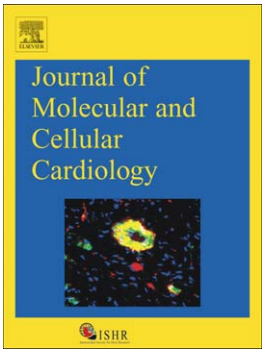
DOI:
10.1016/j.yjmcc.2016.03.010

Document Version
Peer reviewed version



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PII: S0022-2828(16)30057-8
DOI: doi: 10.1016/j.yjmcc.2016.03.010
Reference: YJMCC 8358

To appear in: *Journal of Molecular and Cellular Cardiology*

Received date: 30 October 2015
Revised date: 21 March 2016
Accepted date: 21 March 2016

Please cite this article as: Yamaguchi Osamu, Murakawa Tomokazu, Nishida Kazuhiko, Otsu Kinya, Receptor-mediated mitophagy, *Journal of Molecular and Cellular Cardiology* (2016), doi: 10.1016/j.yjmcc.2016.03.010

Receptor-mediated mitophagy

Osamu Yamaguchi[1,*], Tomokazu Murakawa[1], Kazuhiko Nishida[2], and Kinya Otsu[2]

[1]Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan

[2]Cardiovascular Division, King's College London British Heart Foundation Centre of Excellence, London SE5 9NU, United Kingdom

[*]Correspondence: yamaguti@cardiology.med.osaka-u.ac.jp

Abbreviations

AAA – ATPases associated with diverse cellular activities

ADP – adenosine diphosphate

ANT – adenine nucleotide translocator

Atg – autophagy related

ATP – adenosine triphosphate

BNIP3 – adenovirus E1B-19kDa-interacting protein 3

BNIP3L – adenovirus E1B-19kDa-interacting protein 3 like

CCCP – carbonyl cyanide m-chlorophenyl hydrazine

CUET – coupling of ubiquitin endoplasmic reticulum degradation domain targeting

DNA – deoxyribonucleic acid

Drp1 – dynamin-related protein 1

LC3 – homolog of microtubule-associated protein 1 light chain 3

LIR – LC3-interacting region

MDVs – mitochondria-derived vesicles

Mfn – mitofusin

NIX – NIP3-like protein X

OPA1 – Optic atrophy 1

PINK1 – phosphatase and tensin homolog-induced putative protein kinase 1

ROS – reactive oxygen species

SMURF1 – Smad-ubiquitin regulatory factor 1

VDAC1 – voltage-dependent anion channel 1

Contents

Abstract

Mitochondria are essential organelles that supply ATP through oxidative phosphorylation to maintain cellular homeostasis. Extrinsic or intrinsic agents can impair mitochondria, and these impaired mitochondria can generate reactive oxygen species (ROS) as byproducts, inducing cellular damage and cell death. The quality control of mitochondria is essential for the maintenance of normal cellular functions, particularly in cardiomyocytes, because they are terminally differentiated. Accumulation of damaged mitochondria is characteristic of various diseases, including heart failure, neurodegenerative disease, and aging-related diseases. Mitochondria are generally degraded through autophagy, an intracellular degradation system that is conserved from yeast to mammals. Autophagy is thought to be a nonselective degradation process in which cytoplasmic proteins and organelles are engulfed by isolation membrane to form autophagosomes in eukaryotic cells. However, recent studies have described the process of selective autophagy, which targets specific proteins or organelles such as mitochondria. Mitochondria-specific autophagy is called mitophagy. Dysregulation of mitophagy is implicated in the development of chronic diseases including neurodegenerative diseases, metabolic diseases, and heart failure. In this review, we discuss recent progress in research on mitophagy receptors.

1. Introduction

Autophagy and the ubiquitin/proteasome systems are major degradation pathways for the degradation of intracellular components. Three types of autophagy, i.e., macroautophagy, microautophagy, and chaperone-mediated autophagy, utilize different pathways to deliver cargoes to lysosomes. In macroautophagy, cytosolic proteins and organelles are sequestered by a double-membrane vesicle, isolation membrane, and fused with the lysosome to form autolysosome. The contents of the autolysosomes are then degraded for recycling and generation of ATP [1, 2]. The molecular mechanism of macroautophagy has been well studied and will hereafter be referred to as autophagy. The process of autophagy is regulated by autophagy-related (*Atg*) genes, which were first identified in yeast. The principal role of autophagy is to supply nutrients for survival [3]. In addition, constitutive autophagy in mammals is also important for regulating the quality of proteins and organelles in order to maintain the functions of cell and organs [4, 5]. Recent studies have suggested that autophagy induced protective effects in various organs [6, 7]. Thus, autophagy is thought to function as a cell- and tissue-protective mechanism.

