Mitophagy

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Mitophagy is a selective autophagic process that eliminates unnecessary and/or damaged mitochondria. Therefore, it is a central hormetic mechanism of mitochondrial quality and quantity control, essential for cellular homeostasis. Its dysregulation has been shown to be a key event in metabolic related diseases and it is the target of emerging therapeutical approaches in this field.

Keywords: Mitophagy ; parkin ; PINK1 ; autophagy ; human diseases

1. History

The capacity of the eukaryotic cell to regulate mitochondrial function provides the organisms with key metabolic plasticity, essential for a wide variety of cell functions [1][2]. Hence, maintenance of mitochondrial function relies on the adequate co-regulation of functions that control their turnover, namely mitochondrial biogenesis, which produces new mitochondria and mitophagy which eliminates damaged or unnecessary mitochondria [3]. Insufficient mitophagy leads to the accumulation of poorly functional/damaged mitochondria, with a reduced capacity to synthesize Adenosine triphosphate (ATP^+), that produce high levels of superoxide. This can result in alteration in the cellular pools of intermediate metabolites, with pathological consequences [4]. Poorly functional mitochondria are a well-known hallmark of metabolic and neurodegenerative diseases, which are strongly linked to pathological developments. Alterations in the activity of key mitophagy regulators are central to these processes.

2. Mitophagy, a Type of Autophagy

It has to be highlighted that mitophagy is a type of selected autophagy [5]. Autophagy, literally “the process of the cell eating itself” in Greek, is divided into micro- or macro-autophagy, and chaperone-mediated autophagy, depending on the size of the degraded structure, and can be nonselective or selective, depending on whether any specific cellular component is targeted [6]. Of note, non-selective autophagy is emerging as a primary mechanism in cell death [7]. Early studies suggested that selective autophagy was closely related to (non-selective) macroautophagy, the only apparent difference being an additional step targeting isolation membranes to cargo. However, it has now been well established that, at least in yeast, several components of the canonical macroautophagy pathways are often dispensable for selective autophagy [8]. Therefore, mitophagy is a selective autophagy process that involves isolation within a membrane, sealing, and degradation through the lysosomal pathway of the organelle [9]. However, most subcellular structures, not just mitochondria, are targets of selective autophagy, including Golgi, the endoplasmic reticulum (ER), peroxisomes, ribosomes, the midbody, lipid droplets, and glycogen granules.

Mitophagy, defined as the selective autophagy of damaged mitochondria, was firstly described in yeast, where the presence of a mutated Uth1p in the outer mitochondrial membrane (OMM) was found to block autophagy during starvation [10]. Similar findings were later reported in cultured starved hepatocytes that eliminated damaged mitochondria when exposed to oxidative damage [11]. Commonly, the morphological characteristic feature of mitophagy is considered to be the localization of mitochondria inside an autophagic vacuole, called mitophagosomes [11][12]. However, currently, it is considered that there are three types of mitophagy: type 1, induced by nutrient limitation, type 2, induced by damage signals, and type 3, micro-mitophagy, linked to small mitochondria-derived vesicles [13]. These processes are intrinsically different, because type 1 and type 2 require the fusion of a lysosome to produce an autophagosome encircling mitochondria, while the latter type does not. Mitophagy plays a relevant role in normal development, as recently analyzed and quantified in vitro and in vivo in fluorescent transgenic mouse models (like mt-Keima or mito-QC) [14][15]. However, more generally, this fundamental biological mechanism works in all cells or tissues, being regulated in response to their changing energetic requirements. Some tissues, such as the nervous system, the kidney, the skeletal muscle, the heart, and the liver, show high basal mitophagy activity, while others, such as the spleen and the thymus, display low mitophagy levels[15][16]. The molecular and biochemical pathways involved in mitophagy were first characterized in models of aging

[4], neurodegenerative and psychiatric diseases [17][18], cancer [18], and cardiovascular diseases (CVD) [19]. Basal mitophagy is, for example, vital to maintain synaptic plasticity and to eliminate damaged mitochondria in the brain, while its deranged activity is associated with age-related neuronal damage [20][21].

