A Molecular Approach to Mitophagy and Mitochondrial Dynamics

Seung-Min Yoo, and Yong-Keun Jung*

Global Research Laboratory, School of Biological Sciences, Seoul National University, Seoul 08826, Korea
*Correspondence: ykjung@snu.ac.kr
http://dx.doi.org/10.14348/molcells.2018.2277
www.molcells.org

Mitochondrial quality control systems are essential for the maintenance of functional mitochondria. At the organelle level, they include mitochondrial biogenesis, fusion and fission, to compensate for mitochondrial function, and mitophagy, for degrading damaged mitochondria. Specifically, in mitophagy, the target mitochondria are recognized by the autophagosomes and delivered to the lysosome for degradation. In this review, we describe the mechanisms of mitophagy and the factors that play an important role in this process. In particular, we focus on the roles of mitophagy adapters and receptors in the recognition of damaged mitochondria by autophagosomes. In addition, we also address a functional association of mitophagy with mitochondrial dynamics through the interaction of mitophagy adaptor and receptor proteins with mitochondrial fusion and fission proteins.

Keywords: autophagy, mitochondria, mitochondrial quality control, mitophagy, selective autophagy

INTRODUCTION

Mitochondria are the main site of adenosine tri-phosphate (ATP) synthesis. Mitochondria are also crucial in apoptosis, necrosis, autophagy, stress regulation, production of lipids and carbohydrates, Ca^{2+} storage and innate immunity (Galluzzi et al., 2012). Therefore, mitochondrial dysfunction is associated with many diseases (Suomalainen and Battersby, 2017). Mitochondria undergo constant turnover and their half-life varies from tissue to tissue (Gottlieb and Stotland, 2015). Mitochondria are exposed to damages caused by intra- and extra-mitochondrial events, such as mutated mitochondrial proteins and reactive oxygen species (ROS) (Galluzzi et al., 2012). Mitochondrial quality control systems actively function, both in physiological and pathological conditions, to protect the mitochondria from stress and damage at the protein and organelle level. Damage beyond the protein level activates a bigger response that includes mitochondrial biogenesis, fusion, fission and mitochondrial degradation, also known as mitophagy. In this review, we will focus on recent data regarding the molecular mechanisms of mitochondrial quality control, especially in relation to mitophagy, and describe how these mechanisms can affect each other.

MITOCHONDRIAL QUALITY CONTROL

Mitochondria first respond to stress by attempting to maintain their structure and composition. This is achieved through the action of antioxidants, DNA repair, protein folding and degradation. Mitochondrial DNA is highly exposed to oxidative damage due to the production of ROS during oxidative phosphorylation. DNA damage is repaired by mitochondrial DNA polymerase gamma (encoded by POLG) that has base excision repair and exonuclease function. Mutation of POLG causes neurodegeneration, progressing into Leigh or Alpers-Huttenlocher syndrome (Scheibye-Knudsen et al., 2015). Antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase/glutathione, peroxiredoxins and vitamin E/C, directly protect mitochondria by reducing reactive intermediates (Scheibye-Knudsen et al., 2015).
Mitophagy Adapters and Receptors
Seung-Min Yoo & Yong-Keun Jung

Fig. 1. Mitochondrial quality control. At the protein level, DNA repair proteins (mitochondrial DNA polymerase gamma) repair mitochondrial DNA; Chaperones import nuclear-encoded mitochondrial proteins into the mitochondria and correctly fold misfolded proteins in the matrix; Mitochondrial outer membrane proteins are ubiquitinated by MARCH5 and extracted by P97 to be degraded by the proteasome; Proteases in the mitochondrial matrix and inter membrane space degrade damaged proteins; Antioxidants reduce reactive oxygen species to protect mitochondria. At the organelle level, mitochondria biogenesis occurs upon specific signaling: the mitochondrial network changes mitochondrial shape through fusion and fission to accommodate metabolic requirement; Mitofusin 1/2 and OPA1 mediate fusion in the mitochondrial outer and inner membrane, respectively. MitoPLD converts cardiolipin to phosphatic acid inducing the fusion of mitochondria. When mitochondrial fission occurs, Drp1 binds to its receptors (MFF, MID49, MID51 and FIS1) to form Drp1 oligomers and tightens mitochondria to divide it. Also, increase of short form OPA1 induces mitochondrial fission. Damaged mitochondria are recognized by autophagosome, isolated and then fused with the lysosome to form an autolysosome and finally degraded.

addition, mitochondrial chaperones, such as heat shock protein (Hsp)60 and Hsp70, import nuclear-encoded mitochondrial proteins (NEMP) into the mitochondrial matrix and refold misfolded proteins (Hammerling and Gustafsson, 2014). When misfolded or damaged proteins accumulate, the proteases LON and ClpP, in the matrix, and ATPase associated with diverse cellular activities (AAA), in the membrane, degrade them to maintain normal mitochondria (Cenni and Voos, 2016). Proteins in the outer mitochondrial membrane are ubiquitinated by the E3 ligase MARCH5, and targeted for degradation to the 26S proteasome (Bragoszewski et al., 2017). If the damage is beyond the capacity of these systems, a broader quality control system is activated.

At the organelle level, mitochondria quality is sustained through synthesis of new mitochondria, fusion and fission, and elimination of the damaged ones. Mitochondrial biogenesis requires the delivery of NEMP to the mitochondria and is induced by endogenous (e.g. ROS, nitrogen oxide, carbon monoxide, and H2S), and external signals (e.g. estrogen), which increase the amount of NEMP through nuclear respiration factor-1 (NRF1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and estrogen receptor. Calorie restriction and exercise also stimulate mitochondrial biogenesis through changes in the ratio of oxidized/reduced nicotinamide adenine dinucleotide (NAD+/NADH) and adenosine mono-phosphate (AMP)/ATP, and increase NEMP levels via sirtuins (SIRT) and AMP-activated protein kinase (AMPK) pathways (Suliman and Piantadosi, 2016).