Mitochondria not only generate reactive oxygen species (ROS) as a byproduct of oxidative phosphorylation but also release pro-apoptotic factors, such as cytochrome c and apoptosis-inducing factor, which lead to apoptosis. Thus, quality control of mitochondria by elimination is important to avoid cell death. Damaged mitochondria are selectively degraded by specific autophagic elimination, called mitophagy, which was first investigated in yeast [8-10]. During mitophagy, dysfunctional mitochondria are selectively recognized through the mitophagy receptor, which is expressed on the outer mitochondrial membrane, to be removed [8, 11, 12]. Recent study reveals that the role of mitophagy is not only the

elimination of dysfunctional mitochondria but mitochondrial turnover for metabolic transitioning from carbohydrates to fatty acids in cardiomyocytes during the perinatal period [13]. This observation is similar to the role of Nix-mediated mitophagy in the terminal stages of normal erythrocyte development [14]. Thus, the roles of mitophagy seem to be expanding to include physiological functions other than removal of damaged mitochondria.

The mitochondrial proteins Uth1p, Aup1p, and Atg32, were first identified in yeast and are involved in various mitophagy-related processes [10, 15-17]. Uth1p was the first molecule to be identified as mediating mitophagy in response to rapamycin treatment and nutrient starvation [10]. However, the specific molecules involved in mitophagy and in sensing damaged mitochondria for selective engulfment by autophagosomes in mammalian cells remained unclear. Parkin, an E3 ubiquitin ligase, has been identified as a mitophagy-related factor that promotes selective degradation of damaged mitochondria [18]. Subsequent studies have described various other molecules involved in mitophagy. Some molecules on the outer mitochondrial membrane function as receptors to interact with autophagy-related proteins. Mitochondrial DNA is also degraded during mitophagic processes by DNaseII in lysosomes. Incomplete digestion of mitochondrial DNA induces inflammation in the heart and causes heart failure [19], because mitochondrial DNA may resemble bacterial DNA, which contains unmethylated CpG motifs that can promote inflammatory responses [20-22]. This inflammatory responses are mediated by Toll-like receptor 9, which mounts an innate immune response [23]. Thus, complete execution of mitophagy is important for cardiac protection.

Morphological changes in mitochondria through fission and fusion are also

important steps in mitophagy. The dynamin-like GTPases mitofusin 1/2 (Mfn1/2) [24] on the outer mitochondrial membrane and Optic atrophy 1 (OPA1) [25] on the inner mitochondrial membrane are key regulators that mediate fusion, whereas, dynamin-related protein 1 (Drp1) has a pivotal role in mitochondrial fission [26]. Mitochondrial fragmentation by fission is thought to be one of the important steps during mitophagy both in yeast[27] and mammalian cells [28]. Elongated mitochondria, formed by fusion upon nutrient starvation, are protected from mitophagic degradation [29]. Thus, the size of mitochondria is important for engulfment by isolation membrane during the mitophagic response. Drp1 ablation induces lethal dilated cardiomyopathy in mice [30, 31]. Ablation of Drp1 in adult mouse cardiomyocytes decrease mitochondrial fission, leading to decreased respiratory function [31], on the other hand upregulates the expression level of Parkin to induce mitophagy and cardiac dysfunction [32]. Mitophagy markers, such as mitochondrial p62 and LC3, increase after knockdown of Drp1 [30]. These indicate that mitochondrial fission is not essential step for mitophagy.

The elimination of damaged mitochondria has an important role in the maintenance of cellular homeostasis and tissue function, particularly in heart, because cardiomyocytes are terminally differentiated. Cardiac-specific knockout of Atg5 induces accumulation of abnormal mitochondria and cardiac dysfunction, and Atg5-dependent autophagy in cardiomyocytes is also tissue-protecting mechanism from pressure overload or aging [33, 34].

In this review, we discuss the roles of mitophagic receptors.

2. Atg32 as a mitophagy receptor in yeast

In yeast, mitochondrial degradation is mediated through transport to the vacuole in an autophagy dependent manner [15, 35]. Atg32 was identified by two independent groups as a mitophagy receptor on the outer mitochondrial membrane in yeast by genome-wide screening [16, 17]. Atg32 is a 59-kDa protein that is anchored to the mitochondria via one transmembrane domain; N terminus is exposed to the cytosol, whereas the C terminus is located within the intermembrane space. Atg32 is necessary for mitophagy in post-log phase cells under respiratory growth, but not for canonical nonselective autophagy. Therefore, Atg32 is a mitophagy-specific factor. The expression level of Atg32 protein increases during respiratory growth [16], whereas the antioxidant N-acetylcysteine suppresses Atg32 induction and mitophagy, indicating that oxidative stress is one of the mechanisms to induce Atg32 expression. In Atg32-dependent mitophagy, Atg17, an essential molecule for bulk autophagy, is not required. Atg32 interacts with Atg8, a homolog of LC3 in yeast, via its WXXI motif to induce mitophagy, and with Atg11 via its residues 100-120 [36], which is an essential scaffold protein for selective autophagy in yeast. The WXXI (L) motif is called the Atg8-family interacting motif in yeast[37]. The mitochondrial inner membrane AAA (ATPases associated with diverse cellular activities) protease Yme1 processes Atg32 at the C terminus, and this processing is necessary for interaction with Atg11 and for mitophagic activity [38]. The interaction with Atg11 is also mediated through the phosphorylation of Ser114 on Atg32 by Hog1 and Pbs2, although Atg32 is not a direct substrate of Hog1 [36]. A recent study revealed that casein kinase 2 has a pivotal role in Atg32 phosphorylation at Ser114 and Ser119 [39]. Atg32 mutants, which do not bind to Atg8 or Atg11, cannot induce mitochondrial degradation [16, 17, 40]. Furthermore,

