophagy depends on type and duration, with endurance exercise being more effective in enhancing mitophagy in muscle than resistance training (RT). Indeed, increased levels of the activated form of the mitophagy mediators parkin and PINK1 have been observed in the muscle of adult long-term runners[157]. This was confirmed in a separate study in young and old cyclists who had higher mitochondrial parkin and LC3-II protein levels than age-matched sedentary controls[55]. In addition, protein content of parkin was elevated in moderately trained young males following an 8-week endurance training regimen compared to baseline. Mitophagy gene expression was also induced after a 12-week programme of combined aerobic and RT in patients with dermatomyositis and immune-mediated necrotizing myopathies[158]. No acute activation of mitophagy was

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observed following 2 h of cycling in trained athletes, suggesting that mitophagy activation requires longer exercise regimens[159].

Pure resistance exercise training does not appear to robustly impact mitophagy. RT training (8–10 weeks) did not alter mitophagy biomarkers in the muscle (10-week intervention)[160] or in PBMCs (8-week intervention) of older people[161]. In another clinical study in middle-aged individuals, a 2-week RT programme following leg immobilisation modestly increased *PINK1* gene expression, with no impact on other mitophagy components[162].

Nutritional interventions

There is growing interest in strategies to impact mitophagy by changing dietary habits or implementing diets with specific mitophagy-enhancing supplements as the idea that behavioural changes over the life course can expand health expectancy and prevent major chronic diseases with ageing is gaining traction.

Caloric restriction (CR), defined as a reduction in food intake without malnutrition, is considered one of the most conserved and efficient strategies to impact hallmarks of cellular ageing across species. Preclinical studies on CR showed its beneficial impact on mitochondrial turnover, with an age-dependent upregulation of several proteins involved in mitophagy pathways, such as LC3, ULK1 and sirtuin 1 (SIRT1)[163]. Increased levels of PINK1 and parkin mitophagy mediators have been detected in skeletal muscle of male 10-week-old mice after an 18-month CR regimen[164]. In humans, CR regimens induced mitochondrial function, measured as citrate synthase activity[165,166]. This was associated with increased mitochondrial gene expression in one study[165] and no effects on mitochondrial content in a separate trial[166]. Additional human studies are warranted to investigate a potential involvement of the mitophagy pathway.

Fasting has recently attracted attention as a strategy to protect against ageing and disease. A preclinical study showed increased autophagosomes containing mitochondria in the heart following 48 h of fasting compared to normal feeding[167]. In humans, an 8-h fasting regimen did not change the LC3-II/LC3-I ratio or p62 levels in skeletal muscle[168]. Given the limited number and preliminary nature of these investigations, the impact of fasting on mitophagy remains an open question.

Limited clinical evidence is available on natural compounds improving mitophagy. Urolithin A (UA) is a naturally derived postbiotic shown to increase mitophagy in several organs and conditions in preclinical models[169]. In a clinical study, the impact of UA on mitophagy was measured in the vastus lateralis muscle of middle-aged overweight individuals following 4 months of supplementation[170]. Untargeted muscle proteomics showed the ‘parkin-ubiquitin proteasomal system’ as the most significantly enriched pathway with 500 mg of UA compared to placebo. Targeted protein analyses confirmed a significant increase in the mitophagy marker p-parkin in muscles of participants after 4 months of UA supplementation compared to baseline[170].

UA has also been shown to induce mitophagy in human colon cancer organoids (APTK) and promote expansion of human T memory stem cells[171]. These preclinical data indicate

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the need for further studies on mitophagy boosters to support human immune function and cancer immunotherapies.

Administration of the nicotinamide adenine dinucleotide $(NAD)^+$ booster nicotinamide riboside has been linked to increased mitophagy in several worm and rodent models[84,153,172]. Clinical studies with NAD^+ boosters showed some contrasting data on mitochondrial function, depending on study duration and the population assessed[173–175]. Data on mitophagy biomarkers after administration of NAD^+ enhancers in humans are still lacking. Preclinical data are available on other natural molecules with effects on mitophagy, including spermidine, quercetin and resveratrol, among others[176,177]. A few clinical trials with quercetin show modest[178] or no[179] changes in mitochondrial biogenesis genes. Some clinical studies are available on spermidine supplementation in humans. However, for all these compounds, clinical studies showing their impact on mitophagy are still lacking.

