ANTIOXIDANTS & REDOX SIGNALING
Volume 22, Number 12, 2015

DOI: 10.1089/ars.2014.6204

Discoveries

FORUM REVIEW ARTICLE

Hypoxia Activation of Mitophagy and Its Role in Disease Pathogenesis

Hao Wu[1,2] and Quan Chen[1–3]

Abstract

Significance: Mitochondria utilize most of the oxygen to produce adenosine triphosphate *via* electron transfer coupled with oxidative phosphorylation. Hypoxia undoubtedly induces reduced energy production *via* decreased mitochondrial metabolic activity or altered hypoxia-inducible factor-1- and peroxisome proliferator-activated receptor gamma coactivator 1-dependent mitochondrial biogenesis. Hypoxia may also activate mitophagy to selectively remove damaged or unwanted mitochondria for both mitochondrial quantity and quality control. Increasing evidence has shown that the accumulation of damaged mitochondria is a characteristic of aging and aging-related diseases, such as metabolic disorder, cancer, and neurodegenerative disease. ***Recent Advances:*** Both receptor-dependent and PTEN-induced putative kinase 1-PARKIN-dependent mitophagy have been described. Mitophagy receptors include Atg32 in yeast, as well as NIX/BNIP3L, B-cell lymphoma 2/adenovirus E1B 19-kDa-interacting protein 3 and FUN14 domain containing 1 in mammals. In response to hypoxia or mitochondrial oxidative stress, receptor-mediated mitophagy was found to be activated *via* both transcriptional and post-translational modification. ***Critical Issues:*** To date, the molecular mechanisms by which hypoxia triggers mitophagy and by which mitophagy contributes to the pathogenesis of aging-related diseases remain to be explored. ***Future Directions:*** An improved understanding of the regulation of mitochondrial quality may provide a strategy for treating aging-related diseases by targeting mitochondria and mitophagy pathways. *Antioxid. Redox Signal.* 22, 1032–1046.

Introduction

AUTOPHAGY REFERS TO the catabolic processing of cellular components, including misfolded proteins, protein aggregates, damaged organelles, lipid droplets, and even nuclear components. The to-be-disposed cellular contents are enclosed by a double-membrane structure termed the autophagosome, which fuses with the lysosome for degradation. Subsequently, the breakdown products (such as amino acids, fatty acids, carbohydrates, and even nucleotides) are released and recycled for both biosynthesis and energy generation (97). Autophagy has long been considered a nonselective bulk digestion pathway to eliminate aggregated proteins and organelles in response to energy deprivation and metabolic stress. Increasing evidence has shown that autophagy may be highly selective. Under certain stresses, protein aggregates, organelles, including mitochondria, endoplasmic reticulum (ER), peroxisomes, components of nuclei, lipid droplets, and invading pathogens, are selectively recognized and removed by the autophagy machinery *via* processes referred to as aggrephagy, mitophagy, reticulophagy, pexophagy, nucleophagy, lipophagy, and xenophagy, respectively. Selective autophagy is typically mediated by specific adaptors or receptors (118). Both general and selective autophagy have been extensively reviewed by many outstanding scientists in the field. Here, we summarize the recent advances in mitophagy, with a particular focus on the hypoxic induction of mitophagy. We also discuss the association between mitophagy and diseases, which suggests the therapeutic potential of novel strategies targeting mitochondria and mitophagy.

[1]State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.
[2]University of Chinese Academy of Sciences, Beijing, China.
[3]Tianjin Key Laboratory of Protein Science, College of Life Sciences, Nankai University, Tianjin, China.

General Autophagy

Based on the manner of cellular cargo delivered to the lysosome, there are three distinct modes of autophagy: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy. Microautophagy, which has been described in yeast but rarely in mammalian cells, is defined as the translocation of cytoplasmic components into the lysosome *via* invagination of the lysosomal membrane, resembling the formation of late endosomes/multivesicular bodies (96). In contrast, CMA has been characterized in higher eukaryotes but not in yeast. In CMA, misfolded cytosolic proteins containing the pentapeptide KFERQ are selectively recognized by heat shock cognate protein heat-shock cognate protein 70, and this substrate-chaperone complex is recruited by the lysosomal receptor protein lysosome-associated membrane protein type 2a (63). Macroautophagy refers to the classic autophagy process, in which a double-membrane autophagosome surrounds cellular cargo, fuses with lysosome, and, ultimately, facilitates degradation of the cargo by lysosomal enzymes. As the primary mode of autophagy, macroautophagy is regarded as autophagy.

More than 30 autophagy-related genes (Atg) and corresponding proteins have been identified as participating in autophagy-related processes, including the activation of autophagy signaling cascade, the assembly and expansion of the double-membrane structure, and then fusion between autophagosome and lysosome leading to the lysosomal degradation and the release of autophagic products (98). Starvation has been reported to be the most common trigger of autophagy. Amino-acid or growth factor deprivation induces autophagy primarily *via* the phosphatidylinositol-4,5-bisphosphate 3-kinase-mammalian target of rapamycin (mTOR) pathway, the master sensor that monitors the intracellular nutrient status (58). Specifically, the presence of amino acids, especially branch chain amino acids such as leucine and arginine, facilitate lysosomal localization- and activity-induced activation of mTOR by promoting the formation of the active configuration of the RAG GTPase complex (122, 123, 163). UNC-51-like kinase-1 (ULK1), the mammalian homolog of yeast Atg1, bridges the nutrient sensor mTOR to autophagy initiation *via* phosphorylation/dephosphorylation alteration (37, 52, 67). Furthermore, a low glucose level initiates autophagy *via* AMP-activated protein kinase (AMPK) kinase to regulate ULK1 activity (32, 67, 130). In addition to these highly effective signaling cascade-mediated post-translational modifications, there is also a variety of transcription factors that participate in long-term autophagy regulation. The transcription factor EB, a master transcription factor of lysosomal biogenesis, coordinates this process by inducing the expression of autophagy and lysosomal genes, including *Atg4*, *Atg9*, *Wipi*, and so on (129). Zinc finger protein with KRAB and SCAN domains 3, which belongs to the family of zinc finger transcription factors that contains Kruppel-associated box and SCAN domains, was found to act as an autophagy repressor to prevent the expression of several genes involved in various steps of autophagy and lysosome biogenesis/function (17). Furthermore, the Forkhead box O (FOXO) transcription factors, including FOXO1 (159) and FOXO3 (91), are also master regulators of autophagy.

Despite significant progress in the field, the origin of the autophagosomal membrane remains enigmatic to many autophagy researchers. Several independent groups have shown that these double-membrane structures originate from the ER (121, 153), the Golgi apparatus (152), mitochondria (44), or the plasma membrane (115). Recently, Yoshimori and colleagues demonstrated that the isolation membrane forms at the ER–mitochondria contact site in mammalian cells (47). High-resolution imaging analysis showed that ATG14L, the marker protein of autophagosome/pre-autophagosome, relocalizes to the ER–mitochondria contact site in response to autophagy initiation signaling by binding to the ER-resident SNARE protein syntaxin 17. Once activated, the ULK1 complex translocates to this isolation membrane-forming site to recruit other ATG proteins and autophagy-specific phosphatidylinositol-3-phosphate effectors to induce nucleation. After nucleation, the E3-like ligase complex Atg16L1, composed of the Atg12, Atg5, and Atg16L1 proteins, is recruited to the membrane to mediate the lipidation of microtubule-associated protein 1A/1B-light chain 3 (LC3) and LC3 homolog proteins. Due to the lipidation of LC3 and LC3 homologue proteins, the isolation membrane expands to form a complete autophagosome.

Mitochondrial Autophagy

Mitochondria are cellular powerhouses that produce adenosine triphosphate (ATP) *via* the coupling of electron transport chain activity with oxidative phosphorylation in the inner mitochondrial membrane. In addition to ATP production, mitochondria provide space for key metabolic processes, such as fatty acid oxidation, iron metabolism, the urea cycle, and calcium storage. Research in the past three decades has firmly established that in response to apoptotic stimuli, including DNA damage, chemotherapeutic agents, serum starvation, and UV radiation, the mitochondrial outer membrane becomes permeabilized, releasing apoptogenic factors, especially cytochrome *c*, which binds to apoptotic protease activating factor 1 to form the apoptosome and to activate caspases for apoptosis (85). In addition, reactive oxygen species (ROS) are an inevitable byproduct of oxidative phosphorylation. Excessive ROS accumulation causes mitochondrial oxidative damage and mitochondrial dysfunction and contributes to several pathological processes, including aging (82), apoptosis (140), and cellular injury, during ischemia and reperfusion (56). Therefore, it is critical for the cell to remove unwanted or damaged mitochondria for the maintenance of appropriate mitochondrial quality for cellular health. Mitochondrial autophagy, or mitophagy, which refers to the selective removal of unwanted or damaged mitochondria *via* the autophagic machinery, is considered responsible for the maintenance of mitochondrial quality (76).

Mitophagy was initially detected in hepatocytes treated with glucagon, resulting in the sequestration of mitochondria in lysosomes. In 2007, Lemasters and colleagues found that mitochondria are engulfed by the autophagosome in hepatocytes isolated from green fluorescent protein-LC3 transgenic mice when the cells are maintained in nutrient-deprived medium (66, 117). Therefore, mitophagy was termed to describe selective mitochondrial sequestration by the autophagosome and degradation in the lysosome. Since then, an increasing number of reports have detected mitophagy under a variety of experimental conditions. Similarly, mitophagy has been detected in both physiological processes, such as red

blood cell maturation and sperm-derived mitochondria elimination after fertilization, and pathological events, such as cancer and neurodegenerative disease. Currently, both receptor-dependent and -independent mechanisms of mitophagy have been described.

Receptor-Mediated Mitophagy

Atg32-mediated mitophagy in yeast

Atg32 was found to function as a mitophagy receptor in yeast based on mutant screening (61, 62, 106). Atg32 is a 59-kDa, single-pass mitochondrial outer membrane protein, with its N- and C-terminal domains exposed to cytosol and mitochondrial intermembrane space, respectively. The cytosolic N-terminal domain contains a W/Y X X I/L/V region, the Atg8-family interacting motif (AIM), which interacts with Atg8 (106). In cells cultured in non-fermentable medium, a condition in which mitophagy is induced, yeast lacking Atg32 exhibit deficient mitophagy but predominantly intact starvation-inducing bulk autophagy, confirming Atg32 serving as a specific mitophagy receptor (62, 106). Atg32 is reported to be strongly activated in yeast under respiratory conditions, to which oxidative stress appears to contribute, as the ROS scavenger *N*-acetylcysteine prevents Atg32 induction and subsequent mitophagy (106), possibly due to the restoration of the glutathione pool (28).