the C-terminal intermembrane space domain of Atg32 is not essential for selective-autophagy. The mutant cytosolic domain of Atg32 anchored to peroxisomes can promote peroxisome autophagy (pexophagy) [40]. Atg11 also interacts with Dnm1, a yeast homolog of Drp1, and the fission components are recruited to the mitochondria via this interaction [27]. Thus, there is a close relationship between mitochondrial fission and mitophagy. However, no homologs or functional homologs of Atg11 have been identified in mammalian cells. In mammalian cells, BCL2L13/Bcl-rambo plays an important role in mitophagy, and act as a functional homolog of Atg32 [41]. Full details of the function of BCL2L13 will be described later. The function of this homolog of Atg32 in the heart is not elucidated yet.

3. Expected characteristics of mammalian mitophagy receptors

As Atg32 in yeast, mitophagy receptors in mammalian cells are expected to localize to the outer mitochondrial membrane and interact with LC3 via the LC3-interacting region (LIR) motif in its cytosolic domain for mitophagy. The LIR motif is a tetrapeptide sequence W/YXXL/I, through which LC3 interacts with selective autophagy receptors. Several types of mitophagy receptors or receptor-related factors have been identified in mammalian cells, including NIX/BNIP3L, FUNDC1, PINK1/Parkin, and BCL2L13 (Bcl-rambo) [14, 18, 41-43].

The E3 ubiquitin ligase Parkin and phosphatase and tensin homolog-induced putative protein kinase 1 (PINK1) play important roles in elimination of damaged mitochondria, which lose mitochondrial membrane potential [18, 44-46]. The involvement of PINK1/Parkin during induction of mitophagy will be discussed in detail in another paper in this review series. Defects in the mitophagic pathway also induce cardiac disorders. The expression level of PINK1 is down-regulated in

end-stage heart failure patients. PINK1 knockout mice develop pathological cardiac hypertrophy and left ventricular dysfunction [47]. Although Parkin is the downstream of PINK1, Parkin deficiency has no obvious effect on baseline cardiac phenotypes [48]. It is possible that other E3 ubiquitin ligase to compensate for the deficiency of Parkin.

p62/Sequestome 1 is an important molecule to link aggregated proteins to LC3 [49, 50]. p62 can directly interact with ubiquitin through ubiquitin-binding domain, and with LC3 thorough LC3-interacting region. Depolarized mitochondria are ubiquitylated by Parkin, and then recruited-p62 on mitochondrial outer membrane binds to LC3 to form autophagosome. In this mitophagic process, p62 cooperates with Parkin for the elimination of damaged mitochondria [51]. Whether p62 is an essential molecule for mitophagy is controversial [52]. p62 is absent in lower eukaryotes like yeast. Recently CUET (coupling of ubiquitin endoplasmic reticulum degradation domain targeting) proteins are identified in yeast as a new class of ubiquitin-Atg8 adaptors; Cue5 from yeast and its human homolog Tollip [53]. Overexpression of Tollip promotes the clearance of Huntingtin-derived polyQ proteins in human cell lines.

VDAC1 is a mitochondrial target of Parkin-mediated ubiquitination, and is necessary for PINK1/Parkin-directed autophagy of damaged mitochondria [54]. Parkin is recruited to CCCP-treated mitochondria and associates with VDAC. In the VDACs-deficient cells, the recruitment of Parkin to damaged mitochondria and mitophagy are impaired [55]. VDACs are necessary for efficient elimination of damaged mitochondria thorough recruitment of Parkin from the cytosol. However, p62 is recruited to mitochondria by Parkin in VDAC1-independent manner [52].

Recently, elegant work by Youle's group showed that NDP52 and optineurin,

which were previously linked to xenophagy, are the primary receptors for PINK1/Parkin-mediated mitophagy [56]. PINK1 directly activates mitophagy through recruitment of NDP52 and optineurin. In this process, PINK1-generated phospho-ubiquitin may act as mitophagy signal, and ubiquitin binding by NDP52 is essential for mitophagy. Thus, ubiquitin binding might be one of the expected characteristics of mammalian mitophagy receptors.