3. PINK1

Mitophagy can be phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN)-induced putative kinase 1 (PINK1)-dependent or -independent [22]. PINK1 is a serine/threonine kinase whose levels are normally low, but it is stabilized and accumulates at the OMM in response to mitochondrial damage (mtDNA mutations), increased mitochondrial reactive oxygen species (ROS), depolarization, and the accumulation of misfolded proteins [23]. Accumulated PINK1 is autophosphorylated and activated, and in turn phosphorylates ubiquitin on serine 65, which recruits Parkin from the cytosol to the mitochondrial membrane. Parkin is an E3-ubiquitin ligase that, when recruited and activated, drives the ubiquitination of mitochondrial proteins and hence autophagy [24][25][26]. Recently, an inhibitory mechanism of the pathway has been described, and Ubiquitin carboxyl-terminal hydrolase 30 (USP30) can act as a brake on mitophagy by opposing Parkin-mediated ubiquitination [27]. Importantly, although PINK1 facilitates Parkin recruitment, Parkin can be recruited to depolarized mitochondria and drive mitophagy even in the absence of PINK1 [28]. Some identified targets of Parkin ligase activity at the OMM include Mitofusin 1 and 2 (MFN1/2), voltage dependent anion channel protein 1 (VDAC1), and mitochondrial Rho guanosine triphosphate hydrolases (GTPases) (MIRO) [28]. However, a widespread degradation of OMM has been evidenced by proteomic studies, suggesting that remodeling of the mitochondrial outer membrane proteome is important for mitophagy [26].

Under physiological steady state conditions, PINK1 is imported into the mitochondria through the translocase of the outer mitochondrial membrane (TOMM) complex of the OMM and into the translocase complex (TIMM) of the inner mitochondrial membrane (IMM), where it is cleaved by the mitochondrial processing peptidase (MPP) [29]. Afterwards, PINK1 is also cleaved in its hydrophobic domain, spanning the IMM, by the rhomboid protease presenilin-associated rhomboid-like protein (PARL), generating a 52 kD, N-terminal-deleted form of PINK1 [30]. PARL cleavage releases this PINK1 into the cytosol, where it is targeted by the N-degron type-2 E3 ubiquitin ligases and degraded by the ubiquitin proteasome system (UPS) [31]. This import and degradation cycle maintains PINK1 at very low, almost undetectable, levels on healthy mitochondria. However, mitochondrial import, through the TIMM complex, is affected by membrane depolarization, inhibition of the electron transport chain, genetic or environmental stressors, such as inflammation, and the accumulation of unfolded proteins. Under these adverse conditions, PINK1 processing by PARL is prevented, and uncleaved PINK1 accumulates on the OMM, bound to the TOMM complex [29]. This last event is needed to target PINK1 to selected single damaged mitochondrion [32].

4. Mitochondrial Homeostasis-Related Pathways

It should be highlighted that mitochondrial control through mitophagy is actually part of a more complex homeostatic control process of mitochondria that includes fusion and fission dynamics and mitochondrial biogenesis, with all these processes being interregulated [33]. Of note, mitochondrial fusion is induced upon starvation, and fused mitochondria are particularly resistant to mitophagy, while fragmented/fused organelles with low membrane potential ($\Delta\psi_m$) are more easily targeted into mitophagosomes [34][35]. Accordingly, mitochondrial fusion/fission regulatory cues are also mitophagy modulators [36]. Other regulatory pathways still need to be fully characterized; for example, it has been suggested that mitophagy selectively targets certain mitochondria based on their topology. A recent study reported that serum-starved U2OS osteosarcoma cells formed “donut” mitochondria that exhibited normal inner membrane potential ($\Delta\psi_m$) and were resistant to mitophagy, while swollen mitochondria with low potential were removed [37]. Mitophagy has also been shown to be regulated by changes in mitochondrial subcellular location and changes in cellular bioenergetics through regulators that control the main anabolic and catabolic pathways, as well as mitochondrial biogenesis [38].