Compared to other organelles, mitochondria frequently change their shape under physiologic and pathologic conditions. Specifically, mitochondrial dynamics (fusion and fission) regulates mitochondrial network connectivity, which depends on the specific metabolic needs of the cell (Miettinen and Björklund, 2017; Rambold and Pearce, 2017). The processes controlling mitochondria dynamics are well understood and mediated by specific proteins. Mitochondrial outer membrane fusion is induced by MitoPLD, a member of the phospholipase D family, which converts cardiolipin into phosphatidic acid, and by homo or hetero dimerization of the guanosine triphosphate hydrolases (GTPases) mitofusin (MFN) 1 and 2. In addition to outer membrane, fusion of the mitochondrial inner membrane requires optic atrophy 1 (OPA1), anchored in the inner membrane and exposed to the inter-membrane space. On the other hand, mitochondrial fission occurs when the GTPase dynamin related pro-
protein-1 (Drp1) translocates to the mitochondrial outer membrane to bind its receptors, such as PS1, MFF, MID49 and MID51, and forms a multimeric structure around the fission site of the mitochondrion. While the precise reason is not yet clarified, mitochondrial protein 18 kDa (MTP18) gene activation induces fusion of mitochondria and expression of cleaved OPA1 (S-OPA1) induces fission (Wai and Langer, 2016). Through mechanisms not fully understood, mitochondrial dynamics is also engaged in the repair of a part of the damaged mitochondria. If the mitochondria cannot be restored to a healthy state through these processes, they are eliminated by selective autophagy.

**MITOCHONDRIA RECOGNIZED?**

Autophagy, a lysosome-dependent degradation, is categorized into macroautophagy, microautophagy and chaperone-mediated-autophagy (CMA). Macroautophagy is characterized by the formation of an autophagosome: the phosphatidylinositol 3-kinase (PI3K) class III produces phosphatidylinositol 3-phosphate (PI3P) and induces the formation of the isolation membrane (phagophore), which progressively expands by using two-ubiquitin-like systems till the formation of a double-membrane vesicle (the autophagosome). Cytoplasmic cargos are sequestered into the autophagosome and subsequently degraded through the lysosomes. In microautophagy, cytosolic cargo is directly engulfed by lysosomes while CMA selectively recruits target proteins into the lysosomes through their ubiquitin-binding domain. In contrast, autophagosome-independent mitophagy is induced by mitochondrial depolarization (Matsuda et al., 2010). Mitophagosome formation is mediated by several mitochondrial transmembrane proteins, including Miro 1, leads to their degradation by the proteasome (Chan et al., 2013; Matsumoto et al., 2010; Matsuda et al., 2010).

Unlike non-selective removals of various targets by macroautophagy, targets ranging from proteins to organelles, including aggregated proteins, mitochondria, endoplasmic reticulum, peroxisomes, invaded bacteria, viruses and membranes, are often degraded by selective autophagy (Galuzzi et al., 2017). Among them, mitophagy is the selective removal of mitochondria via autophagy. Then, an important question is how mitochondria are selectively recognized and removed. A report shows that mitochondria-derived vesicles are directly degraded to lysosomes (Lemasters, 2014). Most of the studies, however, have focused on autophagosome-mediated mitophagy and on the importance of mitophagy mediators that interact with LC3. Recently, many studies have investigated the mechanisms of this process. Details on mitophagy are discussed below.

**MECHANISMS OF MITOPHagy: HOW ARE THE MITOCHONDRIA RECOGNIZED?**

The recognition of target mitochondria by the autophagosome occurs through LC3 adapters, in an ubiquitin-dependent and independent pathway, and through the direct interaction of LC3 with its receptors.

**Ubiquitin-mediated interaction of LC3 adapters**

In this pathway, the first event is the ubiquitination of mitochondrial substrates, which are then recognized by LC3 adapters.

**PINK1/Parkin pathway**

PTEN-induced putative kinase 1 (PINK1, PARK6) and Parkin (PARK2) were first identified as genetic factors of Parkinson’s disease in which mitochondrial dysfunction has been suggested as one of the causes (Pickrell and Youle, 2015). Parkin, a cytosolic E3-ubiquitin ligase, and PINK, a mitochondrial serine/threonine-protein kinase, are now known to function in mitophagy (Matsuda et al., 2010; Narendra et al., 2008). Under normal conditions, PINK1 is continuously targeted to the mitochondria through a mitochondrial targeting sequence, degraded by matrix processing peptidases (MPP) and subsequently cleaved by presenilin-associated rhomboid like (PARL), a protease in the mitochondrial inner membrane. Cleaved PINK translocates to the cytosol and is degraded by the proteasome (Matsuda et al., 2010).

Depolarization of the mitochondrial membrane due to damage, reduces the cleavage of PINK1. PINK1 accumulates on the mitochondrial outer membrane through the translocate of the outer membrane (TOM). Mitochondrial pyruvate level can also affect the accumulation of PINK1 by inducing the interaction between PINK1 and TOM (Park et al., 2015). Accumulated PINK1 is autoprophosphorylated and activated, and phosphorylates ubiquitin on serine 65 (Ser65), which recruits Parkin. On the mitochondrial membrane, Parkin is phosphorylated and activated by PINK1, and polyubiquitinates its substrates (Nguyen et al., 2016). Specifically, the polyubiquitination of Parkin substrates, such as voltage-dependent anion channel-1 (VDAC1), MFN 1/2 and MiD49, leads to their degradation by the proteasome (Chan et al., 2011; Tanaka et al., 2010). The degradation of MFN 1/2 induces mitochondrial fission and mitophagy (Tanaka et al., 2010), linking mitochondrial dynamics to mitophagy.