Caldiolipin is a phospholipid on innner mitochondrial membrane. Some mitophagic stimuli can induce externalization of caldiolipin to the mitochondrial surface [57]. Inhibition of externalization of caldiolipin decreased the mitophagic activity. LC3 contains caldiolipin-binding site to interact with caldiolipin directly. The LC3 deletion mutant of caldiolipin-binding site abolished mitophagy, but could induce non-selective autophagy [57]. These results may indicate that the interaction with caldiolipin is also one of the mitophagic receptor-related characters.

PINK1/Parkin also induces the formation of mitochondria-derived vesicles (MDVs) [11, 58, 59], which bud off mitochondria and are transferred to lysosomes to regulate mitochondrial quality control through a pathway that is distinct from common mitophagic pathway described in this review. The MDV pathway is thought to present the process of microautophagy because MDVs are directly transferred to lysosomes. Oxidative stress from within mitochondria induces MDVs in a PINK1/Parkin-dependent manner [60]. Other molecules involved in the formation of MDVs from mitochondria have not yet been identified [11, 58].

4. NIP3-like protein X (NIX/BNIP3L) and BNIP3

NIX/BNIP3L, which is localized to the outer mitochondrial membrane [61], is identified as a adenovirus E1B-19 K-interacting protein with pro-apoptotic activity

[62]. NIX knockout mice develop anemia with reduced mature erythrocytes, which exhibit mitochondrial retention[14]. NIX is highly induced during the late phase of erythrocyte maturation and is involved in the elimination of mitochondria from reticulocytes; this process is essential for red blood cell maturaion [63]. NIX plays a role as a mitophagy receptor through its N-terminal LIR motif, which interacts with LC3 and GABARAP [63, 64]. Mutants in the LIR motif of NIX abolish its interaction with LC3/GABARAP and show decreased mitophagic activity during reticulocytes maturation.

BNIP3 also contains a LIR motif and functions in hypoxia-induced mitophagy [65-67]. BNIP3-mediated mitophagy in cardiomyocytes is independent of mitochondrial permeability transition pore and pro-cell death activity [14, 43, 65]. Phosphorylation of BNIP3 at Ser17 and Ser24 flanking the LIR motif promotes its interaction with LC3 and GATE-16 to induce mitophagy [68]. The molecular basis of phosphorylation of BNIP3 is still unknown. A small GTPase of the Ras superfamily, Rheb, is recruited to the mitochondrial outer membrane under high oxidative phosphorylation activity, and promote mitophagic activity through interaction with NIX/BNIP3L and LC3-II [69].

NIX and BNIP3 have dual roles in the regulation of mitophagy as well as cell death in the heart. The regulatory mechanism of these two roles is not fully understood, but phosphorylation of BNIP3 at serine residues is one of the candidates [68]. Both NIX and BNIP3 mediate the progression of cardiac diseases through cardiomyocyte death [70-72]. NIX localizes to mitochondria in cell lines and induces cytochrome c release, caspase-3 activation, and cell death. Cardiac-specific overexpression of NIX leads lethal dilated cardiomyopathy through cardiomyocyte apoptosis [71]. Induction of BNIP3 to ventricular myocytes provokes cell death

during hypoxia [70]. Cardiac-specific overexpression of BNIP3 also induces cardiomyocyte apoptosis and progressive cardiac dysfunction. Apoptotic cell death is significantly decreased in BNIP3 knockout myocardium 2 days after ischemia-reperfusion injury [72]. In these studies, mitophagic activity in the heart is not examined, therefore it is not elucidated whether mitophagy contributes to the cardiomyocyte apoptosis and cardiac phenotypes. BNIP3 overexpression in adult cardiomyocytes promotes translocation of Drp1 to mitochondria, and BNIP3-mediated translocation of Parkin to mitochondria and ubiquitination is dependent on Drp1. Translocation of Drp1 and Parkin is required for BNIP3-induced autophagy [73].

5. FUNDC1

FUNDC1 also induces mitophagy in mammalian cells [42]. FUNDC1 is an integral mitochondrial outer-membrane protein, and play an important role as a receptor for hypoxia-induced mitophagy. The interaction between BNIP3 and FUNDC1 during hypoxia-induced mitophagy is not clarified yet, however, there might be cooperation between these pathways in regulating mitophagy induced by hypoxia. FUNDC1 interacts with LC3 via the LIR motif, and mutations in this motif inhibit the interaction between FUNDC1 and LC3, impairing the mitophagic activity. The mitophagic activity induced by FUNDC1 is inhibited through its phosphorylation by Src kinase or casein kinase-2 at Tyr18 in the LIR motif and Ser13, respectively [74]. Upon stimulation with hypoxia, Src kinase is inactivated and FUNDC1 is dephosphorylated, thereby inducing mitophagy. Inhibition of both kinases also activates mitophagy. Bcl-xL, which prevent mitochondrial outer membrane permeabilization and apoptosis, inhibits mitophagy through FUNDC1. Bcl-xL