Guanosine triphosphate hydrolases (GTPases) Mitofusin 1 and Mitofusin 2 (MFN1/2) are key players in the control of mitochondrial dynamics (fusion and fission) and orchestrate mitochondrial network connectivity and activity [39]. When mitochondria oxidative phosphorylation (OXPHOS) is activated, they fuse into a network that can cover the whole cell. Conversely, inhibition of mitochondrial OXPHOS activity is linked to the breakdown (fission) of the network into small mitochondrial units that tend to localize close to the nuclei. Fusion is induced by homo or hetero dimerization of MFN1/2, anchored to OMM at their C-termini, which mediate the GTP-dependent merge of separate OMMs. Fusion is also activated by MitoPLD, a member of the phospholipase D family, which converts, the mitochondrial-specific lipid cardiolipin (CL) into phosphatidic acid. CL is predominantly localized into the IMM, but mitochondrial damage leads to its relocalization to the OMM [40]. Fusion of the IMM and cristae organization requires full-length Optic Atrophy Protein 1 (L-OPA1). In cellular stress conditions, L-OPA1 is cleaved to S-OPA1, promoting OMM permeabilization and cytochrome *c*

release [41]. The fission of mitochondrial OMM is also regulated by another GTPase protein, called dynamin-related protein 1 (Drp1) and its receptor proteins fission protein 1 (Fis1), mitochondrial fission factor (Mff) and mitochondrial dynamic proteins 49 and 51 kDa (MiD49 and MiD51) [42]. Intracellular signaling pathways regulate the positioning of Drp1 on the OMM. Once recruited, Drp1 oligomerizes into a ring-like structure that wraps around the mitochondria, which is also marked by the presence of endoplasmic reticulum (ER) and actin cytoskeleton, constricts the mitochondrial membrane and triggers fission [43].

Several related pathways have now been found to link mitochondrial dynamics to mitophagy, since damaged or unnecessary mitochondria should first be fused out and then degraded. In particular, MFN1/2 are extracted from the OMM by a ubiquitin-dependent chaperone and degraded by the proteasome [44]. Ubiquitination and depletion of MFN1/2 prevents the fusion of damaged mitochondria and leads to fragmentation, as fission processes remain functional, which promotes mitophagy [45]. PINK1 phosphorylates MFN2 that then works as a Parkin receptor for culling damaged mitochondria [46].

Although not a necessary element, voltage-dependent anion-selective channel 1 (VDAC1) also plays a relevant role in the control of mitophagy. VDAC1, the most abundant OMM protein, can be considered a mitochondrial porin. It largely controls mitochondrial permeability to a number of metabolites across the OMM and is a key regulatory element in mitochondria-dependent apoptosis [47]. It has been shown to interact with Parkin and become ubiquitinated and to be involved in Parkin recruitment. A recent study on VDAC1′s role in mitophagy revealed that Parkin can induce both mono- and polyubiquitination on VDAC1 [48]. Conversely, defective monoubiquitination leads to the induction of apoptosis, and reduced polyubiquitination hinders mitophagy, suggesting that VDAC1 interaction with Parkin is at a crossroads in terms of the decision to induce mitophagy or apoptosis by damaged mitochondria [49]. Of interest, another study identified an additional functional pathway of VDAC1 in mitophagy control through the cholesterol translocator protein (TSPO) [50]. TSPO facilitates the transfer of cholesterol from the OMM to the IMM, where it serves as a precursor for the synthesis of steroid hormones. It forms a functional complex with VDAC1 and has been shown that its overexpression inhibits mitophagy though an ROS-dependent mechanism that did not prevent the recruitment of Parkin but blocked the ubiquitination of mitochondrial proteins, though a still undefined mechanism.

Parkin also ubiquitinates the mitochondrial outer membrane Rho GTPases (MIRO1/2), which directly interact with PINK1 [51]. These proteins are components of the adaptor complex that anchors mitochondria to motor proteins. Thus, they are involved in the regulation of axonal mitochondrial movement by Ca^{2+} [52]. When Ca^{2+} binds, it causes the dissociation of motor/adaptor complexes from microtubules, thus leading to a mitochondrial movement arrest that facilitates the removal of damaged mitochondria by mitophagy [53]. MIRO serves as a Ca^{2+}-dependent docking site and directly primes Parkin recruitment. However, the role of PINK1 and Parkin in MIRO1 degradation remains controversial. In fact, it has been proposed that MIRO1 ubiquitination, rather than its degradation, is the main signal for mitochondrial arrest [54].

5. LC3

Ubiquitination of the cargo is a critical step in selective autophagy in all cases [55]. The most accepted model is that cargo-bound receptors recruit microtubule-associated protein 1 light chain 3 (LC3) through an LC3-interacting region (LIR), bridging cargo with a preformed, autophagy-generated membrane. In this model, receptors are either integral to the cargo or recruited to the cargo via ubiquitination. A scaffold protein, which recruits additional autophagy-related proteins, may also be involved [56].