**LC3 adapters: p62, OPTN, NDPS2, TAX1BP1 and NBR1**

Five LC3 adapters, sequestosome-1 (p62), optineurin (OPTN), nuclear domain 10 protein 52 (NDP52), TAX1BP1 binding protein 1 (TAX1BP1) and neighbor of BRCA1 gene 1 (NBR1), are recruited to the K63-linked polyubiquitinated substrates on the mitochondria through their ubiquitin-binding domain. These adapters contain an LC3-interacting region (LIR) motif that is recognized by LC3 to recruit the tagged mitochondria to the autophagosome (Lazarou et al., 2015). Studies have shown that OPTN is the most important adapter for the recruitment of the phagophore to the mitochondria (Moore and Holzbaur, 2016). Specifically, Tank-binding kinase 1 (TBK1) is activated after recruitment by OPTN and promotes mitophagy through the phosphorylation of OPTN (Richter et al., 2016). While other adapters were also reported to be phosphorylated by TBK1, only OPTN and NDP52 were common substrates (Heo et al., 2015; Matsumoto et al., 2015; Moore and Holzbaur, 2016; Richter et al., 2016).

Recently, a Parkin-independent role of PINK1 in mitophagy has been uncovered. In this pathway, PINK1 recruits OPTN and NDP52 on the mitochondria, which subsequently recruit autophagy initiation factors, such as ULK1, double FYVE-containing protein 1 (DFCP1) and WD repeat domain phosphoinositide-interacting protein 1 (WIPI1) (Lazarou et al., 2015). In addition, overexpressed synphilin-1, a synuclein alpha-interacting protein, interacts with PINK1 and induces
its accumulation. The PINK1-synphilin1 complex recruits seven in absentia homolog-1 (SIAH-1), an E3 ubiquitin ligase, to increase mitochondrial ubiquitination (Szargel et al., 2016), indicating that E3 ligases other than Parkin target the mitochondria for degradation in different ways.

Ubiquitin-independent interaction of LC3 adapters
Damaged mitochondria can also be recognized by LC3 adapters in an ubiquitin-independent manner.

CHDH and TBC1D15
Choline dehydrogenase (CHDH) is located in the mitochondrial inner and outer membranes under normal conditions. When the mitochondrial membrane potential is disrupted, CHDH accumulates in the mitochondrial outer membrane and interacts with p62 through its Phox and Bem1 (PB1) domain, leading to the formation of the CHDH-p62-LC3 complex that mediates mitophagy (Park et al., 2014). CHDH’s role in mitophagy, shown in gene ablation experiments, is Parkin-independent. In addition, TBC1 domain family member 15 (TBC1D15), a mitochondrial Rab GTPase-activating protein (Rab-GAP), forms a complex with TBC1D17 and migrates to the mitochondrial outer membrane by interacting with mitochondrial fission 1 protein (FIS1). The TBC1D15/17 complex then interacts with LC3 (Yamanou et al., 2014). These adapters sense mitochondrial damage and consequently change their subcellular location or the protein they interact with, guiding the damaged mitochondria to the autophagosome.

Interaction between LC3 and its receptors
Unlike aforementioned LC3 adapters which require additional proteins to interact with LC3, several LC3 receptors are located on the mitochondria: they directly bind to LC3 and recruit the damaged mitochondria to autophagosomes.

NIX/BNIP3L, BNIP3 and FUNDC1
Nip3-like protein X (NIX) and BCL2/Adenovirus E1B 19 kDa Interacting Protein 3 (BNIP3) contain a BH3 domain and interact with LC3 (Hanna et al., 2012; Novak et al., 2010). NIX was initially reported to function in the removal of mitochondria for degradation in different ways.

BNIP3 levels are increased by NIX was initially reported to function in the removal of mitochondria for degradation in different ways.

indicating that E3 ligases other than Parkin target the mitochondria for degradation in different ways.

Ubiquitin-independent interaction of LC3 adapters
Damaged mitochondria can also be recognized by LC3 adapters in an ubiquitin-independent manner.

CHDH and TBC1D15
Choline dehydrogenase (CHDH) is located in the mitochondrial inner and outer membranes under normal conditions. When the mitochondrial membrane potential is disrupted, CHDH accumulates in the mitochondrial outer membrane and interacts with p62 through its Phox and Bem1 (PB1) domain, leading to the formation of the CHDH-p62-LC3 complex that mediates mitophagy (Park et al., 2014). CHDH’s role in mitophagy, shown in gene ablation experiments, is Parkin-independent. In addition, TBC1 domain family member 15 (TBC1D15), a mitochondrial Rab GTPase-activating protein (Rab-GAP), forms a complex with TBC1D17 and migrates to the mitochondrial outer membrane by interacting with mitochondrial fission 1 protein (FIS1). The TBC1D15/17 complex then interacts with LC3 (Yamanou et al., 2014). These adapters sense mitochondrial damage and consequently change their subcellular location or the protein they interact with, guiding the damaged mitochondria to the autophagosome.

Interaction between LC3 and its receptors
Unlike aforementioned LC3 adapters which require additional proteins to interact with LC3, several LC3 receptors are located on the mitochondria: they directly bind to LC3 and recruit the damaged mitochondria to autophagosomes.