Atg32 has been reported to physically associate with Atg8 in a conserved manner *via* its typical AIM, facilitating its function as a mitophagy receptor by directly recruiting the Atg8-containing phagophore to sequester mitochondria. The W86A I89A mutant of Atg32, which lacks the ability to interact with Atg8, exhibits partial but not complete deficiency of mitophagy, suggesting that the AIM-dependent Atg32–Atg8 interaction is important but not essential for mitophagy (74, 106). Furthermore, Atg32 is defined as an Atg11-interacting protein, and the Atg32–Atg11 interaction is thought to be an early step of mitophagy initiation that is distinct from autophagosome formation (74). In addition, Ser114 phosphorylation of Atg32 has been demonstrated to be critically important for Atg32–Atg11 interaction and subsequent mitophagy (2). Casein kinase-2 (CK2) phosphorylates Atg32 at Ser114 and Ser119, increasing the stability of the Atg32–Atg11 interaction and specifically promoting mitophagy but not bulk autophagy or pexophagy. In addition, two mitogen-activated protein kinases, Slt2 and Hog1, are reported to be responsible for Atg32 phosphorylation and mitophagy (2, 93) (Fig. 1).

In addition to Atg32, the mitochondrial outer membrane protein Uth1p (71) and the mitochondrial protein phosphatase homolog Aup1p (136) have also been identified to be involved in mitochondrial clearance in yeast cultures subjected to nutrient starvation or a prolonged stationary phase, respectively. Furthermore, it has been suggested that mitochondrial dynamic is significantly related to mitophagy, as the fragmented mitochondria are more easily sequestered by autophagosomes, and specifically fragmented mitochondria removal is more effective to maintain mitochondrial quality (41, 114, 139). It has been reported that the dynamin-related GTPase Dnm1, which mediates the fission of the outer mitochondrial membrane, is required for mitophagy induced by Mdm38 knockout (103), YPL medium, or lga2 over-expression (101). Recently, Klionsky and colleagues showed that the Dnm1 fission complex can trigger mitophagy *via* an interaction with Atg11-Atg32 (92).

NIX/B-cell lymphoma 2/adenovirus E1B 19-kDa-interacting protein 3-mediated mitophagy

B-cell lymphoma 2 (BCL2)/adenovirus E1B 19-kDa-interacting protein 3 (BNIP3) (9, 116, 151) and NIX (also known as BNIP3 L) (18, 54, 105) were initially identified as BCL2 homology 3 domain (BH3)-only pro-apoptotic proteins with their C-terminal transmembrane domain localizing to the mitochondrial outer membrane. As alternative BH3-only proteins, BNIP3 and NIX confer similar pro-apoptotic activity by heterodimerizing with BCL2 or B-cell lymphoma-extra large (BCL-XL). Over-expression of BNIP3 (27, 45) or NIX (5) triggers protective autophagy under a series of stresses, possibly by disrupting the interaction between BCL2/BCL-XL and Beclin1 (89, 90).

Using NIX knockout mice, several independent groups have shown that NIX deficiency leads to anemia, reticulocytosis, and erythroid-myeloid hyperplasia, and development disorder during erythroid maturation. The clearance of mitochondria during this period is defective in the absence of NIX (124, 127). NIX contains a typical LC3-interacting region (LIR, similar to the AIM in yeast) motif

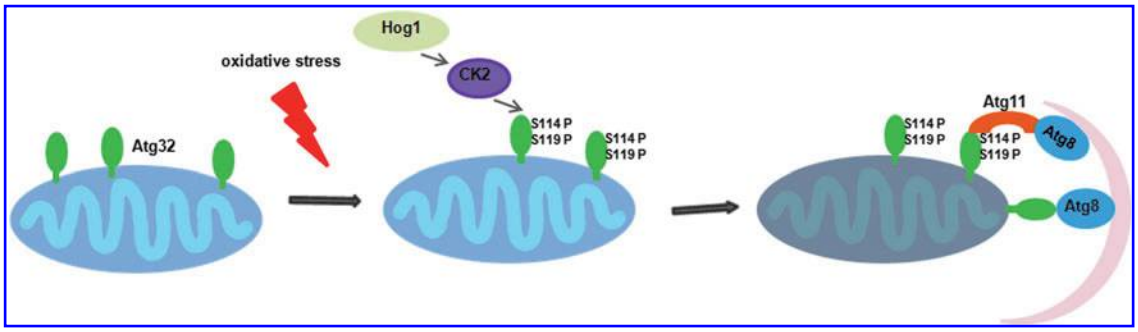

FIG. 1. Atg32-mediated mitophagy in yeast. The single-pass mitochondrial outer membrane protein Atg32 contains an AIM domain and directly interacts with Atg8. When yeast is cultured in non-fermentable medium, Atg32 is phosphorylated *via* the Hog1 MAPK-CK2 pathway at S114 and S119. This phosphorylation enhances the Atg32–Atg11 interaction, the Atg32–Atg11–Atg8 interaction, and the subsequent recognition of mitochondria by the phagophore. AIM, Atg8-family interacting motif; Atg, autophagy-related genes; CK2, casein kinase-2; MAPK, mitogen-activated protein kinases. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

that interacts with the LC3 protein and its homolog GABAA receptor-associated protein (GABARAP) both *in vitro* and *in vivo* (102, 126). It is thought that NIX recruits the autophagosome to mitochondria by directly binding to LC3 and GABARAP. Mutating the LIR motif decreases the interaction between NIX and LC3/GABARAP and abolishes mitophagy to a certain extent. Furthermore, re-introduction of wild-type NIX to $NIX^{-/-}$ reticulocytes rescues mitochondrial clearance to the level in wild-type mice. During erythroid differentiation, NIX is strongly up-regulated (1, 127) and mediates mitochondria removal. In addition to NIX, BNIP3 interacts with LC3 [but not GABARAP (48)] *via* its LIR motif to act as a mitophagy receptor (45, 48, 79, 113) (Fig. 2).

FUN14 domain containing 1–mediated mitophagy

We have recently identified that the mitochondrial outer membrane protein FUN14 domain containing 1 (FUNDC1) functions as a mitophagy receptor in mammalian cells (83). The FUNDC1 protein contains three transmembrane domains, as well as the N-terminus domain exposed to the cytosol, and the C-terminus domain inserted into the mitochondrial outer membrane. Ectopically expressed FUNDC1 induces a significant increase in the colocalization of LC3 puncta with fragmented mitochondria, accompanied by a decrease in mitochondrial mass, the typical phenotype of mitophagy. FUNDC1 was found to contain a characteristic LIR motif at the cytosol-exposed N-terminus. FUNDC1 directly interacts with LC3 and LC3 homologs *via* its LIR domain, and mutation of its LIR domain disrupts this interaction and, subsequently, abolishes mitophagy (Fig. 3).

PTEN-Induced Putative Kinase 1-PARKIN Pathway of Mitophagy

The cytosolic E3 ubiquitin ligase PARKIN, encoded by the *Park2* gene, and the mitochondrial serine/threonine kinase PTEN-induced putative kinase 1 (PINK1), encoded by the *Pink* gene, which are associated with the familial form of Parkinson's disease (26, 72, 104, 141), are reported to regulate mitophagy. Loss-of-function mutation analyses using *Drosophila melanogaster* showed that deficiency of PARKIN or PINK1 results in similar phenotypes, including muscle degeneration and cell death, reduced lifespan, locomotor defects, and male sterility (23, 42, 109). Furthermore, the phenotypes caused by PINK1 loss can be rescued by PARKIN but not *vice versa*, suggesting that PARKIN and PINK1 function *via* identical pathways, with PARKIN acting downstream of PINK1 (150). Research from Youle's group and many other laboratories has demonstrated the function of the identical PINK1-PARKIN pathway in selective mitophagy in mammalian systems (78, 99, 100). Under normal conditions, PINK1 is transported to mitochondria *via* the translocase of the outer mitochondrial membrane (TOM) and the translocase of the inner mitochondrial membrane complexes, processed by presenilin-associated rhomboid-like protease, and, ultimately, degraded. When mitochondria are depolarized by the uncoupler toxin carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone, PINK1 escapes from this processing and accumulates on the mitochondrial outer membrane. Moreover, TOMM7, a component of the TOM complex, serves as a positive regulator to stabilize PINK1 (49). PINK1 accumulation on the mitochondrial outer membrane facilitates the targeting of PARKIN to these depolarized mitochondria. Furthermore, the kinase activity of PINK1 is essential for PARKIN translocation. PINK1 may phosphorylate PARKIN at Ser65, which is the prerequisite for PARKIN translocation and subsequent mitophagy (53, 70, 73, 131). Most recently, it has been reported that phosphorylation of ubiquitin on Ser65 (60, 64, 75) and mitofusin 2 (MFN2) (21) by PINK1 are crucial for the recruitment of PARKIN to mitochondria and its ubiquitin ligase activity. On translocation to the mitochondrial outer membrane, PARKIN ubiquitinates a variety of mitochondrial proteins, including

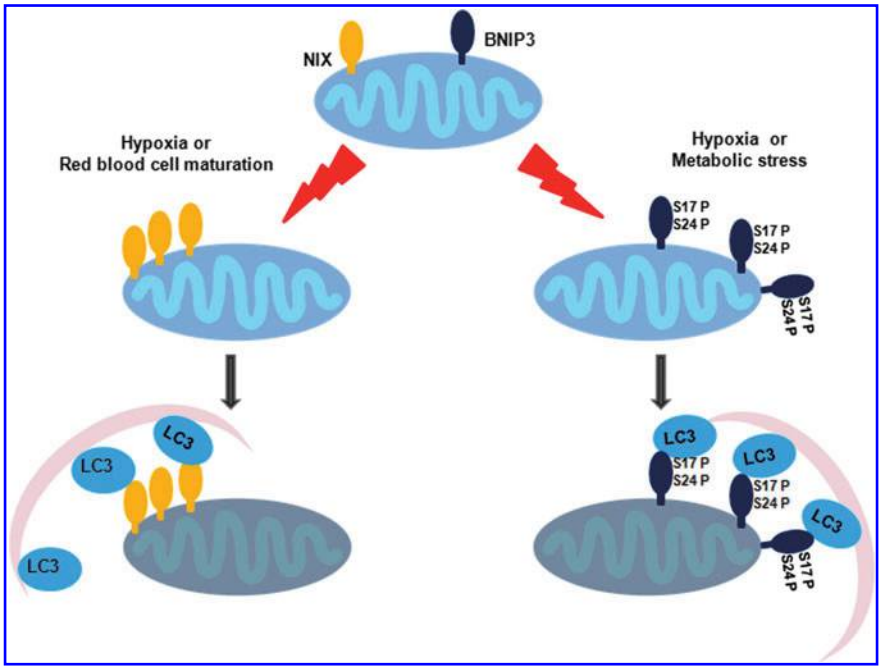

FIG. 2. NIX/BNIP3-mediated mitophagy. The mitochondrial outer membrane proteins NIX and BNIP3 have been identified as pro-apoptotic BH3-only proteins. Both contain an LIR domain and directly interact with LC3 or LC3 homologs. During red blood cell terminal differentiation or hypoxia, NIX and BNIP3 are activated to induce the removal of mitochondria. BNIP3 has recently been demonstrated to be phosphorylated at S17 and S24, which strengthens the BNIP3-LC3 interaction and promotes mitophagy. BH3, BCL2 homology 3 domain; BNIP3, BCL2/adenovirus E1B 19-kDa-interacting protein 3; LC3, microtubule-associated protein 1A/1B-light chain 3; LIR, LC3-interacting region. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