Pharmacological approaches

Drugs specifically targeting mitophagy showed promising data in cell-based and rodent models. These include the ubiquitin-specific peptidase 30 (USP30) inhibitor MTX652, designed to treat kidney diseases. This compound is now undergoing safety and pharmacokinetic studies with a prospect for clinical development. A few PINK1 activators are currently being tested in preclinical models[180]. Other drugs have been reported to modulate mitophagy indirectly in experimental models and include modulators of the NRF2–kelch-like ECH-associated protein 1 (KEAP1) pathway[181,182] and activators of SIRT1 (ref. 183). Some anticancer drugs are also proposed as indirect mitophagy inducers as a consequence of mitochondrial function inhibition and ROS production[136,137]. Finally, AMPK is a well-established regulator of autophagy–mitophagy[35]. AMPK-activating drugs have been shown to promote mitophagy in cell models[184] and also in a clinical trial in patients with T2D. In this particular study, a 3-month treatment with metformin significantly increased expression of both mRNA and proteins related to mitophagy, including MFN2, NIX, parkin, PINK1, LC3-II and lysosome-associated membrane protein 2 (LAMP2)[185].

These preliminary data and recent screening campaigns identifying new mitophagy inducers[83,186] await further data to support the potential to translate mitophagy modulators into clinics.

Conclusions

The biological mechanisms that prevent or remove damage accumulation in macromolecules and cellular organelles, including mitochondria, require plenty of energy to be effective. Mitochondrial OxPhos provides most of this energy through a powerful and sophisticated machinery that needs a coordinated effort of numerous proteins in balanced stoichiometric proportions, assembled in complex architectural structures. Over time, the tuning of such delicate machinery fades, energy production diminishes, and remnants of damaged and fragmented mitochondria accumulate in the cytosol, contributing in many ways to cellular pathology. Mitophagy is at the core of mitochondrial quality-control systems for early detection and effective removal of damaged mitochondria, which is an essential prerequisite to the production of new, healthy mitochondria through biogenesis[187]. Not surprisingly,

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impaired mitophagy has been detected in many chronic diseases and has been linked to accelerated ageing, strengthening the hypothesis that enhancing mitophagy could be highly beneficial and reduce the burden of chronic diseases with ageing.

However, progress in this field is hampered by several limitations. Some critical aspects of mitophagy modulation in humans are not fully understood, and research is needed to identify new druggable targets. There is a scarcity of sensitive and reliable methods to study mitochondrial function as well as autophagy and mitophagy in humans. Even the few methods that are available can only be applied to a limited set of tissues that are accessible in humans, such as circulating blood cells, skin and skeletal muscle. In addition, available methods to assess mitochondrial 'function' are focused on bioenergetics and tend to ignore other biological roles of mitochondria, such as calcium homeostasis, vitamin and amino acid metabolism, steroid synthesis and many others that may contribute to pathology[188]. Finally, mechanisms of mitophagy dysfunction are manifold and may differ among organs and conditions. More precise characterisation and definition of the roots behind mitophagy dysfunction are required to tailor strategies that efficiently revert such mitophagy defects. There is hope that the recent availability of powerful and high-throughput technologies that allow measuring thousands of proteins and metabolites in biofluids could be used to develop autophagy–mitophagy profiles in clinical settings[189]. Further studies are encouraged to translate promising preclinical data on other circulating biomarkers that may be proxies of mitophagy such as extracellular vesicles and in particular MDVs[190]. Because of all these limitations, only a few observational studies and even fewer intervention studies focusing on mitophagy and mitophagy inducers have been performed in humans, and more research in this important area of investigation is needed.

Coordinated efforts between molecular biologists and clinicians are critical to advance our understanding of mitophagy's role in human pathology and physiology and to study strategies to prevent and cure multiple chronic diseases and promote healthy longevity. Newly energized cells may provide many more years of healthy life to people.

