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Review article

Receptor-mediated mitophagy

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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.

Contents

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

Abbreviations: AAA, ATPases associated with diverse cellular activities; ADP, adenosine diphosphate; ANT, adenine nucleotide translocator; Atg, autophagy related; ATP, adenosine triphosphate; BNIP3, adenovirus E1B-19 kDa-interacting protein 3; BNIP3L, adenovirus E1B-19 kDa-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.

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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]. These 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 (Fig. 1).

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] (Fig. 2).

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