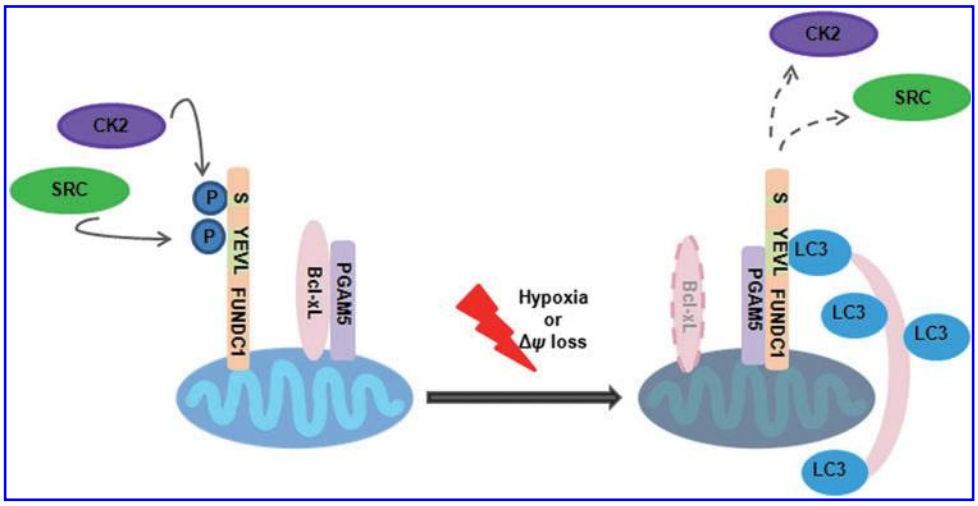

FIG. 3. FUNDC1-mediated mitophagy. The recently identified mitophagy receptor FUNDC1 contains a characteristic LIR domain at its cytosol-exposed N-terminus. FUNDC1 is phosphorylated by SRC and CK2 at Y18 and S13, respectively. The mitochondrially localized phosphatase PGAM5 dephosphorylates FUNDC1 at S13. When cells are maintained under normoxic conditions, SRC and CK2 are constitutively active and phosphorylate Y18 and S13 of FUNDC1, respectively. PGAM5 interacts with BCL-XL, inhibiting its phosphatase activity. This phosphorylation of FUNDC1 status prevents the activity of this mitophagy receptor. During hypoxia, SRC and CK2 become inactivated, and PGAM5 is released, enabling dephosphorylation at S13, due to the rapid degradation of BCL-XL. The dephosphorylation of FUNDC1 at both sites enhances the FUNDC1-LC3 interaction and promotes mitophagy. BCL-XL, B-cell lymphoma-extra large; FUNDC1, FUN14 domain containing 1; PGAM5, phosphoglycerate mutase family member 5. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

dynamin-related protein 1 (143), MFN1/2 (38, 137, 162), voltage-dependent anion channel (39, 132), and components of the TOM complex (15, 125, 154). Ultimately, the hyper-ubiquitination of the mitochondrial outer membrane initiates mitophagy. Recent studies have shown that the targeting of PINK1-PARKIN to depolarized mitochondria arrests the movement of these dysfunctional mitochondria and prevents them from traveling peripherally. PINK1 phosphorylates the GTPase mitochondrial RHO GTPase (Miro), a key component in the recruitment of the kinesin-1 heavy chain (KHC) to the mitochondrial surface. This phosphorylation accelerates PARKIN-dependent Miro degradation, detaches KHC from these depolarized mitochondria, and promotes the accumulation of these mitochondria in somatodendritic regions, where lysosomes are predominantly located (12, 144, 146). As mentioned earlier, PARKIN translocation facilitates MFN1/2 degradation (38, 137). Collectively, the PINK1-PARKIN pathway plays a general role in mitochondrial trafficking and dynamics, including fission and fusion, as well as the highly specific and effective clearance of unwanted mitochondria (Fig. 4).

In addition to the PINK1-PARKIN pathway and these mitophagy receptors, some core autophagy components have been reported to play specific roles in mitophagy regulation. In the absence of Atg7, mitochondrial clearance from reticulocytes is reduced (157). Similarly, in the absence of ULK1, the unique kinase of core autophagy components, mitochondrial clearance from reticulocytes is impaired (77). In stable PARKIN-expressing mouse embryo fibroblast (MEF) cells, CCCP induces the association between the ULK1 complex and clustered mitochondria (55). Although PINK1-PARKIN-pathway-mediated mitophagy and receptor-mediated mitophagy have been reported to occur under distinct experimental conditions, the crosstalk between these pathways should not be ignored. Dorn (30) reported that both aged BNIP3 and NIX knockout mice display accumulation of dysfunctional mitochondria in the heart. Moreover, the BNIP3 and NIX double knockout mice display even further accumulation, suggesting that these two mitophagy receptors perform overlap functions in regulating damaged mitochondrial removal in the aged heart (30). Ectopically expressed BNIP3 in adult myocytes induces the translocation of PARKIN to mitochondria (79). In addition, deficiency of NIX in MEF cells reduces CCCP-induced PARKIN translocation (29). Similarly, knocking down FUNDC1 reduces CCCP-induced PARKIN translocation, demonstrating that these mitophagy receptors cooperate with PINK1-PARKIN pathway to fine-tune the mitophagy process (19).

Hypoxia Activation of Mitophagy

Oxygen is one of the most important metabolic substrates for oxidative phosphorylation inside mitochondria. A low level of oxygen (hypoxia) in cells and tissues, which is characteristic of most tumors, leads to the transcriptional upregulation of a series of genes that participate in angiogenesis, iron metabolism, glucose metabolism, and cell proliferation/survival (65). Hypoxia is currently considered a negative prognostic and predictive factor due to its multiple contributions to chemoresistance, radioresistance, angiogenesis, vasculogenesis, invasiveness, metastasis, resistance to cell death, alteration of metabolism, and genomic instability (147).

Hypoxia signaling and mitochondria

At present, the transcription factor hypoxia-inducible factor-1 (HIF-1) is considered the most important regulator responsible for adaptation of hypoxia. HIF-1, a heterodimeric complex consisting of the hypoxia-induced subunit HIF-1α

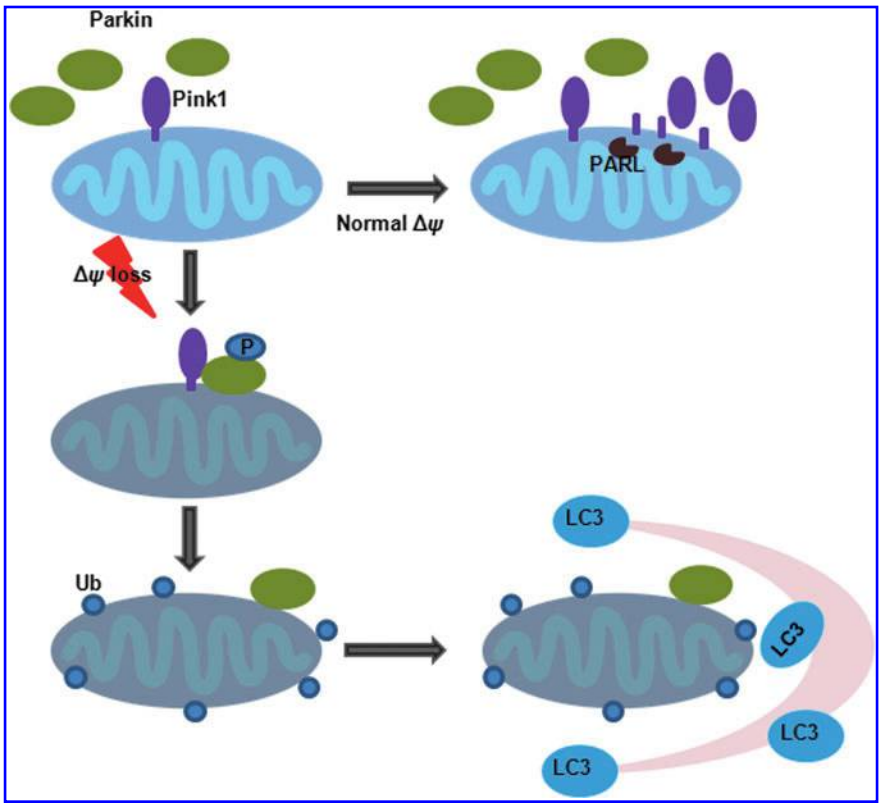

FIG. 4. PARKIN-mediated mitophagy. PINK1 translocates to mitochondria *via* the TOM and TIM complexes. When mitochondria obtain normal membrane potential ($\Delta\psi$), PINK1 is processed by the inner mitochondrial protease PARL and is, ultimately, degraded. When mitochondria lose the membrane potential, PINK1 escapes from PARL and accumulates on the mitochondrial outer membrane *via* its transmembrane domain. The accumulation of PINK1 phosphorylates the E3 ligase Parkin, facilitating its translocation to these mitochondria. Ultimately, PARKIN mediates the hyper-ubiquitination of the mitochondrial outer membrane, inducing the recognition of these damaged mitochondria by an isolation membrane. PARL, presenilin-associated rhomboid-like protease; PINK1, PTEN-induced putative kinase 1; TIM, translocase of the inner mitochondrial membrane; TOM, translocase of the outer mitochondrial membrane. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

and the constitutively expressed subunit HIF-1β, transcriptionally regulate the expression of several genes by binding to the hypoxia response element (HRE) in these hypoxia-responsive genes (142). Since HIF-1β is constitutively expressed, the subunit HIF-1α, which contains an oxygen-dependent degradation domain and is tightly regulated by oxygen, is considered the major regulator of the activity of the HIF-1 complex (112). Under normoxia, the HIF-1α protein is rapidly degraded, resulting in minimal transcriptional activity of the HIF-1 complex. When cells are subjected to hypoxic conditions, HIF-1α becomes stabilized and translocates from the cytosol to the nucleus, where it interacts with HIF-1β to facilitate transcriptional activity. The degradation of HIF-1α under normoxia is dependent on its hydroxylation at two proline residues (P402 and P564) by prolyl hydroxylase domains (PHDs). The hydroxylated HIF-1α is recognized by the E3 ubiquitin ligase complex von Hippel-Lindau tumour suppressor protein, leading to its proteasome-dependent degradation. During hypoxia, PHDs lose their hydroxylase activity due to a lack of O_2, and HIF-1α becomes stabilized (128) (Fig. 5).

In the electron transport chain, oxygen is the terminal acceptor of electrons from cytochrome *c* oxidase. Since mitochondria consume most ($\sim$85–90%) of the O_2 to perform oxidative phosphorylation, hypoxia causes damage to mitochondria and to cells in general. During hypoxia, cytochrome *c* oxidase is unable to transport electrons because of the lack O_2. Hypoxia-activated HIF-1-mediated transcriptional activity converts metabolic activity from aerobic respiration to anaerobic glycolysis by suppressing mitochondrial aerobic metabolic processes, including the tricarboxylic acid cycle and oxidative phosphorylation. In addition, HIF-1 has been reported to initiate the expression of certain genes, including pyruvate dehydrogenase kinase, thereby affecting oxidative phosphorylation (69, 108).