interacts with PGAM5, a mitochondrial phosphatase, as a direct inhibitor to prevent the dephosphorylation of FUNDC1 at Ser13 [75]. Recent study showed that aged PGAM5-deficient mice show Parkinson's disease-like phenotypes [76]. PGAM5 stabilizes the expression of PINK1 on mitochondria, which induce the recruitment of Parkin and mitophagy. This report may indicate the close relationdhip between PGAM5/FUNDC1 and PINK1/Parkin pathway during mitophagy. ULK1, the yeast ATG1 homolog [77, 78] and Ser/Thr kinase translocates to fragmented mitochondria upon mitophagic induction, interacts with FUNDC1 and phosphorylates FUNDC1 at Ser17. The phosphorylation of Ser17 enhances FUNDC1 binding to LC3, inducing mitophagy [79]. MicroRNA-137 (*miR-137*), which is mainly expressed in the brain and regulates neural stem cell determination [80], is hypoxia-responsive microRNAs that targets two mitophagic receptors, NIX and FUNDC1, and significantly inhibits hypoxia-induced mitophagy [81]. Thus, these studies suggest that the level of mitophagic receptor expression can regulate the mitophagic activity induced by hypoxic conditions, and that there exists an interaction or a cooperative mechanism between these two mitophagic pathways. However, FUNDC1 is downregulated during exposure to hypoxic stimuli [42]. The mechanism through which FUNDC1 expression is regulated has not yet been elucidated.

6. Smad-ubiquitin regulatory factor 1 (SMURF1)-meditated autophagy

Orvedahl et al. identified SMURF1 as a selective autophagy factor using genome-wide siRNA screening. SMURF1 is a HECT-domain ubiquitin ligase that targets several cytoplasmic proteins [82] and is recruited to damaged mitochondria, where it promotes mitophagy [83]. SMURF1-deficient cells show diffuse accumulation of fragmented mitochondria. Furthermore, SMURF1-deficient mice

accumulate abnormal mitochondria, which are swollen or fragmented, and contained abnormal cristae structure, in the heart, brain, and liver. However, the function of SMURF1 as a mitophagic receptor on mitochondria is unknown. The interaction between SMURF1 and LC3 has not yet been elucidated.

7. BCL2L13/Bcl-rambo is a novel mitophagy receptor in mammalian cells

As previously described, Atg32 is essential for mitophagy through its interaction with Atg8 and Atg11. Atg32 has a single transmembrane domain anchored to the outer mitochondrial membrane, and contains a WXXI motif binding to Atg8. PINK1 /Parkin mediates mitophagy as described elsewhere. Parkin is expressed in most adult tissues; however, some fetal tissues and cell lines, such as HeLa cells, show little or no endogenous expression of Parkin [44, 84, 85]. Furthermore, Parkin-deficient mice show mild phenotypes, which exhibit grossly normal brain morphology [86], indicating that there may be unknown mechanisms of mitophagy in mammalian cells other than the PINK1/Parkin axis.

Using the molecular profile of Atg32 as a search tool, including mitochondrial localization, WXXL/I motifs, acidic amino acid clusters, and single membrane-spanning topology, we identified BCL2L13 (also known as Bcl-rambo) as a novel mitophagy receptor by screening the UniProt database [41]. BCL2L13 is a protein having 434 amino acids with a C-terminal single transmembrane domain and four conserved BCL2 homology domains (BH1-4) [87]. BCL2L13 is ubiquitously expressed [87] and localized in the outer mitochondrial membrane, and the N-terminus of BCL2L13 is exposed to the cytosol. Previous reports have revealed the pro-apoptotic function of BCL2L13 through its membrane-anchored C-terminal domain, independent of the BH domains. However, BCL2L13 does not induce

apoptosis in HEK293 cells, even when overexpressed. Analysis of the sequence of mouse BCL2L13 revealed two WXXL/I motifs at positions 147-150 and 273-276. A yeast two-hybrid assay and glutathione S-transferase affinity isolation assay have shown that BCL2L13 can bind to LC3. Mutants of BCL2L13 in two WXXL/I motif analysis have shown that the second WXXL/I motif at residues 273-276, but not the first WXXL/I motif at residues 147-150, is a functional LIR motif that interacts with LC3.

Surprisingly, overexpression of BCL2L13 induces mitochondrial fragmentation in HEK293 cells, whereas the knockdown of BCL2L13 induces mitochondrial elongation, indicating that endogenous BCL2L13 has an important role in mitochondrial fission. Binding to LC3 via the second LIR motif is not required for BCL2L13-induced fragmentation. However, all BH1-4 domains are involved in mitochondrial fission. Mitochondrial localization of BCL2L13 with the transmembrane domain is also essential for mitochondrial fission. BCL2L13 can induce mitochondrial fission, even in cells in which Drp1, which is a central molecule in mitochondrial fission, is knocked down. However, the molecular mechanisms of mitochondrial fragmentation without Drp1 have not been unraveled.