NIX/BNIP3L, BNIP3 and FUNDC1
Nip3-like protein X (NIX) and BCL2/Adenovirus E1B 19 kDa Interacting Protein 3 (BNIP3) contain a BH3 domain and interact with LC3 (Hanna et al., 2012; Novak et al., 2010). NIX was initially reported to function in the removal of mitochondria for degradation in different ways.
Mitophagy Adapters and Receptors
Seung-Min Yoo & Yong-Keun Jung

Mitochondrial inner membrane protein (Wei et al., 2017). Parkin-mediated degradation of mitochondrial outer membrane proteins results in rupture of the mitochondrial outer membrane, leading to exposure of PHB2 to LC3, and subsequent mitophagy. The function of PHB2 is crucial for the selective elimination of paternal mitochondria in C. elegans (Wei et al., 2017). However, the detailed mechanism responsible for this process remains to be elucidated.

Cardiolipin, a membrane lipid in the mitochondrial inner membrane, can also function as a LC3 receptor in mitophagy. Cardiolipin is translocated from the inner membrane to the outer membrane in the presence of external toxic signals, such as 6-hydroxydopamine (6-OHDA), rotenone and 1-methyl-4-phenylpyridinium (MPP+). Externalized cardiolipin directly interacts with the N-terminal helices of LC3 (Chu et al., 2013). This interaction seems to be LC3 subtype-specific. In vitro experiments revealed that cardiolipin directly interacts with gamma-aminobutyric acid receptor-associated protein (GABARAP), a member of the LC3 family, but does not translocate GABARAP to the mitochondria in rotenone-treated cells, implying that cardiolipin interacts with GABARAP in different autophagic processes (Anton et al., 2016). These data indicate that mitochondrial inner membrane components also participate in mitophagy.

FUNCTIONAL ASSOCIATION OF MITOCOCHONDRIAL DYNAMICS AND MITOPHAGY

Changes in mitochondrial shape and size are associated to the fission and fusion of the mitochondria according to the cell’s needs (Trotta and Chipuk, 2017). Since the size of the autophagosome in mammalian cells is approximately 500-1500 nm (Wang and Klonsky, 2003), it is reasonable to predict that a huge mitochondria network should be fragmented through fission prior to mitophagy. In that regard, it has been reported that either mitochondrial fission-related factors or degradation of mitochondrial fusion-related factors are required for mitophagy (Shirihai et al., 2015). Proteins with a role in mitochondrial dynamics are thus implicated in mitophagy to function together with the aforementioned LC3 adapters/receptors. That is, Drp1, a mitochondrial fission protein, interacts with over-expressed FUNDC1 and BCL2L13, and both FUNDC1 and BNIP3 require Drp1 for mitochondrial fragmentation and Parkin-mediated mitophagy (Lee et al., 2011; Wu et al., 2016). In contrast, a recent report showed that mitophagy occurs without mitochondrial fission (Yamashita and Kanki, 2017). Thus, the role of Drp1 in mitophagy needs to be further addressed.

In addition, OPA1, a mitochondrial fusion protein, may also play a role as a mitophagic factor. BNIP3 induces mitochondrial fragmentation through its interaction with OPA1 (Landes et al., 2010). Ablation of PHB2 leads to loss of OPA1 long isoform, resulting in an abnormal cristae morphogenesis and mitochondrial fragmentation (Merkwirth et al., 2008). A recent report has shown that the fusion of the inner membrane of the mitochondria requires the binding of OPA1 with cardiolipin and high concentration of cardiolipin in the inner membrane (Ban et al., 2017). Additionally, TBC1D15 mediates the autophagic encapsulation of mitochondria downstream of Parkin activation, upon interaction with FIS1 (Yamano et al., 2014). Thus, it is likely that mitophagy is functionally associated with mitochondrial dynamics, through the interaction between mitochondrial dynamics factors and LC3 adapter/receptors. The details of the interplay between mitochondrial dynamics and mitophagy remain, however, to be further assessed.

PHYSIOLOGIC ROLE OF MITOPHAGY

To understand the molecular mechanism of mitophagy, CCCP has widely been used to disrupt the membrane potential of the mitochondria and induce mitophagy, in vitro. Despite its great contribution to the elucidation of mitophagy, the conditions it creates are different from physiologic conditions. Mitophagy constantly occurs in basal conditions (Bingol et al., 2014) and is induced under specific physiological conditions. During development, NIX is responsible for the removal of mitochondria from maturing erythrocytes (Sand- oval et al., 2008), and Parkin and mitochondrial E3 ubiquitin protein ligase 1 (MUL1) are required for degradation of paternal mitochondria after fertilization in mice (Rojansky et al., 2016). Mitophagy is also involved in cellular differentiation. PINK1-dependent mitophagy affects pluripotency and differentiation state in stem cells (Vazquez-Martin et al., 2016). Mitophagy appears to contribute to metabolic changes in the differentiation of retinal ganglion cells (Esteban-Martinez et al., 2017) and the transition from beige to white adipocytes (Wrighton, 2016). In addition, mitophagy plays a role in the activation of the NOD-like receptor protein 3 (NLRP3) inflammasome (Lazarou, 2015) and FUNDC1-dependent mitophagy decreases the activation of platelet following acute ischemia/reperfusion injury (Zhang et al., 2016). Together, these reports show diverse roles of mitophagy in development and cell differentiation.