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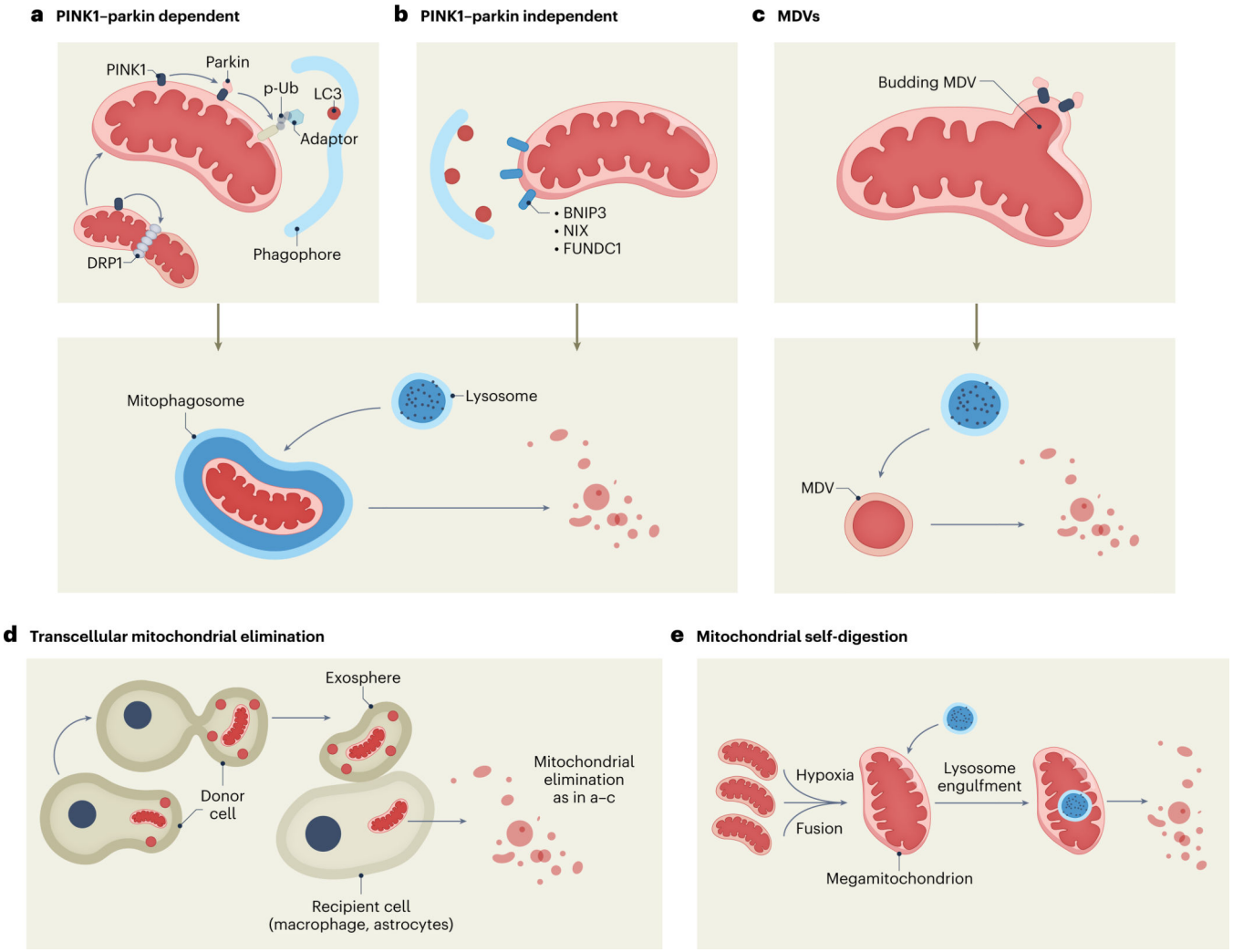


Fig. 1 |. Schematic representation of mitophagy pathways.

a,**b**, Canonical mitophagy pathways classified into PINK1–parkin dependent (**a**) and PINK1–parkin independent (**b**) depending on surface receptors expressed. The PINK1–parkin pathway involves stabilisation of PINK1, mitochondrial recruitment of parkin and generation of p-Ub chains on mitochondrial proteins. Adaptor proteins including optineurin, NDP52 and p62 bind p-Ub chains and are recognized by LC3. PINK1–parkin-independent mitophagy receptors, including BNIP3, NIX and FUNDC1, bind LC3 without involvement of PINK1–parkin. LC3 mediates phagophore formation to generate mitophagosomes. Damaged mitochondrial compartments can be segregated by upstream mitochondrial fission via DRP1. These mitophagy processes conclude with the fusion of lysosome vesicles to mitophagosomes for final degradation and recycling (middle). **c**, Noncanonical mitophagy via MDVs; MDVs derive from buddings of mitochondrial portions in a process that requires PINK1–parkin and concludes with the fusion of lysosome vesicles to MDVs for final degradation and recycling (middle). **d**, Noncanonical transcellular mitochondrial elimination. Mitochondria are compartmentalized into exospheres deriving from the extracellular matrix of a donor cell. Exospheres containing mitochondria can be