It was thought that hypoxia decreases the ROS level due to the low level of O_2 and the diminished mitochondrial respiration. However, the ROS level has been reported by several groups to increase during hypoxia. Chandel *et al.* reported progressive increases in ROS at 5%, 3%, and 1% oxygen and demonstrated that this increased ROS level is vital for hypoxia-induced HIF-1α stability and subsequent HIF-1 transcriptional activity (16). In addition, independent groups have demonstrated that hypoxia increases the level of nitric oxide (NO) (25), which competitively inhibits the interaction between mitochondrial enzyme cytochrome *c* oxidase and O_2 (10, 24). Similar to ROS, NO has been demonstrated to stabilize HIF-1α during hypoxia (95). Furthermore, several studies have shown that hypoxia also affects mitochondrial Ca^{2+} flux (11, 86, 111), mitochondrial morphology (22, 84), and the mitochondrial membrane potential (36).

Hypoxia-induced autophagy

Hypoxia has long been known to trigger autophagy both *in vivo* and *in vitro*. In 2007, Tracy *et al.* showed that hypoxia triggers autophagy-dependent cell death in MEF cells *via* the induction of BNIP3. Repressing BNIP3 suppresses autophagy and cell death, suggesting that the pro-apoptotic protein BNIP3 plays a central role in hypoxia-induced autophagy and autophagic cell death (138). A similar phenomenon and mechanism were confirmed by independent groups in glioma, breast cancer cells, and other systems (3, 33, 155). However, Mazure and colleagues reported that hypoxia induces protective autophagy in an HIF-1-dependent manner *via* the induction of BNIP3 and NIX. Inhibition of this

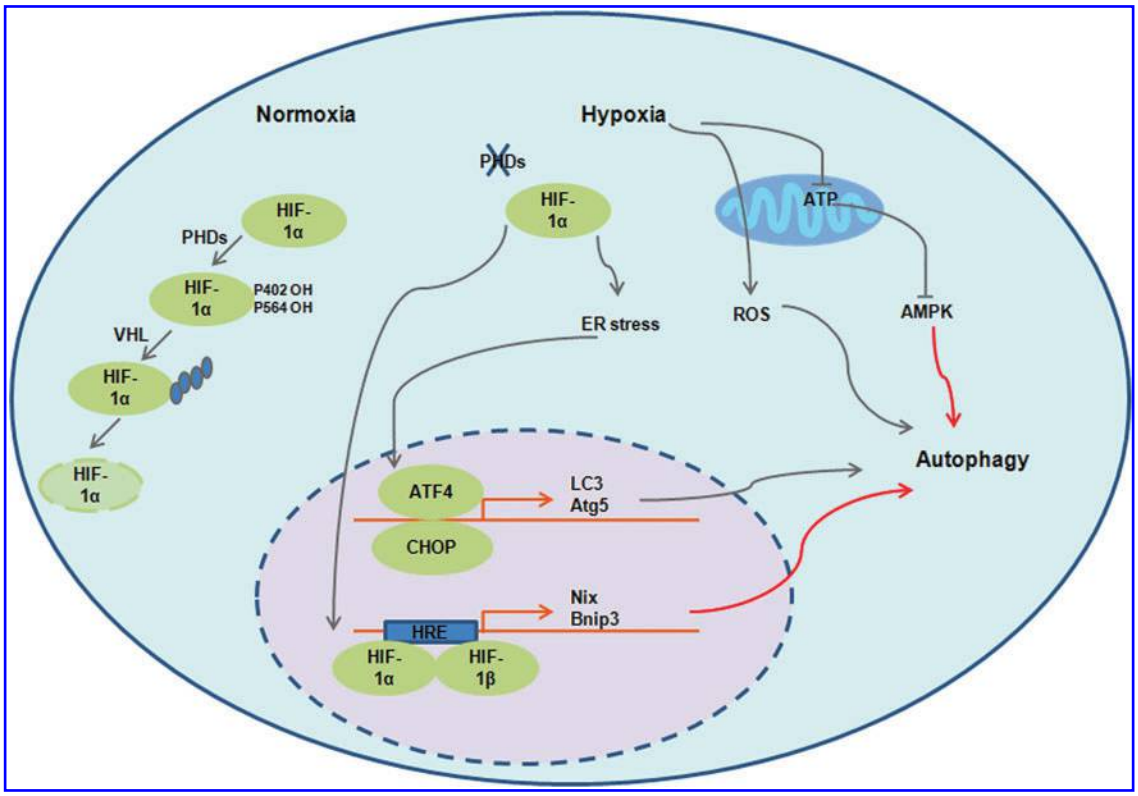

FIG. 5. Hypoxia signaling and hypoxia-induced autophagy. Hypoxia signaling and hypoxia-induced autophagy are primarily mediated by the oxygen-sensitive transcription factor HIF-1α. During normoxia, HIF-1α is hydroxylated by PHDs, and the hydroxylated HIF-1α is ubiquitinated by the E3 ligase complex VHL, leading to its degradation. During hypoxia, PHDs are inactivated, stabilizing HIF-1α expression. Then, HIF-1α translocates to the nucleus and interacts with HIF-1β to form the HIF-1 complex, which binds to HRE regions to promote the expression of specific genes, such as Nix and Bnip3, promoting autophagy. HIF-1α has been demonstrated to induce endoplasmic reticulum stress and, subsequently, autophagy either directly or indirectly *via* the transcriptional control of LC3 and Atg5. Hypoxia has also been reported to regulate autophagy *via* the ROS level and the AMP/ATP ratio. ATP, adenosine triphosphate; HIF-1, hypoxia-inducible factor-1; HRE, hypoxia response element; PHD, prolyl hydroxylase domain; ROS, reactive oxygen species; VHL, von Hippel-Lindau tumor suppressor protein. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

autophagy by knocking down Atg5 or Beclin1 increases cell death (5) (Fig. 5).

In addition to BNIP3 and NIX, ER stress and the unfolded protein response (UPR) pathway have been found to participate in hypoxia-induced autophagy. Harris and colleagues showed that severe hypoxia up-regulates LC3 expression and promotes cell survival-related autophagic activity, which is dependent on activating transcription factor 4 (ATF4), the transcriptional factor involved in double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK)-mediated UPR signaling (120). Almost simultaneously, Wouters and colleagues confirmed this concept. The UPR-related transcription factors ATF4 and CCAAT-enhancer-binding protein homologous protein bind to LC3 and Atg5 promoters, respectively. The levels of the core autophagic components LC3 and Atg5, along with cell-protective autophagy, are increased in hypoxic tumor cells both *in vivo* and *in vitro*, which is dependent on PERK signaling (119). Furthermore, mTOR may participate in hypoxia-induced autophagy (7, 80) (Fig. 5).

Hypoxia-induced mitophagy

As mentioned earlier, hypoxia leads to alterations in mitochondria, including decreased oxidative phosphorylation, cytochrome *c* oxidase activity, and increased ROS production. Due to these damages, mitophagy is undoubtedly induced during hypoxia. However, several independent studies have demonstrated that hypoxia stimulates mitochondrial biogenesis *via* various mechanisms, including nitric oxide synthase (NOS) (43) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (160). PGC-1α is elevated in heart tissue and cell lines subjected to hypoxia, and this up-regulation is dependent on AMPK signaling. Moreover, it has been reported that NO and neuronal NOS (nNOS), but not endothelial NOS (eNOS), are vital for hypoxia-induced PGC-1α expression and mitochondrial biogenesis. In contrast, hypoxia has been reported to suppress mitochondrial biogenesis, which is dependent on the inhibitory effect of FOXO3A on c-Myc transcription factor activity, suppressing the expression of mitochondria-associated genes (34, 57). Collectively, this dual effect of hypoxia on mitochondria (hypoxia inducing both mitochondrial biogenesis and mitophagy) suggests that the O_2 level and the O_2 sensor HIF-1 act as the principal effectors that maintain the homeostasis between the cellular energy demand and redox homeostasis (Fig. 6).

It remains unclear whether the PINK1-PARKIN pathway or the mitophagy receptor Atg32 participates in the regulation of hypoxia-induced mitophagy. Increasing evidence

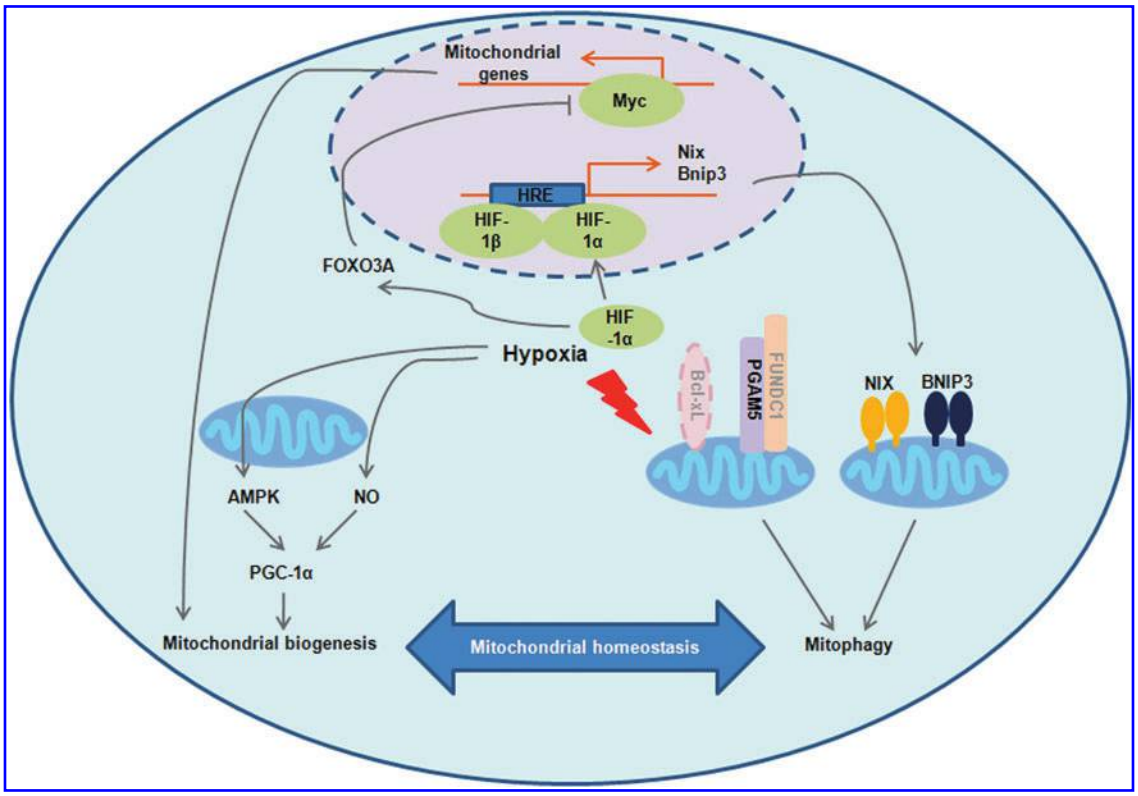

FIG. 6. Hypoxia-mediated regulation of mitochondrial homeostasis. In response to hypoxia, the decreased level of O_2 triggers mitochondrial biogenesis *via* the hypoxia-induced activation of the AMPK/PGC-1α pathway or NO production *via* the hypoxia-induced activation of NOS. It has also been reported that hypoxia-stabilized HIF-1α suppresses mitochondrial biogenesis by inhibiting the expression of mitochondria-associated genes *via* the FOXO3A-mediated inhibition of the transcription factor Myc. In contrast, hypoxia and hypoxia-stabilized HIF-1α are involved in hypoxia-induced mitophagy. At the transcriptional level, the mitophagy receptors NIX and BNIP3 are up-regulated, inducing the removal of dysfunctional mitochondria. Alternatively, the hypoxia-induced degradation of BCL-XL releases the phosphatase PGAM5, facilitating the dephosphorylation of FUNDC1 at Ser13, which activates its mitophagic activity. Hypoxia-induced mitochondrial biogenesis and mitophagy ensure the homeostasis between cellular energy demands and redox homeostasis. AMPK, AMP-activated protein kinase; FOXO, forkhead box O; NO, nitric oxide; NOS, nitric oxide synthase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