In mitochondria, following OMM remodeling mediated by proteasomal degradation of ubiquitinated proteins, adaptor proteins that bind ubiquitin (Ub) are recruited for the transport of depolarized mitochondria to the perinuclear region through a microtubule-dependent mechanism [57]. These adaptors interact with microtubule-associated protein 1 light chain 3 (LC3), which in turn promotes the sequestration of damaged mitochondria into autophagosomes. Finally, the autophagosomes fuse with the lysosomes, leading to the degradation of damaged mitochondria [5]. Five mitochondrial cargo-bound receptors (LC3 adapters) that contain an LIR motif that is recognized by LC3 [58] are recruited to the polyubiquitinated substrates on the mitochondria through their ubiquitin-binding domain: sequestosome-1 (p62), optineurin (OPTN), nuclear domain 10 protein 52 (NDP52), Trans-activating transcriptional regulatory protein of HTLV-1 (TAX1) binding protein 1 (TAX1BP1), and neighbor of Breast Cancer 1 (BRCA1) gene 1 (NBR1). Of note, it has been shown that OPTN [59] is largely dependent on its activation by Tank-binding kinase 1 (TBK1), a key signaling regulator of innate immunity, which highlights the interplay between mitophagy and the regulation of the immune system [60].

6. Ubiquitin Independent Mitophagy

It has been demonstrated that autophagy and mitophagy are upregulated in cells lacking PINK1 [61]. Damaged mitochondria can also be recognized by LC3 adapters in a ubiquitin-independent manner. These adapters directly sense mitochondrial damage and consequently change their subcellular location or the protein they interact with, guiding the damaged mitochondria to the autophagosome. The best characterized systems involved in the programmed mitochondrial clearance or mitochondrial elimination in the context of a developmental program are the B-Cell CLL/Lymphoma 2 (BCL2)/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and BCL2/adenovirus E1B 19 kDa-interacting protein 3-like (NIX/BNIP3L) pathways [62]. The current evidence suggests that both BNIP3 and NIX play an important role in oxygen sensing, inducing mitophagy in response to hypoxia, and can also directly promote the depolarization of mitochondria, as well as the fusion with cellular membranes. BIP3 and its homolog NIX are transmembrane OMM proteins. Their cytoplasmic N-terminal portion can interact with LC3-related molecules, targeting mitochondria for degradation by autophagy. BNIP3 is able to interact directly with PINK1, stabilizing it and promoting its ability to recruit Parkin, and its activity involves Drp1-mediated mitochondrial fission [63].

Other Parkin-independent mechanisms include those mediated by receptors, such as FUN14 domain-containing protein 1 (FUNDC1), another mitochondrial OMM protein sensitive to hypoxia [64]. Choline dehydrogenase (CHDH) is located in the IMM and OMM under normal conditions. When the mitochondrial membrane potential is disrupted, CHDH accumulates in the OMM and interacts with p62 through its Phox and Bem1 (PB1) domain, leading to the formation of the CHDH-p62-LC3 complex that mediates mitophagy [65]. TBC1 domain family member 15 (TBC1D15), a mitochondrial Rab GTPase activating protein, forms a complex with TBC1D17 and migrates to the mitochondrial outer membrane by interacting with Fis1. The TBC1D15/17 complex then interacts with LC3 [66]. Bcl2 like 13 (BCL2L13) is the mammalian homologue of Autophagy-related protein 32 Atg32, the only mitophagy receptor found in yeast [67]. Like other LC3 receptors, BCL2L13 locates on the OMM and binds to LC3 via the LC3-interacting region. FK506-binding protein 8 (FKBP8), located on the OMM, was identified as an LC3 interacting protein using yeast two-hybrid screening [68]. Remarkably, specific IMM components have also being shown to participate in mitophagy. Prohibitin 2 (PHB2) is a IMM protein [69] that becomes exposed to LC3 following Parkin-mediated degradation of OMM proteins. CL, as mentioned above, a membrane lipid in the IMM, can also function as an LC3 receptor in mitophagy when translocated from the IMM to the OMM in the presence of external depolarizing toxins [70]. Of note, the nutrient deprivation sensor, adenosine monophosphate activated protein kinase (AMPK), has also been shown to induce Parkin-independent mitophagy through the phosphorylation and activation of TBK1 [71].

The mitophagy main regulatory pathways have been summarized in Figure 1.