Western blot analysis to detect LC3 conversion from LC3-I to LC3-II, immunocytochemical analysis to determine co-localization of mitochondria and lysosomes, and ultrastructural analysis by electron microscopy have collectively shown that BCL2L13 induces mitophagy. BCL2L13-induced mitophagy is mediated through its interaction with LC3 via the second LIR motif. BCL2L13 can induce mitophagy in Atg32-deficient yeast, and the second LIR motif is essential for BCL2L13-induced mitophagy in yeast, suggesting that BCL2L13 is a functional homolog of Atg32. BCL2L13 is the first molecule that has been shown to be able to

rescue mitophagy in the context of Atg32 deficiency. BCL2L13 fails to induce mitophagy in Atg7-deficient yeast, indicating that BCL2L13-induced mitophagy is mediated through common autophagy machinery. The physiological significance of BCL2L13 has been elucidated in BCL2L13-knockdown experiments. Carbonyl cyanide m-chlorophenyl hydrazine (CCCP) induces loss of mitochondrial membrane potential, leading to mitophagy. The protein expression level of BCL2L13 is increased after the addition of CCCP. Knockdown of endogenous BCL2L13 attenuates CCCP-induced mitochondrial fragmentation and mitophagy. This indicates an important role of BCL2L13 during CCCP-induced mitochondrial fission and mitochondrial elimination by mitophagy. Moreover, phosphorylation of Ser114 of Atg32, which is close to the Atg8-family interacting motif, is an important step mediating the interaction between Atg32 and Atg11 [36]. Similarly, BCL2L13 is also phosphorylated at Ser272, which is close to the second LIR motif (residues 273-276). The BCL2L13S272A mutant induces mitochondrial fragmentation, but also decreases the interaction with LC3 and the mitophagic activity of the cells, as assessed by western blotting of LC3. Thus, while phosphorylation of BCL2L13 may regulate its mitophagic activity, the kinase for BCL2L13 is unknown. Prosurvival Bcl-2 family proteins, such as Bcl-2, Bcl-xL and Mcl-1, antagonize Parkin/PINK1-dependent mitophagy in a Beclin-1-independent manner [88]. Translocation of Parkin to damaged mitochondria is decreased by prosurvival Bcl-2 family proteins, and increased by BH3-only proteins. BCL2L13 can induce mitophagy in Parkin-deficient cells [41], and the crosstalk between BCL2L13 and Parkin during mitophagic process is not known.

The molecular mechanisms of BCL2L13-induced mitochondrial fission and mitophagy are not entirely clear. We hypothesize that BCL2L13 on the outer

mitochondrial membrane recruits LC3 to the surface of the mitochondria via LIR-mediated interactions. Although the specific components of the complex involved in mitophagy have not been identified, BCL2L13 may bind to an unidentified mammalian homolog of Atg11 as a scaffold protein, similar to the mechanism observed in yeast. In yeast, Atg11 can interact with Dnm1 [27] to induce mitochondrial fission to be manageable for mitophagy. No mamalian homolog of Atg11 has been identified to date [89], and whether BCL2L13 can induce mitophagy, even in Atg32 and Atg11 double-deficient yeast, is still unclear. In other words, it is not clear whether BCL2L13 has dual functions of Atg32 and Atg11 in mitophagy. Interestingly, the dual roles of BCL2L13 in mitochondrial fission and mitophagy, facilitated by binding with LC3, are expected to contribute to effective cooperation of the two processes. BCL2L13 contains a C-terminal intermembrane space domain, suggesting that BCL2L13 can interact with other mitochondrial proteins. The regulation of the receptor function of BCL2L13, allowing it to sense changes in the integrity and status of the mitochondria, such as the loss of mitochondrial membrane potential, may be mediated through the interaction with such factors. It has been reported that BCL2L13 can bind to adenine nucleotide translocator (ANT) and suppress its ADP-ATP-dependent translocation activity to induce mitochondrial permeability transition [90]. This interaction may one of the regulatory mechanisms of BCL2L13, however, much more work is required to clarify the molecular mechanisms and functions of BCL2L13. For example, it is not clear how the BH domains of BCL2L13 regulate mitochondrial fission. BCL2L13 does not interact with BCL2 family proteins, and the conserved BH domains of BCL2L13 are not essential for the induction of cell death [87]. Identification of novel binding proteins for the BH domains of BCL2L13 may shed light on the molecular mechanism

underlying mitochondrial fission. In Drp1-knockdown HEK293 cells overexpressing BCL2L13, elongated mitochondria persist, and the levels of fragmented mitochondria are reduced, suggesting that there may ba an interaction between these two pathways. Interactions between BCL2L13 and other know factors involved in mitochondrial fission or fusion must be examined. Moreover, the kinase(s) or phosphatas(s) regulating phosphorylation at Ser272 of BCL2L13 should be defined. These enzymes may sense mitochondrial membrane potential. Finally, it is important to examine whether BCL2L13 may have physiological roles in vivo. To date, the involvement of BCL2L13 in mitophagy in the heart at baseline and pathophysiological role to induce cardiac diseases are not known. Analysis of genetically modified animal models, such as BCL2L13-knockout mice, will provide fundamental information about the in vivo role of this protein in mitochondrial quality control under physiological and pathological conditions not only in the heart but in other tissues.