shows that hypoxia-induced mitophagy occurs *via* the HIF-1α-induced expression of BNIP3 and NIX, the two mitophagy receptors (4, 155, 156). Both BNIP3 and NIX contain an HRE motif in their promoter region. In addition to the transcriptional regulation, the post-translational regulation of BNIP3 has been reported. Brady and colleagues showed that phosphorylation of serine residues 17 and 24, which flank the BNIP3 LIR domain, promotes the interaction between BNIP3 and LC3, increasing the maturation and autophagic degradation of mitochondria (161) (Fig. 2).

The molecular mechanism by which hypoxia initiates FUNDC1-mediated mitophagy has begun to be understood. Hypoxia affects the reversible phosphorylation of this mitophagy receptor. Tyr18, which is located in the LIR motif, is phosphorylated by SRC kinase under normoxia, and dephosphorylation occurs before hypoxia-induced mitophagy due to the inactivation of SRC kinase. Ser13 is phosphorylated by CK2 under normoxia and becomes dephosphorylated by the mitochondrially localized phosphatase phosphoglycerate mutase family member 5 (PGAM5) in response to hypoxia. Under normoxic conditions, PGAM5 interacts with BCL-XL, which blocks its phosphatase activity. When cells encounter hypoxic conditions, rapid BCL-XL degradation induces PGAM5 release and activation to dephosphorylate FUNDC1 at Ser13. When dephosphorylated at both sites, FUNDC1 displays a significantly higher affinity to LC3, which induces a strong interaction between FUNDC1 and LC3, resulting in specific mitophagy to remove these damaged mitochondria (149). Inactivating either SRC or CK2 alone using pharmacological inhibitors or a knockdown approach is not sufficient to initiate mitophagy, but inhibition of both kinases strongly induces mitophagy (19). This two-part mechanism results in the fine-tuning of mitophagy during hypoxia (19, 83, 149) (Fig. 3).

Mitophagy and Diseases

Hypoxia has been associated with several types of human diseases, such as tumors (50), neurodegenerative diseases (110), and metabolic disorders (35). Many of these diseases display characteristic energy metabolism defects due to the accumulation of dysfunctional mitochondria. As the most important mechanism to maintain appropriate mitochondrial quality and quantity, mitophagy is suggested to play pivotal roles in the pathogenesis of these diseases. It remains under debate whether defective mitophagy plays a causal role in the

pathogenesis of diseases or the accumulation of dysfunctional mitochondria is merely a consequence of an accompanying cellular event associated with these diseases (20, 87).

Mitophagy and cancer

In the early 1920s, Warburg found that cancer cells display a metabolic shift from oxidative phosphorylation to glycolysis, which is referred to as the ''Warburg effect'' (145). Extensive studies in recent decades have revealed the role of mitochondrial dysfunction anaerobic glycolysis in cancer cells. Autophagy was suggested to play opposing roles in tumorigenesis depending on the cellular context. Autophagy may suppress tumorigenesis by eliminating the harmful macromolecules or diminishing ROS production during the early onset of tumorigenesis. Alternatively, autophagy may promote tumorigenesis by sustaining tumor cell survival under the hypoxic conditions of the tumor during the late stage of tumor growth. Furthermore, in addition to the alleviation of oxidative stress by ROS scavengers and the maintenance of mitochondrial genetic stability, mitophagy, as the primary mechanism for the removal of damaged mitochondria, emerges as a key repressor of carcinogenesis. SH3-domain GRB2-like endophilin B1, which is also known as BIF-1 (SH3GLB1), a reported component of the Beclin1 complex (133), regulates the post-Golgi trafficking of membrane-integrated ATG9A during autophagy (135). SH3GLB1-deficient mice are susceptible to the development of spontaneous tumors, indicating that SH3GLB1 serves as a tumor suppressor (133). In addition, SH3GLB1 was recently found to mediate the removal of dysfunctional mitochondria *via* mitophagy in Myc-induced lymphoma cells. Loss of SH3GLB1 suppresses mitophagy, inhibits caspase-3 activation, and promotes Myc-induced genomic instability and lymphoma development, emphasizing the tumor-suppressive role of mitophagy (134). In contrast, mitophagy appears to promote tumorigenesis. Oncogenic K-Ras induces mitophagy during cell transformation, and this mitophagy may serve as an important cellular strategy to overcome cellular energy deficiency due to insufficient glucose import by expediting glycolysis, thereby promoting cancer development (68). The accumulation of dysfunctional mitochondria due to deficient mitophagy likely contributes to the Warburg effect. It remains to be determined whether abnormal receptor-mediated mitophagy is causally associated with cancer.

Mitophagy and neurodegenerative diseases

Neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and Huntington's disease, are a large group of disabling disorders of the nervous system, characterized by the progressive loss of neuronal structure or function. The high energy demands of neurons, due to their numerous neuronal processes, is critically dependent on O_2 supply and mitochondrial integrity (107). Studies in past decades showed that mitochondrial damage in neurons and the subsequent induction of neuronal death are strongly associated with the pathogenesis of neurodegenerative disease (31, 81). The extensive examination of PINK1-PARKIN-mediated mitophagy has revealed a very close relationship between mitophagy deficiency and Parkinson's disease, as discussed earlier. However, conclusive evidence of the role of PINK1-PARKIN-mediated mitophagy in Parkinson's disease pathogenesis has yet to be presented, as most of the reported studies were performed using cultured cells, and Pink1 or Parkin knockout mice failed to faithfully recapitulate Parkinson's disease (59). In addition to Parkinson's disease, mitochondrial damage and mitophagy deficiency appear to be related to Alzheimer's disease. Specifically, β-amyloid fragments have been found to accumulate in mitochondria and disrupt mitochondrial function, inducing oxidative stress (13, 14, 88). Furthermore, mitochondrial dysfunction, including loss of the mitochondrial membrane potential, decreased respiratory ability, and changes in mitochondrial structure, are detected in Huntington's disease patients (8). A recent study demonstrated a primary defect in the ability of autophagic vacuoles to recognize cytosolic cargo in cells isolated from humans with Huntington's disease, as well as an abnormal mitochondrial turnover rate in these cells, indicating a protective role of mitophagy and a mitophagy defect in Huntington's disease pathogenesis (94).

Mitophagy and metabolic disorder

Mitophagy is suggested to finely tune metabolic progress by regulating mitochondrial mass. The accumulation of damaged mitochondria and mitophagy deficiency are observed in metabolic syndrome (148). Adipose-specific Atg7 knockout mice exhibit a lean phenotype, with $\sim$20% of the adipose mass of wild-type mice. This result is likely attributed to increased β-oxidation, reduced rates of hormone-induced lipolysis, decreased plasma concentrations of leptin, and higher levels of basal physical activity. These mutant mice are also resistant to high-fat diet-induced obesity and insulin resistance. High levels of mitochondria are detected in both white and brown adipose tissue in these mutant mice, demonstrating the association between the autophagy-mediated regulation of mitochondrial mass and metabolic disorder (158). Deficiency of Bnip3, an identified mitophagy receptor, leads to the obese phenotype, including increased lipogenesis and reduced β-oxidation in the liver, accompanied by elevated mitochondrial mass and loss of mitochondrial function (40). In addition, PARKIN-mediated mitophagy has been found to maintain insulin secretion and protect mice from type 1 diabetes (51).

Perspectives

Mitochondrial function is the principal oxygen consumer. Under hypoxia, the immediate response of the cell may be to reduce or even stop the reactions that utilize oxygen. Mitochondria are able to sense this hypoxic signaling to perform subsequent responses. On the one hand, the decrease in ATP and increase in NO activate AMPK or PGC-1α, respectively, which may activate mitochondrial biogenesis as a compensatory response for the loss of energy. On the other hand, mitochondria as cargoes are subject to destruction *via* mitophagy in response to both oxidative damage and hypoxic conditions. Currently, the mechanisms underlying hypoxia-induced activation of mitophagy, including transcriptional regulation of NIX or BNIP3 by HIF-1, are understood to be distinct. Hypoxia also immediately activates mitophagy *via* the reversible phosphorylation of mitophagy receptors, such as FUNDC1. Specifically, hypoxia promotes the degradation of BCL-XL, which releases PGAM5, facilitating its activation and dephosphorylation of FUNDC1. It is clear that

hypoxia plays opposing roles in both mitochondrial biogenesis and mitophagy, depending on the acuteness and duration of the hypoxic conditions and the cellular context. The precise mechanism by which mitochondria sense and integrate these distinct cellular or environmental cues to establish mitochondrial homeostasis requires further investigation. As the predominant mechanism that regulates mitochondrial quantity and quality, mitophagy has been suggested to play a protective role in aging-related diseases. Although the causal association between mitophagy and the occurrence of these diseases remains elusive, the hypoxia-induced activation of mitophagy should be explored to search for preventive or therapeutic strategies to treat these diseases.

Acknowledgments

Work in the authors' laboratories was supported by the 973 program project (No. 2011CB910903 and No. 2013CB531200) from the MOST and Natural Science Foundation of China (81130045, 31271529, and 31301175).