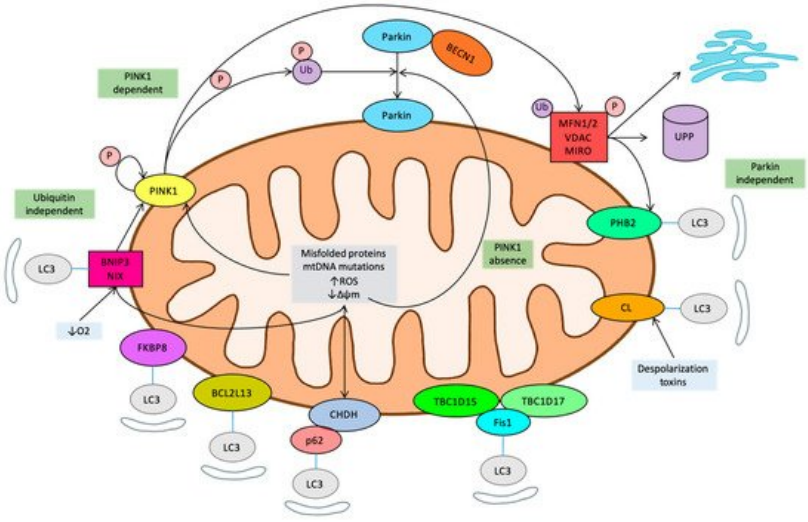

Figure 1. Summary of main mitophagy pathways.

7. Novel Regulatory Pathways

Recent studies have also shown the physiological relevance of LC3-independent mitophagy. In particular, mitophagy can be driven by Rab9-associated autophagosomes, through the formation of a protein complex that involves Rab9, Unc-51-like kinase 1 (ULK1), and Drp1 [72].

Additional, novel pathways that impact mitophagy continue to be identified almost daily. For example, it has been demonstrated that, several ligases may regulate mitophagy, such as SMAD-specific E3 (SMURF1) [73], while the

autophagy protein Coiled-coil myosin-like BCL2-interacting protein (BECN1)/Beclin 1, which plays a central role in autophagosome formation and maturation, has been shown to interact with Parkin and does not require its translocation to mitochondria [74].

Recently, a number of studies have focused on evidence linking mitophagy to ER stress, through the specialized ER-mitochondrial contact regions (MAMs) that regulate Ca^{2+} fluxes and control the induction of apoptosis [75]. PINK1 controls mitochondrial Ca^{2+} efflux [76][77], while, in turn, PINK1 gene expression has also been shown to be sensitive to Ca^{2+} fluxes [78]. The role of MAMS as key regulators of mitophagy is now well established, as they have been shown to be indispensable in the autophagy process, with many proteins that are directly involved in autophagy located in MAMs. In fact, in response mitophagy stimuli PINK1 and Beclin 1 have been shown to relocalize at MAMs where they further promote the association of mitochondria with ER, and autophagosome formation [79]. Although the mechanisms involved remain to be clearly elucidated [80], its physiological relevance has been clearly demonstrated, particularly in the context of Parkinson's disease [81].

8. Implications for human diseases and therapeutical approaches

The central relevance of mitophagy in all diseases related to metabolic control is now well established. As mitophagy is required to control metabolic homeostasis or remove damaged or unnecessary mitochondria, it prevents mitochondrial malfunction and subsequent molecular events such as oxidative stress that lead to disease development. In diseased states, mitophagy can sometimes partially compensate other deficits alleviating them, but when mitochondrial activity is compromised, mitophagy can actually play a detrimental role. This is specially evidenced in diseases where normal mitophagy activity is compromised by genetic or regulatory events. These results have boosted pharmacological research in the field, since there are several potentially druggable targets along the mitophagy pathway. Some of these molecules are already showing promising results and more will certainly come soon. Several natural dietary compounds, such as polyphenols, flavonoids, spermidine, or trehalose, which restore normal mitophagy fluxes in the elderly [82][83], could help to control the inflammasome and to prevent neurodegeneration having a direct impact on apoptosis and the caspase activation cascade [84][85]. Perhaps more importantly, lifestyle interventions can promote cardiovascular health, boosting mitophagy [86][87]. The future could also bring new findings on novel, non-canonical mechanisms of mitochondrial quality control, such as the recently described mitochondrion-derived vesicles, characterized in hypoxic neurons and cardiomyocytes [88].

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