8. Conclusions and perspectives

Cardiomyocytes must have many mitochondria in order to generate sufficient amounts of ATP for contraction. Moreover, cardiomyocytes are terminally differentiated cells that cannot divide to dilute toxic factors produced by mitochondria, such as ROS. Therefore, mitochondrial quality is important, and so mitochondrial quality control mechanisms such as mitophagy are candidate for pathophisiological processes. Some mitophagic receptors have been identified in mammalian cells as described; however, the full details of the mechanisms underlying mitophagy have not been elucidated. Thus, further studies are needed to identify novel targets associated with mitophagy for the treatment of cardiovascular

diseases.

Acknowledgments

We appreciate all members we have worked with for their cooperation. This work was supported by JSPS KAKENHI Grant Number 15K09140.

Disclosures

The authors declare no competing financial interests.

Figure Legends

Figure 1. Summary scheme indicates the molecular mechanisms for mitophagy of Atg32 in yeast and BCL2L13 in mammalian cells as a functional homolog of Atg32. The mitophagic activities of Atg32 and BCL2L13 are regulated by phosphorylation at serine. The homolog of scaffold protein Atg11 has not been identified in mammalian cells.

Figure 2. The schema indicates the molecular mechanisms for mitophagy mediated by molecules on mitochondria. NIX, BIP3, and FUNDC1 interact with LC3 via LIR motif to induce mitophagy. FUNDC1 and BNIP3-dependent mitophagy is induced by hypoxia. The externalization of caldiolipin from inner mitochondrial membrane to outer mitochondrial membrane induces mitophagy by binding to LC3 directly via caldiolipin-binding site. The interaction between LC3 and SMURF1 is not elucidated.

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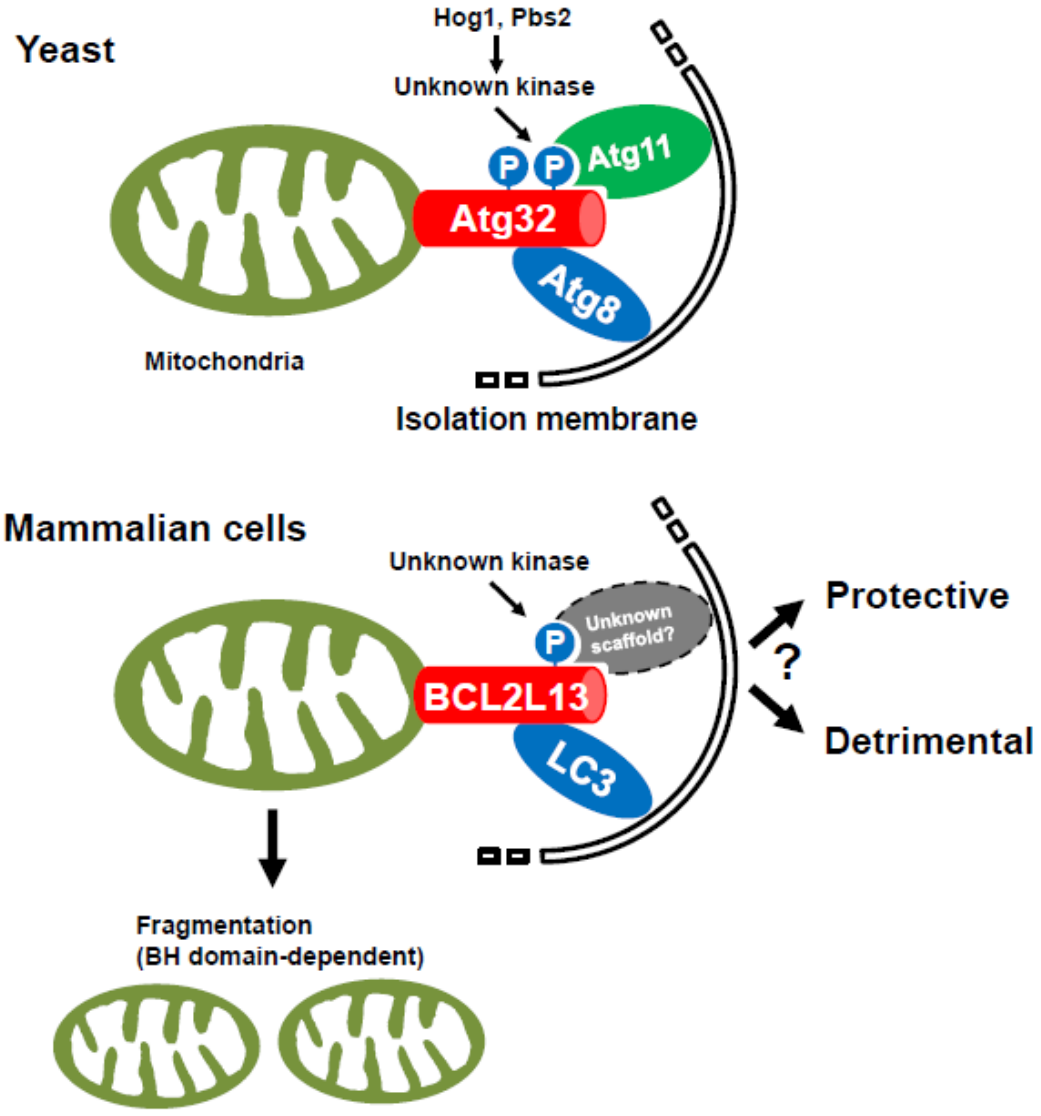