References

1. Aerbajinai W, Giattina M, Lee YT, Raffeld M, and Miller JL. The proapoptotic factor Nix is coexpressed with Bcl-xL during terminal erythroid differentiation. *Blood* 102: 712–717, 2003.
2. Aoki Y, Kanki T, Hirota Y, Kurihara Y, Saigusa T, Uchiumi T, and Kang D. Phosphorylation of serine 114 on Atg32 mediates mitophagy. *Mol Biol Cell* 22: 3206–3217, 2011.
3. Azad MB, Chen Y, Henson ES, Cizeau J, McMillan-Ward E, Israels SJ, and Gibson SB. Hypoxia induces autophagic cell death in apoptosis-competent cells through a mechanism involving BNIP3. *Autophagy* 4: 195–204, 2008.
4. Band M, Joel A, Hernandez A, and Avivi A. Hypoxia-induced BNIP3 expression and mitophagy: *in vivo* comparison of the rat and the hypoxia-tolerant mole rat, *Spalax ehrenbergi*. *FASEB J* 23: 2327–2335, 2009.
5. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouyssegur J, and Mazure NM. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol* 29: 2570–2581, 2009.
6. This reference has been deleted.
7. Blagosklonny MV. Hypoxia, MTOR and autophagy: converging on senescence or quiescence. *Autophagy* 9: 260–262, 2013.
8. Bossy-Wetzel E, Petrilli A, and Knott AB. Mutant huntingtin and mitochondrial dysfunction. *Trends Neurosci* 31: 609–616, 2008.
9. Boyd JM, Malstrom S, Subramanian T, Venkatesh LK, Schaeper U, Elangovan B, Dsaeipper C, and Chinnadurai G. Adenovirus-E1b 19-Kda and Bcl-2 proteins interact with a common set of cellular proteins. *Cell* 79: 341–351, 1994.
10. Brown GC. Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. *Biochim Biophys Acta* 1504: 46–57, 2001.
11. Buckler KJ and Vaughan-Jones RD. Effects of hypoxia on membrane potential and intracellular calcium in rat neonatal carotid body type I cells. *J Physiol* 476: 423–428, 1994.
12. Cai Q, Zakaria HM, Simone A, and Sheng ZH. Spatial parkin translocation and degradation of damaged mitochondria via mitophagy in live cortical neurons. *Curr Biol* 22: 545–552, 2012.
13. Cardoso SM, Santos S, Swerdlow RH, and Oliveira CR. Functional mitochondria are required for amyloid beta-mediated neurotoxicity. *FASEB J* 15: 1439–1441, 2001.
14. Casley CS, Land JM, Sharpe MA, Clark JB, Duchen MR, and Canevari L. Beta-amyloid fragment 25–35 causes mitochondrial dysfunction in primary cortical neurons. *Neurobiol Dis* 10: 258–267, 2002.
15. Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL, Hess S, and Chan DC. Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Hum Mol Genet* 20: 1726–1737, 2011.
16. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, and Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci U S A* 95: 11715–11720, 1998.
17. Chauhan S, Goodwin JG, Chauhan S, Manyam G, Wang J, Kamat AM, and Boyd DD. ZKSCAN3 is a master transcriptional repressor of autophagy. *Mol Cell* 50: 16–28, 2013.
18. Chen G, Cizeau J, Velde CV, Park JH, Bozek G, Bolton J, Shi L, Dubik D, and Greenberg A. Nix and Nip3 form a subfamily of pro-apoptotic mitochondrial proteins. *J Biol Chem* 274: 7–10, 1999.
19. Chen G, Han Z, Feng D, Chen Y, Chen L, Wu H, Huang L, Zhou C, Cai X, Fu C, Duan L, Wang X, Liu L, Liu X, Shen Y, Zhu Y, and Chen Q. A Regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. *Mol Cell* 54: 362–377, 2014.
20. Chen H and Chan DC. Mitochondrial dynamics—fusion, fission, movement, and mitophagy—in neurodegenerative diseases. *Hum Mol Genet* 18: R169–R176, 2009.
21. Chen Y and Dorn GW, 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science* 340: 471–475, 2013.
22. Chiche J, Rouleau M, Gounon P, Brahimi-Horn MC, Pouyssegur J, and Mazure NM. Hypoxic enlarged mitochondria protect cancer cells from apoptotic stimuli. *J Cell Physiol* 222: 648–657, 2010.
23. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA, and Guo M. *Drosophila* pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* 441: 1162–1166, 2006.
24. Cooper CE. Nitric oxide and cytochrome oxidase: substrate, inhibitor or effector? *Trends Biochem Sci* 27: 33–39, 2002.
25. Cooper CE and Giulivi C. Nitric oxide regulation of mitochondrial oxygen consumption II: molecular mechanism and tissue physiology. *Am J Physiol Cell Physiol* 292: C1993–C2003, 2007.
26. Cruts M, Theuns J, and Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. *Hum Mutat* 33: 1340–1344, 2012.
27. Daido S, Kanzawa T, Yamamoto A, Takeuchi H, Kondo Y, and Kondo S. Pivotal role of the cell death factor BNIP3 in ceramide-induced autophagic cell death in malignant glioma cells. *Cancer Res* 64: 4286–4293, 2004.
28. Deffieu M, Bhatia-Kissova I, Salin B, Galinier A, Manon S, and Camougrand N. Glutathione participates in the regulation of mitophagy in yeast. *J Biol Chem* 284: 14828–14837, 2009.

29. Ding WX, Ni HM, Li M, Liao Y, Chen X, Stolz DB, Dorn GW, 2nd, and Yin XM. Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming. *J Biol Chem* 285: 27879–27890, 2010.
30. Dorn GW, 2nd. Mitochondrial pruning by Nix and BNip3: an essential function for cardiac-expressed death factors. *J Cardiovasc Transl Res* 3: 374–383, 2010.
31. Dubinsky JM. CNS mitochondria in neurodegenerative disorders. *Antioxid Redox Signal* 7: 1089–1091, 2005.
32. Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, Asara JM, Fitzpatrick J, Dillin A, Viollet B, Kundu M, Hansen M, and Shaw RJ. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 331: 456–461, 2011.
33. Farrall AL and Whitelaw ML. The HIF1alpha-inducible pro-cell death gene BNIP3 is a novel target of SIM2s repression through cross-talk on the hypoxia response element. *Oncogene* 28: 3671–3680, 2009.
34. Ferber EC, Peck B, Delpuech O, Bell GP, East P, and Schulze A. FOXO3a regulates reactive oxygen metabolism by inhibiting mitochondrial gene expression. *Cell Death Differ* 19: 968–979, 2012.
35. Formenti F, Constantin-Teodosiu D, Emmanuel Y, Cheeseman J, Dorrington KL, Edwards LM, Humphreys SM, Lappin TR, McMullin MF, McNamara CJ, Mills W, Murphy JA, O'Connor DF, Percy MJ, Ratcliffe PJ, Smith TG, Treacy M, Frayn KN, Greenhaff PL, Karpe F, Clarke K, and Robbins PA. Regulation of human metabolism by hypoxia-inducible factor. *Proc Natl Acad Sci U S A* 107: 12722–12727, 2010.
36. Frezza C, Zheng L, Tennant DA, Papkovsky DB, Hedley BA, Kalna G, Watson DG, and Gottlieb E. Metabolic profiling of hypoxic cells revealed a catabolic signature required for cell survival. *Plos One* 6: e24411, 2011.
37. Ganley IG, Lam DH, Wang JR, Ding XJ, Chen S, and Jiang XJ. ULK1 center dot ATG13 center dot FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem* 284: 12297–12305, 2009.
38. Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, and Taanman JW. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum Mol Genet* 19: 4861–4870, 2010.
39. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, and Springer W. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 12: 119–131, 2010.
40. Glick D, Zhang W, Beaton M, Marsboom G, Gruber M, Simon MC, Hart J, Dorn GW, 2nd, Brady MJ, and Macleod KF. BNip3 regulates mitochondrial function and lipid metabolism in the liver. *Mol Cell Biol* 32: 2570–2584, 2012.
41. Gomes LC, Di Benedetto G, and Scorrano L. During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. *Nat Cell Biol* 13: 589–598, 2011.
42. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, and Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila* parkin mutants. *Proc Natl Acad Sci U S A* 100: 4078–4083, 2003.
43. Gutsaeva DR, Carraway MS, Suliman HB, Demchenko IT, Shitara H, Yonekawa H, and Piantadosi CA. Transient hypoxia stimulates mitochondrial biogenesis in brain subcortex by a neuronal nitric oxide synthase-dependent mechanism. *J Neurosci* 28: 2015–2024, 2008.
44. Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK, and Lippincott-Schwartz J. Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 141: 656–667, 2010.
45. Hamacher-Brady A, Brady NR, Logue SE, Sayen MR, Jinno M, Kirshenbaum LA, Gottlieb RA, and Gustafsson AB. Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. *Cell Death Differ* 14: 146–157, 2007.
46. This reference has been deleted.
47. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, Amano A, and Yoshimori T. Autophagosomes form at ER-mitochondria contact sites. *Nature* 495: 389–393, 2013.
48. Hanna RA, Quinsay MN, Orogo AM, Giang K, Rikka S, and Gustafsson AB. 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, 2012.
49. Hasson SA, Kane LA, Yamano K, Huang CH, Sliter DA, Buehler E, Wang C, Heman-Ackah SM, Hessa T, Guha R, Martin SE, and Youle RJ. High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy. *Nature* 504: 291–295, 2013.
50. Hockel M and Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 93: 266–276, 2001.
51. Hoshino A, Ariyoshi M, Okawa Y, Kaimoto S, Uchihashi M, Fukai K, Iwai-Kanai E, Ikeda K, Ueyama T, Ogata T, and Matoba S. Inhibition of p53 preserves Parkin-mediated mitophagy and pancreatic beta-cell function in diabetes. *Proc Natl Acad Sci U S A* 111: 3116–3121, 2014.
52. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, Iemura S, Natsume T, Takehana K, Yamada N, Guan JL, Oshiro N, and Mizushima N. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* 20: 1981–1991, 2009.
53. Iguchi M, Kujuro Y, Okatsu K, Koyano F, Kosako H, Kimura M, Suzuki N, Uchiyama S, Tanaka K, and Matsuda N. Parkin-catalyzed ubiquitin-ester transfer is triggered by PINK1-dependent phosphorylation. *J Biol Chem* 288: 22019–22032, 2013.
54. Imazu T, Shimizu S, Tagami S, Matsushima M, Nakamura Y, Miki T, Okuyama A, and Tsujimoto Y. Bcl-2/E1B 19 kDa-interacting protein 3-like protein (Bnip3L) interacts with Bcl-2/Bcl-x(L) and induces apoptosis by altering mitochondrial membrane permeability. *Oncogene* 18: 4523–4529, 1999.
55. Itakura E, Kishi-Itakura C, Koyama-Honda I, and Mizushima N. Structures containing Atg9A and the ULK1 complex independently target depolarized mitochondria at initial stages of Parkin-mediated mitophagy. *J Cell Sci* 125: 1488–1499, 2012.
56. Jaeschke H, Smith CV, and Mitchell JR. Reactive oxygen species during ischemia reflow injury in isolated perfused rat-liver. *J Clin Invest* 81: 1240–1246, 1988.
57. Jensen KS, Binderup T, Jensen KT, Therkelsen I, Borup R, Nilsson E, Multhaupt H, Bouchard C, Quistorff B, Kjaer A, Landberg G, and Staller P. FoxO3A promotes metabolic adaptation to hypoxia by antagonizing Myc function. *EMBO J* 30: 4554–4570, 2011.
58. Jewell JL, Russell RC, and Guan KL. Amino acid signalling upstream of mTOR. *Nat Rev Mol Cell Biol* 14: 133–139, 2013.

