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Review

Molecular machinery of macroautophagy and its deregulation in diseases

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ABSTRACT

Macroautophagy maintains cellular homeostasis through targeting cytoplasmic contents and organelles into autophagosomes for degradation. This process begins with the assembly of protein complexes on isolation membrane to initiate the formation of autophagosome, followed by its nucleation, elongation and maturation. Fusion of autophagosomes with lysosomes then leads to degradation of the cargo. In the past decade, significant advances have been made on the identification of molecular players that are implicated in various stages of macroautophagy. Post-translational modifications of macroautophagy regulators have also been demonstrated to be critical for the selective targeting of cytoplasmic contents into autophagosomes. In addition, recent demonstration of distinct macroautophagy regulators has led to the identification of different subtypes of macroautophagy. Since deregulation of macroautophagy is implicated in diseases including neurodegenerative disorders, cancers and inflammatory disorders, understanding the molecular machinery of macroautophagy is crucial for elucidating the mechanisms by which macroautophagy is deregulated in these diseases, thereby revealing new potential therapeutic targets and strategies. Here we summarize current knowledge on the regulation of mammalian macroautophagy machineries and their disease-associated deregulation.

1. Introduction

Autophagy is regarded as a "self-digestion" process, which degrades a cell's own cytoplasmic content through lysosomes, for the maintenance of cellular homeostasis [1]. There are at least three types of autophagy, including chaperone-mediated autophagy (CMA), microautophagy and macroautophagy. They differ in terms of their mechanism for directing the cytoplasmic content to the lysosomes, where the engulfed content is degraded by lysosomal proteases into macromolecules and are released back into the cytoplasm [2,3]. In CMA, recognition of a specific consensus sequence on the targeted proteins by a lysosomal chaperone directs the proteins into the lysosomes; whereas in microautophagy, invagination leads to the direct engulfment of cytoplasmic content by lysosome [3]. In macroautophagy, the cytoplasmic content is enveloped into a double-membraned vesicle called autophagosome, through non-specific encircling of the bulk cytoplasm or a selective process for targeted proteins, organelles, protein aggregates and intracellular pathogens. The autophagosome then fuses with lysosome to form an autolysosome to dispose of its content. Precise regulation of macroautophagy is required to facilitate selective engulfment and degradation when needed, in addition to preventing undesired removal of cytoplasmic contents.

Macroautophagy at its optimal level ensures cell homeostasis, while its deregulation compromises cell survival [4–6]. Macroautophagy deregulation has been demonstrated in many diseases such as neurodegenerative disorders, cancers and inflammatory disorders [3,7,8]. Interestingly, the macroautophagy pathway is deregulated via distinct mechanisms in each disease. In neurodegenerative disorders, impaired macroautophagic engulfment of cytosolic content and mitochondria by autophagosomes are respectively observed in models of Huntington's disease [9] and Parkinson's disease [10–13], whereas autophagosome degradation is defective in Alzheimer's disease models [14]. In inflammatory disorder like cystic fibrosis, the machinery for autophagosome nucleation is mislocalized, which in turn inhibits macroautophagy [15]. Understanding the molecular control of macroautophagy is therefore imperative for dissecting the contribution of macroautophagy deregulation in such diverse diseases. In this review, we focus our discussion on the molecular regulation of mammalian macroautophagy, and discuss how its deregulation is linked to various diseases.

2. Molecular machinery of macroautophagy and its regulation

During the last decade, research has focused on how mammalian macroautophagy is initiated and regulated in the cell [2,16]. Since the macroautophagy machineries in organisms such as yeast, *Caenorhabditis elegans* and *Drosophila* are highly conserved with those in mammals [17–20], researchers have performed molecular and genetic screens in these organisms to identify macroautophagy regulators, leading to the

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subsequent identification of mammalian counterparts of these regulators [21,22]. Macroautophagy involves a battery of molecular players to initiate autophagosome formation, as well as the nucleation, elongation, maturation, and degradation of autophagosomes. Macroautophagy regulators are also modified post-translationally through ubiquitination, phosphorylation and acetylation, which facilitate the delivery of cytoplasmic contents into autophagosomes and provide an additional level of control over macroautophagy. Furthermore, distinct molecular machineries have been identified for selective targeting of certain cytoplasmic contents into autophagosomes, as well as to mediate subtypes of macroautophagy, further highlighting the complexity of macroautophagy regulation in the cell.