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taken up by receiving cells, and mitochondria released in the cytosol can be degraded as described in Fig. 2a–c. **e**, Noncanonical mitochondrial self-digestion, a process activated by hypoxia and leading to fusion of mitochondria into dysfunctional megamitochondria. The latter intake whole-lysosomal vesicles that release their degrading content, causing digestion of mitochondria from their inside.

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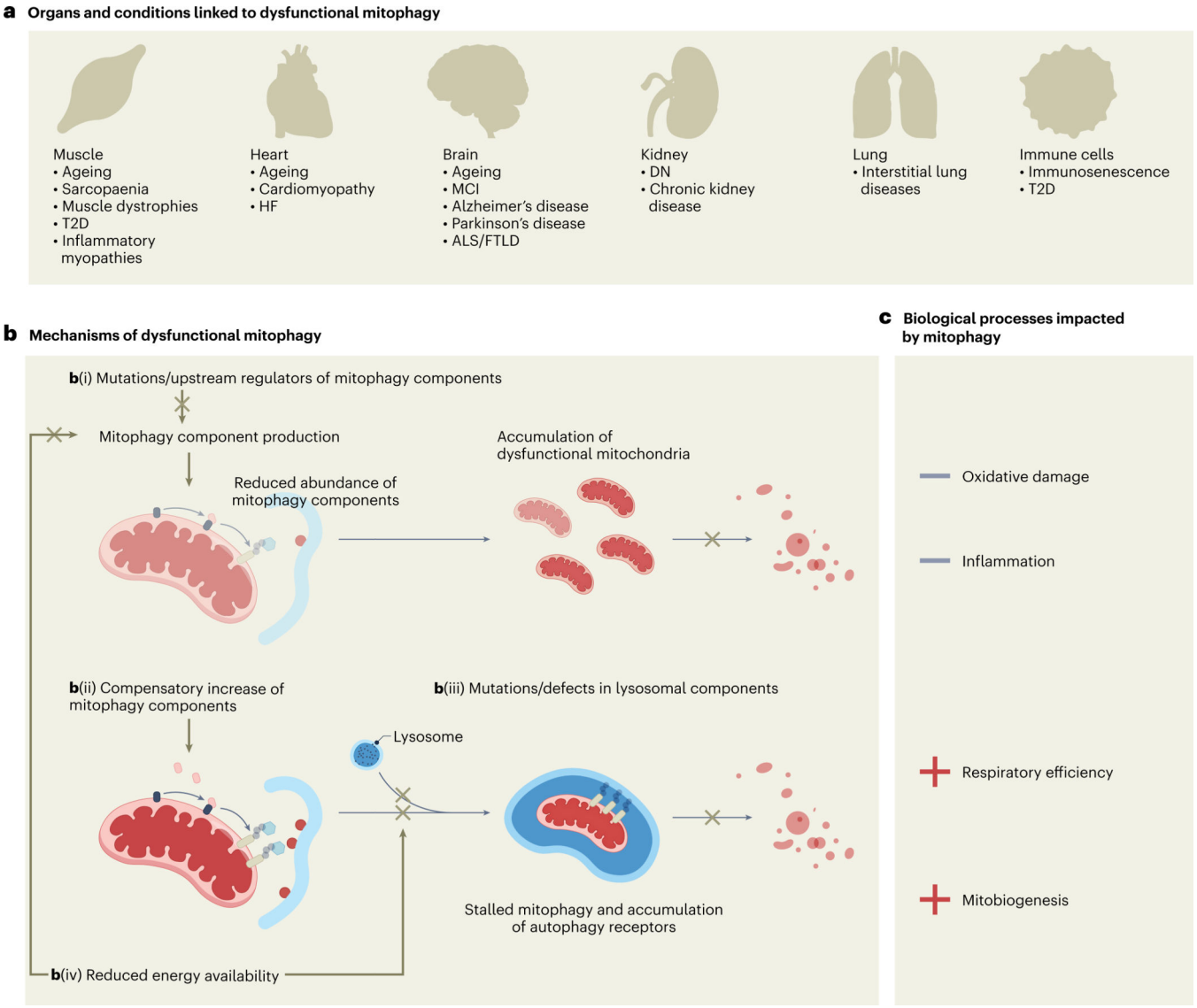