MITOPHAGY AND HUMAN DISEASES

A dysfunction in the mitochondria quality control and/or mitophagy is associated with many pathological conditions: one of the most studied examples are some neurodegenerative diseases (Rodolfo et al., 2017). Parkinson and PINK1 are genetic factors in Parkinson’s disease and play a protective role in neurons through mitophagy (Ashrafi et al., 2014). In Alzheimer disease progression, Parkin is involved in the removal of mitochondria (Ye et al., 2015) and its overexpression alleviates the symptoms of the disease (Martin-Maestro et al., 2016). In Huntington’s disease, mitophagy is altered. Mutant huntingtin acts as a scaffold inducing selective autophagy (Martinez-Vicente et al., 2010) or negatively affecting mitochondrial delivery to the lysosome (Wong and Holzbaur, 2014). The details of this process are unclear. Mitochondrial dysfunction is also observed in amyotrophic lateral sclerosis (ALS), though the role of autophagy in this disease is uncertain (Rodolfo et al., 2017). Mutant superoxide dismutase-1 (SOD1), involved in ALS, induces mitochondrial fragmentation and inhibits mitochondrial retrograde axonal transport (Magrane et al., 2014). In addition, mutant SOD1 binds to p62, OPTN and LC3 (Rodolfo et al., 2017).
Fig. 2. Mitochondria recognition by LC3 adaptors and receptors during mitophagy. (A) In healthy mitochondria, PINK1 is targeted to the mitochondria and is cleaved by the proteases PARL and MPP in the matrix and mitochondrial inner membrane, respectively. Cleaved PINK1 is degraded in the cytosol through the proteasome. (B) In damaged mitochondria, PINK1 accumulates on the mitochondrial outer membrane and is activated through autophosphorylation. Activated PINK1 phosphorylates ubiquitin on its substrates. Phosphorylated ubiquitin-substrates interact with OPTN or NDP52 to subsequently recruit initiation factors of autophagy or Parkin, an E3 ubiquitin ligase, which is then activated by PINK1 for polyubiquitination. K63-linked polyubiquitinated substrates are recognized by five LC3 adapters (p62, NDP52, OPTN, TAX1BP1 and NBR1) to interact with LC3 on autophagosomes through the LIR motif. TBK1 phosphorylates OPTN to facilitate the recognition by LC3 and NIX is polyubiquitinated to be recognized by LC3 through NBR1. K27-linked Miro and K48-linked VDAC1 and MFN 1 and 2 are degraded by proteasome. The ubiquitin-independent LC3 adapters CHDH and TBC1D15 recognize p62 and FIS1, respectively. (C) Under hypoxic condition, homodimerized BNIP3 is phosphorylated at Ser17 and 24, and NIX is phosphorylated at Ser81 to facilitate the interaction with LC3. FUNDC1 is dephosphorylated at Ser13 by PGAM5 and phosphorylated at Ser17 by ULK1. Phosphorylation of Ser272 in BCL2L13 enhances the interaction with LC3. PHB2 in the mitochondrial inner membrane interacts with LC3 when PHB2 LIR is exposed to the cytosol following Parkin-mediated rupture of the mitochondrial outer membrane. Cardiolipin translocates to the mitochondrial outer membrane and promotes mitophagy through direct interaction with LC3.

Thus, reduced targeting of ubiquitinated mitochondria to autophagosomes might contribute to ALS pathology.

Mitophagy is also associated with cancer (Drake et al., 2017). Metabolic-reprogramming in cancer cells is associated with decreased mitophagy and increased mitochondrial biogenesis (Vazquez et al., 2013) and inhibition of mitophagy reduces cell stemness in cell self-renewal (Drake et al., 2017). In relation to inflammation-induced cell death, p62-mediated elimination of damaged mitochondria attenuates inflammation by reducing NLRP3 inflammasome activation in macrophages (Zhong et al., 2016). When DNA is damaged, mitophagy is induced through the poly (ADP-ribose) polymerase-1 (PARP1)-SIRT1-BNIP3/NIX pathway to limit the generation of ROS or inflammasomes, which are harmful to...
the cell (Drake et al., 2017). In cardiovascular diseases, mitophagy factors are either protective or damaging. It was shown that Parkin and PGAM5 prevent cardiac damage, while FUNDC1, MFN1 and MFN2 in the absence of Bnip3 have an opposite effect. Additionally, Parkin and PINK1 have a protective role in atherosclerosis (Bravo-San Pedro et al., 2017). Likewise, the role of mitophagy in lung disease is not clear. The absence of PINK1 has a protective effect on mitophagy-dependent necroptosis in chronic obstructive pulmonary disease (COPD) (Mizumura et al., 2014), but also induces idiopathic pulmonary fibrosis (IPF). Mitophagy reduces mitochondrial ROS, which modulate senescence in human bronchial epithelial cells. Thus, the role of mitophagy in lung disease depends on the cell type (Cloonan and Choi, 2016).

In acute kidney injury, mitochondrial dysfunction precedes renal dysfunction. Mitophagy protects renal cells against damage in acute kidney injury and metabolic acidosis, a cause of chronic kidney disease. A high-calorie diet causes renal dysfunction. Mitophagy protects renal cells against failure in the removal of dysfunctional mitochondria induces mitochondrial dynamics.

Mitophagy also affects non-alcoholic fatty liver disease (NAFLD). NAFLD is relieved by the thyroid hormone (TH), which stimulates mitophagy and mitochondrial biogenesis. Bnip3, Nix, Ulk1, p62 and LC3 mRNA levels are increased by TH (Sinha and Yen, 2016). Additionally, activation of AMPK contributes to NAFLD reduction through mitophagy (Smith et al., 2016). Of course, there are active ongoing researches to show the roles of mitophagy in the pathogenesis in other diseases as well.