59. Jiang P and Mizushima N. Autophagy and human diseases. *Cell Res* 24: 69–79, 2014.
60. Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, Banerjee S, and Youle RJ. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol* 205: 143–153, 2014.
61. Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA, Du Z, Geng JF, Mao K, Yang ZF, Yen WL, and Klionsky DJ. A genomic screen for yeast mutants defective in selective mitochondria autophagy. *Mol Biol Cell* 20: 4730–4738, 2009.
62. Kanki T, Wang K, Cao Y, Baba M, and Klionsky DJ. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev Cell* 17: 98–109, 2009.
63. Kaushik S, Bandyopadhyay U, Sridhar S, Kiffin R, Martinez-Vicente M, Kon M, Orenstein SJ, Wong E, and Cuervo AM. Chaperone-mediated autophagy at a glance. *J Cell Sci* 124: 495–499, 2011.
64. Kazlauskaite A, Kondapalli C, Gourlay R, Campbell DG, Ritorto MS, Hofmann K, Alessi DR, Knebel A, Trost M, and Muqit MM. Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem J* 460: 127–139, 2014.
65. Ke Q and Costa M. Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* 70: 1469–1480, 2006.
66. Kim I, Rodriguez-Enriquez S, and Lemasters JJ. Selective degradation of mitochondria by mitophagy. *Arch Biochem Biophys* 462: 245–253, 2007.
67. Kim J, Kundu M, Viollet B, and Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 13: 132–141, 2011.
68. Kim JH, Kim HY, Lee YK, Yoon YS, Xu WG, Yoon JK, Choi SE, Ko YG, Kim MJ, Lee SJ, Wang HJ, and Yoon G. Involvement of mitophagy in oncogenic K-Ras-induced transformation: overcoming a cellular energy deficit from glucose deficiency. *Autophagy* 7: 1187–1198, 2011.
69. Kim JW, Tchernyshyov I, Semenza GL, and Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 3: 177–185, 2006.
70. Kim Y, Park J, Kim S, Song S, Kwon SK, Lee SH, Kitada T, Kim JM, and Chung J. PINK1 controls mitochondrial localization of Parkin through direct phosphorylation. *Biochem Biophys Res Commun* 377: 975–980, 2008.
71. Kissova I, Deffieu M, Manon S, and Camougrand N. Uth1p is involved in the autophagic degradation of mitochondria. *J Biol Chem* 279: 39068–39074, 2004.
72. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, and Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392: 605–608, 1998.
73. Kondapalli C, Kazlauskaite A, Zhang N, Woodroof HI, Campbell DG, Gourlay R, Burchell L, Walden H, Macartney TJ, Deak M, Knebel A, Alessi DR, and Muqit MM. PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol* 2: 120080, 2012.
74. Kondo-Okamoto N, Noda NN, Suzuki SW, Nakatogawa H, Takahashi I, Matsunami M, Hashimoto A, Inagaki F, Ohsumi Y, and Okamoto K. Autophagy-related protein 32 acts as autophagic degron and directly initiates mitophagy. *J Biol Chem* 287: 10631–10638, 2012.
75. Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, Kimura Y, Tsuchiya H, Yoshihara H, Hirokawa T, Endo T, Fon EA, Trempe JF, Saeki Y, Tanaka K, and Matsuda N. Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* 510: 162–166, 2014.
76. Kubli DA and Gustafsson AB. Mitochondria and mitophagy the yin and yang of cell death control. *Circ Res* 111: 1208–1221, 2012.
77. Kundu M, Lindsten T, Yang CY, Wu J, Zhao F, Zhang J, Selak MA, Ney PA, and Thompson CB. Ulk1 plays a critical role in the autophagic clearance of mitochondria and ribosomes during reticulocyte maturation. *Blood* 112: 1493–1502, 2008.
78. Lazarou M, Jin SM, Kane LA, and Youle RJ. Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. *Dev Cell* 22: 320–333, 2012.
79. Lee Y, Lee HY, Hanna RA, and Gustafsson AB. Mitochondrial autophagy by Bnip3 involves Drp1-mediated mitochondrial fission and recruitment of Parkin in cardiac myocytes. *Am J Physiol Heart Circ Physiol* 301: H1924–H1931, 2011.
80. Leontieva OV, Natarajan V, Demidenko ZN, Burdelya LG, Gudkov AV, and Blagosklonny MV. Hypoxia suppresses conversion from proliferative arrest to cellular senescence. *Proc Natl Acad Sci U S A* 109: 13314–13318, 2012.
81. Lin MT and Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443: 787–795, 2006.
82. Liochev SI. Reactive oxygen species and the free radical theory of aging. *Free Radic Biol Med* 60: 1–4, 2013.
83. Liu L, Feng D, Chen G, Chen M, Zheng Q, Song P, Ma Q, Zhu C, Wang R, Qi W, Huang L, Xue P, Li B, Wang X, Jin H, Wang J, Yang F, Liu P, Zhu Y, Sui S, and Chen Q. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat Cell Biol* 14: 177–185, 2012.
84. Liu X and Hajnoczky G. Altered fusion dynamics underlie unique morphological changes in mitochondria during hypoxia-reoxygenation stress. *Cell Death Differ* 18: 1561–1572, 2011.
85. Liu X, Kim CN, Yang J, Jemmerson R, and Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 86: 147–157, 1996.
86. Lopez-Barneo J. Oxygen-sensing by ion channels and the regulation of cellular functions. *Trends Neurosci* 19: 435–440, 1996.
87. Lu H, Li G, Liu L, Feng L, Wang X, and Jin H. Regulation and function of mitophagy in development and cancer. *Autophagy* 9: 1720–1736, 2013.
88. Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, Trinchese F, Liu S, Gunn-Moore F, Lue LF, Walker DG, Kuppusamy P, Zewier ZL, Arancio O, Stern D, Yan SS, and Wu H. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* 304: 448–452, 2004.
89. Maiuri MC, Criollo A, Tasdemir E, Vicencio JM, Tajeddine N, Hickman JA, Geneste O, and Kroemer G. BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X-L. *Autophagy* 3: 374–376, 2007.
90. Maiuri MC, Zalckvar E, Kimchi A, and Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 8: 741–752, 2007.
91. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, Burden SJ, Di Lisi R, Sandri C, Zhao J,

Goldberg AL, Schiaffino S, and Sandri M. FoxO3 controls autophagy in skeletal muscle *in vivo*. *Cell Metab* 6: 458–471, 2007.
92. Mao K, Wang K, Liu X, and Klionsky DJ. The scaffold protein Atg11 recruits fission machinery to drive selective mitochondria degradation by autophagy. *Dev Cell* 26: 9–18, 2013.
93. Mao K, Wang K, Zhao M, Xu T, and Klionsky DJ. Two MAPK-signaling pathways are required for mitophagy in *Saccharomyces cerevisiae*. *J Cell Biol* 193: 755–767, 2011.
94. Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, de Vries R, Arias E, Harris S, Sulzer D, and Cuervo AM. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. *Nat Neurosci* 13: 567–576, 2010.
95. Mateo J, Garcia-Lecea M, Cadenas S, Hernandez C, and Moncada S. Regulation of hypoxia-inducible factor-1alpha by nitric oxide through mitochondria-dependent and -independent pathways. *Biochem J* 376: 537–544, 2003.
96. Mijaljica D, Prescott M, and Devenish RJ. Microautophagy in mammalian cells revisiting a 40-year-old conundrum. *Autophagy* 7: 673–682, 2011.
97. Mizushima N. Autophagy: process and function. *Genes Dev* 21: 2861–2873, 2007.
98. Mizushima N, Yoshimori T, and Ohsumi Y. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 27: 107–132, 2011.
99. Narendra D, Tanaka A, Suen DF, and Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* 183: 795–803, 2008.
100. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, Cookson MR, and Youle RJ. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* 8: e1000298, 2010.
101. Nieto-Jacobo F, Pasch D, and Basse CW. The mitochondrial Dnm1-like fission component is required for lgA2-induced mitophagy but dispensable for starvation-induced mitophagy in *Ustilago maydis*. *Eukaryot Cell* 11: 1154–1166, 2012.
102. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, Rogov V, Lohr F, Popovic D, Occhipinti A, Reichert AS, Terzic J, Dotsch V, Ney PA, and Dikic I. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep* 11: 45–51, 2010.
103. Nowikovsky K, Reipert S, Devenish RJ, and Schweyen RJ. Mdm38 protein depletion causes loss of mitochondrial K+/H+ exchange activity, osmotic swelling and mitophagy. *Cell Death Differ* 14: 1647–1656, 2007.
104. Nuytemans K, Theuns J, Cruts M, and Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. *Hum Mutat* 31: 763–780, 2010.
105. Ohi N, Tokunaga A, Tsunoda H, Nakano K, Haraguchi K, Oda K, Motoyama N, and Nakajima T. A novel adenovirus E1B19K-binding protein B5 inhibits apoptosis induced by Nip3 by forming a heterodimer through the C-terminal hydrophobic region. *Cell Death Differ* 6: 314–325, 1999.
106. Okamoto K, Kondo-Okamoto N, and Ohsumi Y. Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev Cell* 17: 87–97, 2009.
107. Palikaras K and Tavernarakis N. Mitophagy in neurodegeneration and aging. *Front Genet* 3: 297, 2012.
108. Papandreou I, Cairns RA, Fontana L, Lim AL, and Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 3: 187–197, 2006.
109. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, and Chung J. Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature* 441: 1157–1161, 2006.
110. Peers C, Dallas ML, Boycott HE, Scragg JL, Pearson HA, and Boyle JP. Hypoxia and neurodegeneration. *Ann N Y Acad Sci* 1177: 169–177, 2009.
111. Premkumar DR, Adhikary G, Overholt JL, Simonson MS, Cherniack NS, and Prabhakar NR. Intracellular pathways linking hypoxia to activation of c-fos and AP-1. *Adv Exp Med Biol* 475: 101–109, 2000.
112. Pugh CW, O'Rourke JF, Nagao M, Gleadle JM, and Ratcliffe PJ. Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit. *J Biol Chem* 272: 11205–11214, 1997.
113. Quinsay MN, Thomas RL, Lee Y, and Gustafsson AB. Bnip3-mediated mitochondrial autophagy is independent of the mitochondrial permeability transition pore. *Autophagy* 6: 855–862, 2010.
114. Rambold AS, Kostelecky B, Elia N, and Lippincott-Schwartz J. Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. *Proc Natl Acad Sci U S A* 108: 10190–10195, 2011.
115. Ravikumar B, Moreau K, Jahreiss L, Puri C, and Rubinsztein DC. Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* 12: 747–757, 2010.
116. Ray R, Chen G, Vande Velde C, Cizeau J, Park JH, Reed JC, Gietz RD, and Greenberg AH. BNIP3 heterodimerizes with Bcl-2/Bcl-X-L and induces cell death independent of a Bcl-2 homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites. *J Biol Chem* 275: 1439–1448, 2000.
117. Rodriguez-Enriquez S, Kim I, Currin RT, and Lemasters JJ. Tracker dyes to probe mitochondrial autophagy (mitophagy) in rat hepatocytes. *Autophagy* 2: 39–46, 2006.
118. Rogov V, Dotsch V, Johansen T, and Kirkin V. Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. *Mol Cell* 53: 167–178, 2014.
119. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, Keulers T, Mujcic H, Landuyt W, Voncken JW, Lambin P, van der Kogel AJ, Koritzinsky M, and Wouters BG. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest* 120: 127–141, 2010.
120. Rzymski T, Milani M, Pike L, Buffa F, Mellor HR, Winchester L, Pires I, Hammond E, Ragoussis I, and Harris AL. Regulation of autophagy by ATF4 in response to severe hypoxia. *Oncogene* 29: 4424–4435, 2010.
121. Saitoh T, Fujita N, Hayashi T, Takahara K, Satoh T, Lee H, Matsunaga K, Kageyama S, Omori H, Noda T, Yamamoto N, Kawai T, Ishii K, Takeuchi O, Yoshimori T, and Akira S. Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. *Proc Natl Acad Sci U S A* 106: 20842–20846, 2009.
122. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, and Sabatini DM. Ragulator-Rag complex targets

mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* 141: 290–303, 2010.
123. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, and Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320: 1496–1501, 2008.
124. Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, and Wang J. Essential role for Nix in autophagic maturation of erythroid cells. *Nature* 454: 232–235, 2008.
125. Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, Gygi SP, and Harper JW. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature* 496: 372–376, 2013.
126. Schwarten M, Mohrluder J, Ma PX, Stoldt M, Thielmann Y, Stangler T, Hersch N, Hoffmann B, Merkel R, and Willbold D. Nix directly binds to GABARAP a possible crosstalk between apoptosis and autophagy. *Autophagy* 5: 690–698, 2009.
127. Schweers RL, Zhang J, Randall MS, Loyd MR, Li W, Dorsey FC, Kundu M, Opferman JT, Cleveland JL, Miller JL, and Ney PA. NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proc Natl Acad Sci U S A* 104: 19500–19505, 2007.
128. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell* 148: 399–408, 2012.
129. Settembre C, Di Malta C, Polito VA, Garcia-Arencibia M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P, Sardiello M, Rubinsztein DC, and Ballabio A. TFEB links autophagy to lysosomal biogenesis. *Science* 332: 1429–1433, 2011.
130. Shang LB, Chen S, Du FH, Li S, Zhao LP, and Wang XD. Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. *Proc Natl Acad Sci U S A* 108: 4788–4793, 2011.
131. Shiba-Fukushima K, Imai Y, Yoshida S, Ishihama Y, Kanao T, Sato S, and Hattori N. PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. *Sci Rep* 2: 1002, 2012.
132. Sun Y, Vashisht AA, Tchieu J, Wohlschlegel JA, and Dreier L. Voltage-dependent anion channels (VDACs) recruit Parkin to defective mitochondria to promote mitochondrial autophagy. *J Biol Chem* 287: 40652–40660, 2012.
133. Takahashi Y, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, Liang C, Jung JU, Cheng JQ, Mule JJ, Pledger WJ, and Wang HG. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* 9: 1142–1151, 2007.
134. Takahashi Y, Hori T, Cooper TK, Liao J, Desai N, Serfass JM, Young MM, Park S, Izu Y, and Wang HG. Bif-1 haploinsufficiency promotes chromosomal instability and accelerates Myc-driven lymphomagenesis via suppression of mitophagy. *Blood* 121: 1622–1632, 2013.
135. Takahashi Y, Meyerkord CL, Hori T, Runkle K, Fox TE, Kester M, Loughran TP, and Wang HG. Bif-1 regulates Atg9 trafficking by mediating the fission of golgi membranes during autophagy. *Autophagy* 7: 61–73, 2011.
136. Tal R, Winter G, Ecker N, Klionsky DJ, and Abeliovich H. Aup1p, a yeast mitochondrial protein phosphatase homolog, is required for efficient stationary phase mitophagy and cell survival. *J Biol Chem* 282: 5617–5624, 2007.
137. Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, and Youle RJ. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* 191: 1367–1380, 2010.
138. Tracy K, Dibling BC, Spike BT, Knabb JR, Schumacker P, and Macleod KF. BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy. *Mol Cell Biol* 27: 6229–6242, 2007.
139. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, and Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 27: 433–446, 2008.
140. Ueda S, Masutani H, Nakamura H, Tanaka T, Ueno M, and Yodoi J. Redox control of cell death. *Antioxid Redox Signal* 4: 405–414, 2002.
141. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, and Wood NW. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304: 1158–1160, 2004.
142. Wang GL, Jiang BH, Rue EA, and Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci U S A* 92: 5510–5514, 1995.
143. Wang H, Song P, Du L, Tian W, Yue W, Liu M, Li D, Wang B, Zhu Y, Cao C, Zhou J, and Chen Q. Parkin ubiquitinates Drp1 for proteasome-dependent degradation: implication of dysregulated mitochondrial dynamics in Parkinson disease. *J Biol Chem* 286: 11649–11658, 2011.
144. Wang XN, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, and Schwarz TL. PINK1 and parkin target miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell* 147: 893–906, 2011.
145. Warburg O. On the origin of cancer cells. *Science* 123: 309–314, 1956.
146. Weihofen A, Thomas KJ, Ostaszewski BL, Cookson MR, and Selkoe DJ. Pink1 forms a multiprotein complex with miro and milton, linking Pink1 function to mitochondrial trafficking. *Biochemistry* 48: 2045–2052, 2009.
147. Wilson WR and Hay MP. Targeting hypoxia in cancer therapy. *Nat Rev Cancer* 11: 393–410, 2011.
148. Wrighton KH. Metabolism: putting energy into mitophagy. *Nat Rev Mol Cell Biol* 14: 324, 2013.
149. Wu H, Xue D, Chen G, Han Z, Huang L, Zhu C, Wang X, Jin H, Wang J, Zhu Y, Liu L, and Chen Q. The BCL2L1 and PGAM5 axis defines hypoxia-induced receptor-mediated mitophagy. *Autophagy* 10: 1712–1725, 2014.
150. Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, Yang L, Beal MF, Vogel H, and Lu B. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. *Proc Natl Acad Sci U S A* 103: 10793–10798, 2006.
151. Yasuda M, Theodorakis P, Subramanian T, and Chinnadurai G. Adenovirus E1B-19K/BCL-2 interacting protein BNIP3 contains a BH3 domain and a mitochondrial targeting sequence. *J Biol Chem* 273: 12415–12421, 1998.
152. Yen WL, Shintani T, Nair U, Cao Y, Richardson BC, Li ZJ, Hughson FM, Baba M, and Klionsky DJ. The conserved oligomeric Golgi complex is involved in double-membrane vesicle formation during autophagy. *J Cell Biol* 188: 101–114, 2010.

153. Yla-Anttila P, Vihinen H, Jokita E, and Eskelinen EL. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* 5: 1180–1185, 2009.
154. Yoshii SR, Kishi C, Ishihara N, and Mizushima N. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. *J Biol Chem* 286: 19630–19640, 2011.
155. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, and Semenza GL. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem* 283: 10892–10903, 2008.
156. Zhang J and Ney PA. Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. *Cell Death Differ* 16: 939–946, 2009.
157. Zhang J, Randall MS, Loyd MR, Dorsey FC, Kundu M, Cleveland JL, and Ney PA. Mitochondrial clearance is regulated by Atg7-dependent and -independent mechanisms during reticulocyte maturation. *Blood* 114: 157–164, 2009.
158. Zhang Y, Goldman S, Baerga R, Zhao Y, Komatsu M, and Jin S. Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. *Proc Natl Acad Sci U S A* 106: 19860–19865, 2009.
159. Zhao Y, Yang J, Liao WJ, Liu XY, Zhang H, Wang S, Wang DL, Feng JN, Yu L, and Zhu WG. Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. *Nat Cell Biol* 12: 665–675, 2010.
160. Zhu L, Wang Q, Zhang L, Fang Z, Zhao F, Lv Z, Gu Z, Zhang J, Wang J, Zen K, Xiang Y, Wang D, and Zhang CY. Hypoxia induces PGC-1alpha expression and mitochondrial biogenesis in the myocardium of TOF patients. *Cell Res* 20: 676–687, 2010.
161. Zhu Y, Massen S, Terenzio M, Lang V, Chen-Lindner S, Eils R, Novak I, Dikic I, Hamacher-Brady A, and Brady NR. 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, 2013.
162. Ziviani E, Tao RN, and Whitworth AJ. *Drosophila* parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci U S A* 107: 5018–5023, 2010.
163. Zoncu R, Bar-Peled L, Efeyan A, Wang SY, Sancak Y, and Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H+-ATPase. *Science* 334: 678–683, 2011.

Address correspondence to:
Dr. Quan Chen
State Key Laboratory of Biomembrane and Membrane Biotechnology
Institute of Zoology
Chinese Academy of Sciences
Beijing 100101
China

E-mail: chenq@ioz.ac.cn

Date of first submission to ARS Central, November 21, 2014; date of acceptance, December 18, 2014.

Abbreviations Used

AIM = Atg8-family interacting motif
AMPK = AMP-activated protein kinase
ATF4 = activating transcription factor 4
Atg = autophagy-related genes
ATP = adenosine triphosphate
BCL2 = B-cell lymphoma 2
BCL-XL = B-cell lymphoma-extra large
BH3 = BCL2 homology 3 domain
BNIP3 = BCL2/adenovirus E1B 19-kDa-interacting protein 3
CHOP = CCAAT-enhancer-binding protein homologous protein
CK2 = casein kinase-2
CMA = chaperone-mediated autophagy
ER = endoplasmic reticulum
FOXO = forkhead box O
FUNDC1 = FUN14 domain containing 1
GABARAP = GABAA receptor-associated protein
HIF-1 = hypoxia-inducible factor-1
HRE = hypoxia response element
KHC = kinesin-1 heavy chain
LC3 = microtubule-associated protein 1A/1B-light chain 3
LIR = LC3-interacting region
MAPK = mitogen-activated protein kinases
MEF = mouse embryo fibroblast
MFN1/2 = mitofusin 1/2
Miro = mitochondrial RHO GTPase
mTOR = mammalian target of rapamycin
NO = nitric oxide
NOS = nitric oxide synthase
PARL = presenilin-associated rhomboid-like protease
PERK = double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase
PGAM5 = phosphoglycerate mutase family member 5
PGC-1α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PHD = prolyl hydroxylase domain
PINK1 = PTEN-induced putative kinase 1
ROS = reactive oxygen species
SH3GLB1 = SH3-domain GRB2-like endophilin B1
TIM = translocase of the inner mitochondrial membrane
TOM = translocase of the outer mitochondrial membrane
ULK1 = UNC-51-like kinase-1
UPR = unfolded protein response
VDAC = voltage-dependent anion channel
VHL = von Hippel-Lindau tumor suppressor protein

This article has been cited by:

1. Yin Fei, Cadenas Enrique. 2015. Mitochondria: The Cellular Hub of the Dynamic Coordinated Network. *Antioxidants & Redox Signaling* **22**:12, 961-964. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Huifang Wei, Lei Liu, Quan Chen. 2015. Selective removal of mitochondria via mitophagy: distinct pathways for different mitochondrial stresses. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* . [CrossRef]