Fig. 2 |. Disease conditions associated with altered mitophagy.

a, Altered mitophagy has been linked to multiple diseases affecting different organs in humans, including skeletal muscle, heart, brain, liver and the immune system. FTLD, frontotemporal lobar degeneration. **b**, Different mechanisms associated with dysfunctional mitophagy have been described. **b**(i), Mutations of mitophagy components or deficits in upstream mitophagy regulators causing impaired synthesis and activity of mitophagy machinery and leading to accumulation of non-recycled, dysfunctional organelles. **b**(ii), Compensatory increase of certain mitophagy components in response to mitochondrial dysfunction. **b**(iii), Downstream blockage of lysosomal-mediated degradation, with consequent accumulation of mitophagy receptors and mitophagosome vesicles. **b**(iv), Reduced energy availability likely contributing to the mechanisms above by impairing the cellular ability to synthesize and activate mitophagy machinery. **c**, Impact of mitophagy on biological processes.

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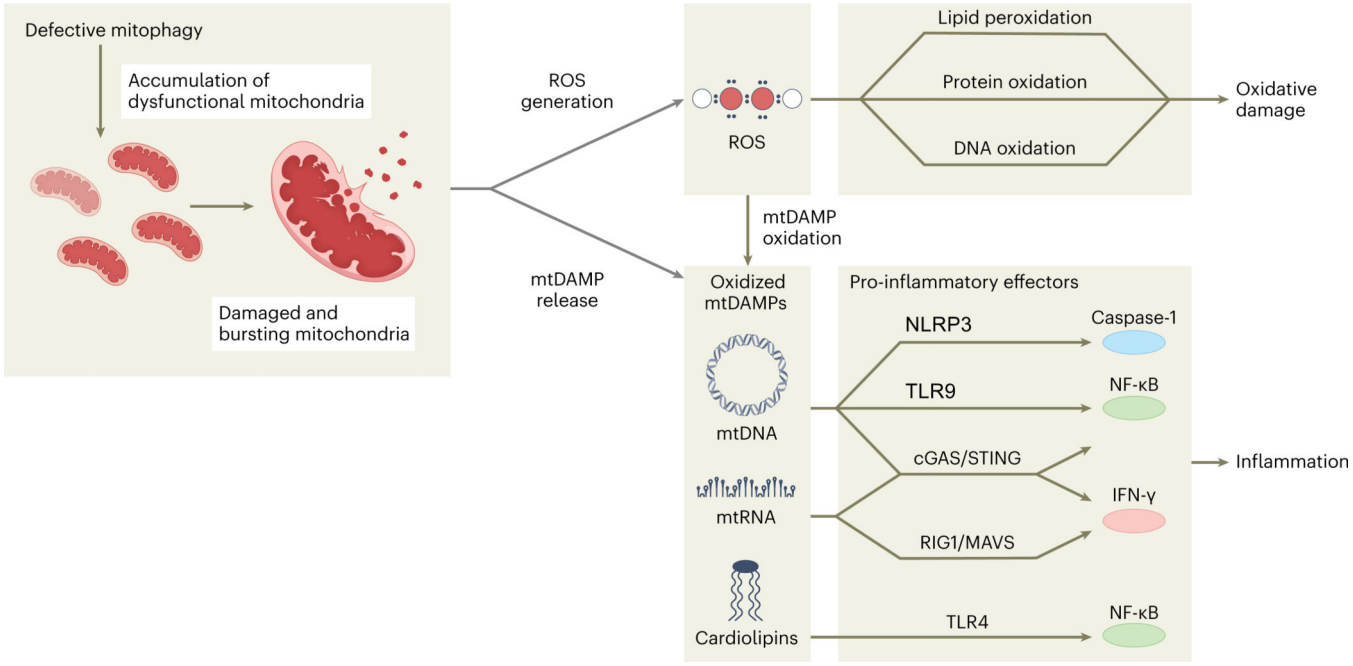


Fig. 3 |. Mitophagy dysfunction links to sterile inflammatory responses.