CONCLUSION

Failure in the removal of dysfunctional mitochondria induces cellular stress and is linked to human diseases. The function of mitophagy-regulating proteins depends on the tissues and the stimuli they are exposed to. Many LC3 adaptors and receptors were originally reported to function independently of Parkin. However, some of them, such as Nix, participate in Parkin-mediated mitophagy (Gao et al., 2015). NDP52, FUNDCl, Bcl2l13, FBKP8 and TBC1D15 are also Parkin substrates under certain conditions (Sarraf et al., 2013). Importantly, mitochondrial dynamics and mitophagy affect each other to maintain the quality of mitochondria. Here again, mitochondrial dynamic factors, such as Miro 1 and 2, Drp1 and FIS1 are also Parkin substrates (Sarraf et al., 2013). Therefore, future studies on mitophagy need also to consider the molecular connection among proteins involved in mitophagy, and among proteins involved in mitophagy and mitochondrial dynamics.

ACKNOWLEDGMENTS

This work was supported by a Bio & Medical Technology Development Program of the National Research Foundation (NRF-2017M3A9G707352) and a Global Research Laboratory grant (NRF-2010-00341) funded by the Ministry of Science & ICT, Korea.

REFERENCES

Anton, Z., Landajuela, A., Hervás, J.H., Montes, L.R., Hernandez-Tiedra, S., Velasco, G., Goni, F.M., and Alonso, A. (2016). Human Atg16b-cardiolipin interactions in mitophagy: Specific properties of LC3B, GABARAPL2 and GABARAP. Autophagy 12, 2386-2403.

Ashrafi, G., Schleie, J.S., Levoie, M.J., and Schwarz, T.L. (2014). Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. J. Cell Biol. 206, 655-670.

Ban, T., Ishihara, T., Kohno, H., Saita, S., Ichimura, A., Maenaka, K., Oka, T., Miha, K., and Ishihara, N. (2017). Molecular basis of selective mitochondrial fusion by heterotypic action between OPA1 and cardiolipin. Nat. Cell Biol. 19, 856-863.

Bhujabal, Z., Birigsottit, A.B., Sjottom, E., Brenne, H.B., Overvatn, A., Habssov, S., Kirkin, V., Lamark, T., and Johansen, T. (2017). FBKP8 recruits LC3A to mediate Parkin-independent mitophagy. EMBO Rep. 18, 947-961.

Bingol, B., Tea, J.S., Phu, L., Reichelt, M., Bakalarski, C.E., Song, Q., Foreman, O., Kirkpatrick, D.S., and Sheng, M. (2014). The mitochondrial deubiquitinsase USP30 opposes parkin-mediated mitophagy. Nature 510, 370-375.

Bragoszewski, P., Turek, M., and Chacinska, A. (2017). Control of mitochondrial biogenesis and function by the ubiquitin-proteasome system. Open Biol. 7.

Bravo-San Pedro, J.M., Kroemer, G., and Galluzzi, L. (2017). Autophagy and mitophagy in cardiovascular disease. Circ. Res. 120, 1812-1824.

Cenini, G., and Voos, W. (2016). Role of mitochondrial protein quality control in oxidative stress-induced neurodegenerative diseases. Curr. Alzheimer Res. 13, 164-173.

Chan, N.C., Salazar, A.M., Pham, A.H., Sveredoski, M.J., Kolawa, N.J., Graham, R.L., Hess, S., and Chan, D.C. (2011). Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. Hum. Mol. Genet. 20, 1726-1737.

Chen, G., Han, Z., Feng, D., Chen, Y., Chen, L., Wu, H., Huang, L., Zhou, C., Cai, X., Fu, C., et al. (2014). A regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. Mol. Cell 54, 362-377.

Chen, Z., Liu, L., Cheng, Q., Li, Y., Wu, H., Zhang, W., Wang, Y., Sehgal, S.A., Siraj, S., Wang, X., et al. (2017). Mitochondrial E3 ligase MARCHE5 regulates FUNDC1 to fine-tune hypoxic mitophagy. EMBO Rep. 18, 495-509.

Chu, C.T., Ji, J., Dagda, R.K., Jiang, J.F., Tyurina, Y.Y., Kapralov, A.A., Tyurin, V.A., Yanamala, N., Shrivastava, I.H., Mohammad, D., et al. (2013). Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat. Cell Biol. 15, 1197-U1168.

Cloonan, S.M., and Choi, A.M. (2016). Mitophagy in lung disease. J. Clin. Invest. 126, 809-820.

Drake, L.E., Springer, M.Z., Poole, L.P., Kim, J.C., and Macleod, K.F. (2017). Expanding perspectives on the significance of mitophagy in cancer. Semin Cancer Biol. 47, 110-124.

Esteban-Martinez, L., Sierra-Filandri, E., McGeal, R.S., Salazar-Roa, M., Marino, G., Seco, E., Durand, S., Enot, D., Grana, O., Malumbres, M., et al. (2017). Programmed mitophagy is essential for the glycolytic switch during cell differentiation. EMBO J. 36, 1688-1706.

Galluzzi, L., Kepp, O., Trojel-Hansen, C., and Kroemer, G. (2012). Mitochondrial control of cellular life, stress, and death. Circ. Res. 111, 1198-1207.

Galluzzi, L., Baehe, F.H., Ballabio, A., Boya, P., Bravo-San Pedro, J.M., Cecconi, F., Choi, A.M., Chu, C.T., Codogno, P., Colombo, M.I.,...
Matsuda, N., Sato, S., Shiba, K., Okatsu, K., Saisho, K., Gautier, C.A., Sou, Y.S., Saiki, S., Kawajiri, S., Sato, F., et al. (2010). PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J. Cell Biol. 189, 211-221.

Matsumoto, G., Shimogori, T., Hattori, N., and Nukina, N. (2015). TBK1 controls autophagosome engulfment of polyubiquitinated mitochondria through p62/SQSTM1 phosphorylation. Hum. Mol. Genet. 24, 4429-4442.