Figure 1

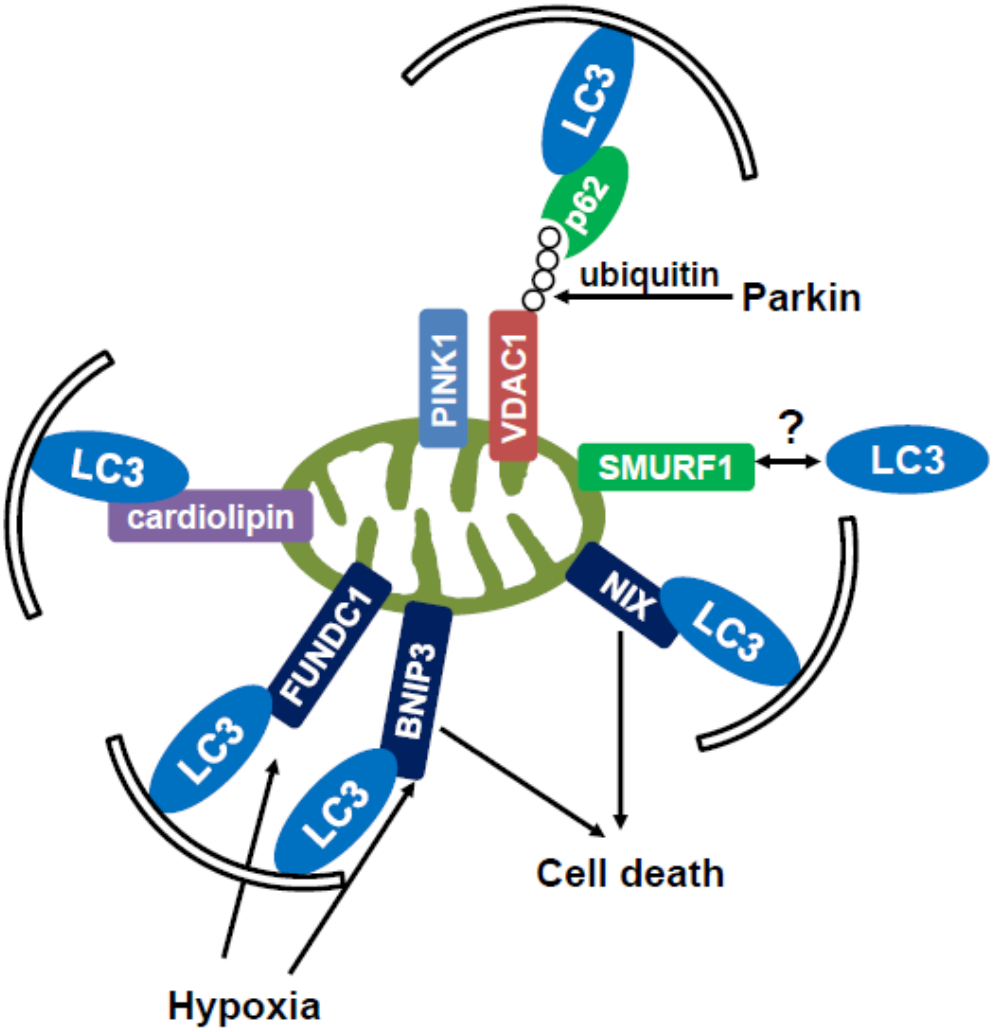

Figure 2

Highlights

1) Mitochondrial elimination is mediated through mitophagy.
2) Mitophagy is important for maintaining cellular and organ homeostasis.
3) Receptors on the outer mitochondrial membrane play an important role in mitophagy.
4) BCL2L13 has roles both in mitochondrial fission and as a mitophagy receptor.