Deficits in mitophagy lead to the accumulation of damaged and ROS-producing mitochondria that may burst and release their content into the cytoplasm or at the extracellular level. Excessive ROS released by low-quality mitochondria lead to lipid peroxidation and protein and nucleic acid oxidation, causing cellular oxidative damage. Bulk ROS production also oxidizes released mitochondrial components named mtDAMPs. mtDAMPs include mtDNA, mtRNA and cardiolipins. mtDAMPs and oxidized mtDAMPs are recognized by different innate immunity receptors and can activate downstream signalling pathways leading to activation of innate immune-triggered inflammation. mtDNA can activate the NLRP3 inflammasome, TLR9 and the DNA-sensing cGAS–STING pathway. NLRP3 inflammasome activation culminates in caspase-1 activation and pro-inflammatory signalling, while activation of TLR9 and the cGAS–STING pathway leads to NF-κB-driven cytokine production. cGAS–STING can also elicit an interferon γ (IFN-γ) response. mtRNA can be recognized as ‘non-self’ by the mtRNA-sensing MAVS–RIG1 complex and trigger the interferon γ response. Finally, cardiolipins herniated from the inner mitochondrial membrane can bind to TLR4 and activate pro-inflammatory signals through NF-κB.

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Table 1 |

Studies investigating mitophagy's role in human pathophysiology

Condition/population	Tissue/organ/system	Intervention	Changes in mitophagy biomarkers	Mechanisms of dysfunctional mitophagy	References
Muscle					
Skeletal muscle					
Ageing	Vastus lateralis	N/A	↓ Parkin/VDAC ratio (proteins)	Reduced mRNA and protein expression of mitophagy components	55
			↓ *PARK2, ATG7, BECN1, BNIP3* (mRNA)		60
			↑ p62 and BNIP3 (proteins)	Increased protein levels of mitophagy receptors negatively correlated with muscle volume	61
			= Parkin, BNIP3 and p62 (proteins)	No changes in mitophagy components	56
Muscle dystrophies	Vastus lateralis or N/A		↓ *PARK2, PINK1, BNIP3, SQSTM1* (mRNA)	Reduced mRNA expression of mitophagy components	48
Sporadic inclusion body myositis	N/A		↑ BNIP3 and LC3 colocalization (proteins)	Increased protein levels of mitophagy components	62
Chronic HF	Diaphragm		↑ p62 and LC3-I/LC3-II, MFN2 and DRP1 (proteins)	Increased protein levels of mitophagy receptors and mediators of mitochondrial dynamics	65
Peripheral artery disease	Gastrocnemius		↑ Dysfunctional mitochondria	N/A	66
Ageing	Vastus lateralis	Long-term endurance exercise	↑ p-PINK1 and p-parkin (proteins)	Increased protein levels of total and activated (that is, phosphorylated) mitophagy mediators by endurance exercise	158
Health			↑ Parkin (protein)		56
Health		Acute endurance exercise	= Parkin and p62/SQSTM1 (protein, both fasted and fed state) ↓ LC3B-II/LC3B-I (protein only in fasted state) ↑ *PARK2* (mRNA only in fed state) ↑ *BNIP3* and *BNIP3L* (mRNA, only in fed state)	No change in mitophagy components except for BNIP3 in fed state after acute exercise	160
Ageing	Vastus lateralis	Resistance exercise	NS	No significant changes in protein expression of mitophagy receptors with resistance exercise	161
			NS		163
	PBMCs		NS		162
Inflammatory myopathies	Vastus lateralis	Combined aerobic and RT	↑ *FOXO1, LAMP2, PGC1-a, TFEB* (mRNA)	Exercise-induced increased mRNA expression of genes related to autophagy/mitochondrial and lysosomal biogenesis	159
Health	Vastus lateralis	Fasting	NS	No significant changes in protein expression of mitophagy receptors following fasting	169
Ageing	Vastus lateralis	Urolithin A	↑ Parkin-Ub proteasomal system (protein sets) ↑ p-parkin (protein)	UA-mediated enrichment of mitophagy-associated protein sets from muscle proteomics Increased protein levels of activated p-parkin	171