Merkworth, C., Dargazani, S., Tatsuta, T., Geimer, S., Lower, B., Wunderlich, F.T., von Kleist-Retzow, J.C., Waisman, A., Westermann, B., and Langer, T. (2008). Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. Gene Dev. 22, 476-488.

Miettinen, T.P., and Björklund, M. (2017). Mitochondrial Function and Cell Size: An Allometric Relationship. Trends Cell Biol. 27, 393-402.

Mizumura, K., Cloonan, S.M., Nakahira, K., Bhashyam, A.R., Cervo, M., Kitada, T., Glass, K., Owen, C.A., Mahmood, A., Washko, G.R., et al. (2014). Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. J. Clin. Invest. 124, 3987-4003.

Moore, A.S., and Holzbaur, E.L. (2016). Spatiotemporal dynamics of autophagy receptors in mitophagy. Autophagy 12, 1957-2051.

Murakawa, T., Yamaguchi, O., Hashimoto, A., Hikosu, S., Takeda, T., Oka, T., Yasui, H., Ueda, H., Akazawa, Y., Nakayama, H., et al. (2015). Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. Nat. Commun. 6, 7527.

Nah, J., Yuan, J., and Jung, Y.K. (2015). Autophagy in neurodegenerative diseases: from mechanism to therapeutic approach. Mol. Cells 38, 381-389.

Narendra, D., Tanaka, A., Suen, D.F., and Youle, R.J. (2015). The roles of PINK1, parkin, and MAPK-signaling pathways are required for mitophagy. Autophagy 11, 332-343.

Park, S., Choi, S.G., Yoo, S.M., Nah, J., Jeong, E., Kim, H., and Jung, Y.K. (2015). Pyruvate stimulates mitophagy via PINK1 stabilization. Cell Signal. 27, 1824-1830.

Park, S., Choi, S.G., Yoo, S.M., Son, J.H., and Jung, Y.K. (2014). Choline dehydrogenase interacts with SQSTM1/p62 to recruit LC3 and stimulate mitophagy. Autophagy 10, 1906-1920.

Pickrell, A.M., and Youle, R.J. (2015). The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson’s disease. Neuron 85, 257-273.

Rambold, A.S., and Pearce, E.L. (2017). Mitochondrial dynamics at the interface of immune cell metabolism and function. Trends Immunol. pii: S1471-4906(17)30170-9.

Richter, B., Sliter, D.A., Herhaus, L., Stolz, A., Wang, C.X., Beli, P., et al. (2017). Molecular definitions of autophagy and related processes. EMBO J. 36, 1811-1836.

Gao, F., Chen, D., Si, J.M., Hu, Q.S., Qin, Z.H., Fang, M., and Wang, G.H. (2015). The mitochondrial protein BNIP3L is the substrate of PARK2 and mediates mitophagy in PINK1/PARK2 pathway. Hum. Mol. Genet. 24, 2528-2538.

Gottlieb, R.A., and Stotland, A. (2015). MitoTimer: a novel protein for monitoring mitochondrial turnover in the heart. J. Mol. Med. (Berl). 93, 271-278.

Hammerling, B.C., and Gustafsson, A.B. (2014). Mitochondrial quality control in the myocardium: cooperation between protein degradation and mitophagy. J. Mol. Cell Cardiol. 75, 122-130.

Hanna, R.A., Quinsay, M.N., Orogo, A.M., Giang, K., Rikka, S., and Gustafsson, A.B. (2012). Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy. J. Biol. Chem. 287, 19094-19104.

Heo, J.M., Oriduea, A., Paulo, J.A., Rinehart, J., and Harper, J.W. (2015). The PINK1-PARKIN mitochondrial ubiquitination pathway drives a program of OPTN/NIP52 recruitment and TBK1 activation to promote mitophagy. Mol. Cell 60, 7-20.

Hirot, Y., Yamashita, S., Kunihara, Y., Jin, X.L., Aihara, M., Saigusa, T., Kang, D.C., and Kanki, T. (2015). Mitophagy is primarily due to alternative autophagy and requires the MAPK1 and MAPK14 signaling pathways. Autophagy 11, 332-343.

Landes, T., Emeorin, L.J., Courilleau, D., Rojo, M., Belenguer, P., and Arnaune-Pelloquin, L. (2010). The BH3-only Bnip3 binds to the dynamin Opa1 to promote mitochondrial fragmentation and apoptosis by distinct mechanisms. EMBO Rep. 11, 459-465.

Mao, K., Wang, K., Zhao, M., Xu, T., and Klionsky, D.J. (2011). Two mammalian cells. Nat. Cell Biol. 14, 177-185.

Magrane, J., Cortez, C., Gan, W.B., and Manfredi, G. (2014). Abnormal mitochondrial transport and morphology are common pathological determinants in SOD1 and TDP43 ALS mouse models. Hum. Mol. Genet. 23, 1413-1424.

Mao, K., Wang, K., Zhao, M., Xu, T., and Klionsky, D.J. (2011). Two MAPK-signaling pathways are required for mitophagy in Saccharomyces cerevisiae. J. Cell Biol. 193, 755-767.

Martin-Maestro, P., Gargini, R., Perry, G., Avila, J., and Garcia-Escudero, V. (2016). PARK2 enhancement is able to compensate mitophagy alterations found in sporadic Alzheimer’s disease. Hum. Mol. Genet. 25, 792-806.

Martinez-Vicente, M., Tallozcy, Z., Wong, E., Tang, G.M., Koga, H., Kaushik, S., de Vries, R., Arias, E., Harris, S., Sulzer, D., et al. (2010). Cargo recognition failure is responsible for inefficient autophagy in Huntington’s disease. Nat. Neurosci. 13, 567-574.
Mitophagy Adapters and Receptors
Seung-Min Yoo & Yong-Keun Jung

Zaffagnini, G., Wild, P., Martens, S., Wagner, S.A., et al. (2016). Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. Proc. Natl. Acad. Sci. USA 113, 4039-4044.