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Condition/population	Tissue/organ/system	Intervention	Changes in mitophagy biomarkers	Mechanisms of dysfunctional mitophagy	References
Type 2 diabetes	PBMCs	Metformin	↑ Parkin, PINK1, LAMP2, LC3-II and NIX (both mRNA and proteins)	Increased protein level of mitophagy and lysosome components with metformin	186
Cardiac muscle					
Idiopathic dilated cardiomyopathy	Cardiomyocytes	N/A	↓ Parkin (mRNA and protein)	Reduced protein level of mitophagy component	69
Heart failure		N/A	↓ PINK1 (protein)	Reduced protein level of mitophagy component	71
Heart failure		N/A	AMPK isoform shift (protein)	Potential reduced capacity to activate PINK1	72
Brain					
Ageing	Hippocampus	N/A	↑ p-Ub granules	Increased level and aggregation of mitophagy components	75
MCI, MD and AD	Serum	N/A	↓ ATG5 and parkin (protein)	Reduced protein levels of mitophagy components	77
AD	Hippocampus	N/A	↓ PINK (protein)	Reduced protein levels of mitophagy components	78
AD	Hippocampus, frontal cortex	N/A	↑ p-Ub granules and parkin (protein)	Increased protein levels and aggregation of mitophagy components	83
PD	Plasma	N/A	↓ p-Ub (protein) (patients with loss-of-function PINK1)	Reduced plasma levels of mitophagy component	92
	Whole body	N/A	*PARK2, PARK7* mutations	Loss of function of mitophagy components	87
ALS	Whole body	N/A	*TBK1, SQSTM1, OPTN1, VCP* mutations	Loss of function of mitophagy and lysosomal components	94,95
FTLD	Whole body	N/A	*TBK1, SQSTM1, OPTN1, VCP* mutations	Loss of function of mitophagy and lysosomal components	76
Immune system					
T lymphocytes					
Ageing	CD8+	N/A	↓ LC3 (protein)	Reduced levels of macroautophagy component	103
	CD4+	N/A	↑ Mitophagosomes	Stalled mitophagy associated with reduced mitochondrial respiration	105
RBCs					
Sickle cell disease	Mature RBCs and reticulocytes	N/A	↓ NIX and PINK1 (proteins)	Reduced protein level of mitophagy receptors	109
Metabolic disorders					
Type 2 diabetes	Muscle and adipose tissue	N/A	↓ *PINK1* (mRNA)	Reduced gene expression of mitophagy receptor	126
Type 2 diabetes DN	PBMCs	N/A	↓ *PINK1, PRKN* (mRNA) ↓ Mitochondrial respiration (in DN only)	Reduced mRNA levels of mitophagy components associated with reduced mitochondrial respiration in DN	127

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Condition/population	Tissue/organ/system	Intervention	Changes in mitophagy biomarkers	Mechanisms of dysfunctional mitophagy	References
Lung					
Interstitial lung diseases	Lung	N/A	↓ PINK1 (protein) ↑ Oxidized mtDNA release	Reduced protein level of mitophagy component associated with increased circulating oxidized mtDNA levels	147
	Bronchoalveolar lavage, plasma	N/A	↑ mtDNA		
Cancer					
Breast, ovarian and colon	Cancer biopsies	N/A	Parkin mutation ↓ Parkin (protein)	Loss of function of mitophagy component	130,131
Breast		N/A	↑ *PRKN*, *BNIP3*, *BECN1* and *LC3* (mRNA)	Increased mRNA expression of mitophagy components	133
Endometrial cancer		N/A	↑ *BNIP3* (mRNA)	Increased mRNA levels of mitophagy receptor	134

Abbreviations: BNIP3L, BNIP3 like; FOXO1, forkhead box protein O1; FTLD, frontotemporal lobar degeneration; MD, mixed dementia; N/A, not available; NS, no significant changes; OPTN1, optineurin; PARK, Parkinson disease protein; PGC1-α, peroxisome proliferator-activated receptor-gamma coactivator 1 alpha; RBC, red blood cell; VCP, valosin-containing protein; VDAC, voltage-dependent anion-selective channel.