Rodolfo, C., Campello, S., and Cecconi, F. (2017). Mitophagy in neurodegenerative diseases. Neurochem. Int. pii: S0197-0186(17)30087-6.

Roijansky, R., Cha, M.Y., and Chan, D.C. (2016). Elimination of paternal mitochondria in mouse embryos occurs through autophagic degradation dependent on PARKN and MUL1. Elife 5, pii: e17896.

Saita, S., Shirane, M., and Nakayama, K.I. (2013). Selective escape of proteins from the mitochondria during mitophagy. Nat. Commun. 4, 1410.

Sandoval, H., Thiagarajan, P., Dasgupta, S.K., Schumacher, A., Prchal, J.T., Chen, M., and Wang, J. (2008). Essential role for Nix in autophagic maturation of erythroid cells. Nature 454, 232-235.

Sarraf, S.A., Raman, M., Guarani-Pereira, V., Sowa, M.E., Huttlin, E.L., Gygi, S.P., and Harper, J.W. (2013). Landscape of the PARKN-dependent ubiquitylation in response to mitochondrial depolarization. Nature 496, 372-376.

Scheibye-Knudsen, M., Fang, E.F., Croteau, D.L., Wilson, D.M., 3rd (2014). BNIP3 interacting with LC3 triggers excessive mitophagy in delayed neuronal death in stroke. CNS Neurosci. Ther. 20, 1045-1055.

Shinaihi, O.S., Song, M.S., and Dorn, G.W. (2015). How mitochondrial dynamism orchestrates mitophagy. Circ. Res. 116, 1835-1849.

Sinha, R.A., and Yen, P.M. (2016). Thyroid hormone-mediated autophagy and mitochondrial turnover in NALFD. Cell Biosci 6.

Smith, B.K., Marcinko, K., Desjardins, E.M., Lally, J.S., Ford, R.J., and Steinberg, G.R. (2016). Treatment of nonalcoholic fatty liver disease: modulation of serines 17 and 24 in the LC3-interacting region of autophagy and mitochondrial turnover in NAFLD. Cell Biosci 6.

Sowter, H.M., Ratcliffe, P.J., Watson, P., Greenberg, A.H., and Harris, A.L. (2001). HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. Cancer Res. 61, 291, 21616-21629.

Trotta, A.P., and Chipuk, J.E. (2017). Mitochondrial dynamics as regulators of cancer biology. Cell Mol. Life Sci. 74, 1999-2017.

Vazquez-Martin, A., Van den Haute, C., Cufi, S., Corominas-Faja, B., Cuyas, E., Lopez-Bonet, E., Rodriguez-Gallego, E., Fernandez-Arroyo, S., Joven, J., Baekelandt, V., et al. (2016). Mitophagy-driven mitochondrial rejuvenation regulates stem cell fate. Aging (Albany NY) 8, 1330-1352.

Vazquez, F., Lim, J.H., Chim, H., Bhalla, K., Girnun, G., Pierce, K., Clish, C.B., Grantner, S.R., Wildlund, H.R., Spiegelman, B.M., et al. (2013). PGCl alpha expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. Cancer Cell 23, 287-301.

Wai, T., and Langer, T. (2016). Mitochondrial dynamics and mitochondrial quality control as a therapeutic target. Pharmacol Rev. 68, 980-981.

Ye, X., Sun, X., Starovoytov, V., and Cai, Q. (2015). Parkin-mediated mitophagy in mutant hAPP neurons and Alzheimer’s disease patient brains. Hum. Mol. Genet. 24, 2938-2951.

Yu, Y., Zheng, Y., Zhang, X., Chen, Y., Wu, X., Wu, J., Shen, Z., Jiang, L., Wang, L., Yang, W., et al. (2017). BNP3/NIX-mediated mitophagy protects against ischemic brain injury independent of PARK2. Autophagy 13, 1754-1766.

Zhang, T., Xue, L., Li, L., Tang, C., Wan, Z., Wang, R., Tan, J., Tan, Y., Han, H., Tian, R., et al. (2016). BNP3 Protein Suppresses PIN1 Kinase Proteolytic Cleavage to Promote Mitophagy. J. Biol. Chem. 291, 21616-21629.

Zhang, W., Ren, H., Xu, C., Zhu, C., Wu, H., Liu, D., Wang, J., Liu, L., Li, W., Ma, Q., et al. (2016). Hypoxic mitophagy regulates mitochondrial quality and platelet activation and determines severity of I/R heart injury. Elife 5, pii: e21407.

Zhong, Z., Umemura, A., Sanchez-Lopez, E., Liang, S., Shalappour, S., Wong, J., He, F., Boassa, D., Perkins, G., Ali, S.R., et al. (2016). NfkappaB restricts inflammasome activation via elimination of damaged mitochondria. Cell 164, 896-910.

Zhou, H., Zhu, P., Guo, J., Hu, N., Wang, S., Li, D., Hu, S., Ren, J., Cao, F., and Chen, Y. (2017). Ripk3 induces mitochondrial apoptosis via inhibition of FUNDC1 mitophagy in cardiac I/R injury. Redox. Biol. 12, 498-507.

Zhu, Y., Massen, S., Terenzi, M., Lang, V., Chen-Lindner, S., Eils, R., Novak, I., Dikic, I., Harnacher-Brady, A., and Brady, N.R. (2013). Modulation of serines 17 and 24 in the LC3-interacting region of Bnip3 determines pro-survival mitophagy versus apoptosis. J. Biol. Chem. 288, 1099-1113.