2.1. Initiation of autophagosome formation

The origin of autophagosome, as the starting point of macroautophagy, has been characterized as a small crescent-shaped structure called isolation membrane or phagophore. Various intracellular membranes have been suggested as the source of isolation membrane, including the endoplasmic reticulum [23], golgi apparatus [24], mitochondria [25], as well as the plasma membrane [26]. However, the mechanisms underlying the recruitment of these membranes for autophagosome formation remain unclear.

Initiation of macroautophagy involves the assembly of ULK protein complex comprising ULK1, Atg13, FIP200 and Atg101 at the isolation membranes, where this complex works with other autophagy-related gene (Atg) proteins to initiate autophagosome formation [27] (Fig. 1). The assembly of ULK complex is facilitated by a Ras-like small G protein RalB and the exocyst complex [28], and its activation requires dissociation from the negative regulator mTOR complex 1 (mTORC1) [29] during cellular stress such as starvation. Alternatively, macroautophagy can also be induced via mTOR-independent mechanisms, such as through altering the transcription of macroautophagy genes or reducing the cellular level of inositol 1,4,5-trisphosphate (IP_3) [30,31]. For example, macroautophagy is induced when IP_3 production is reduced by treatment with IP_3 receptor antagonists [32], pharmacological inhibition of inositol monophosphatase activity or inositol synthesis [33], as well as by lowering cyclic adenosine monophosphate (cAMP) level [30]. Interestingly, the reduced IP_3 level further decreases intracytosolic calcium ion concentration and calpain activity to lower the cAMP level, resulting in a feedback loop for stimulating macroautophagy [30,31]. It will be interesting to further elucidate the downstream signaling mechanism by which IP_3 inhibits macroautophagy.

Atg5 and Atg12 are also key macroautophagy regulators present on the isolation membranes [34]. Interaction of Atg5 with Atg16L1 at the isolation membranes facilitates autophagosome formation, as revealed by the observation that forced localization of Atg16L1 to the plasma membrane promotes on-site autophagosome formation [35,36]. Proteins such as phosphoinositide 3-phosphatase Jumpy and WIPI-1 are also localized to the isolation membranes [37], and further studies will elucidate how these proteins are implicated in the initiation of autophagosome formation.

2.2. Autophagosome nucleation and elongation

Autophagosome formation proceeds with nucleation and elongation of the isolation membranes to generate vesicular structures [2,38] (Fig. 1). Nucleation of isolation membranes requires the formation of a large protein complex, known as Beclin 1/Class III phosphatidylinositol-3-kinase (PI3K) complex, coordinated by the interactions of several proteins including Beclin 1, UV irradiation resistance-associated tumor suppressor gene (UVRAG), Atg14, B-cell leukemia/lymphoma-2 (Bcl-2), p150, ambra1, endophilin B1, and PI3K Vacuolar protein sorting 34 (Vps34), which then activates PI3K to produce phosphatidylinositol-3-phosphate [2,31]. Beclin 1/PI3K complex formation and PI3K activity are inhibited when Beclin 1 is bound to Bcl-2 [39], but are stimulated upon UVRAG recruitment to the complex [40]. Ambra1 also directly binds Beclin 1 to regulate Beclin 1/PI3K complex formation [41]. Interestingly, Atg14 and UVRAG are located in the Beclin 1-PI3K complex in a mutually exclusive manner [42] and Atg14 competes with UVRAG for Beclin 1 to promote autophagosome formation [43]. These findings suggest a coordinated role of specific components within the Beclin 1/P13K complex to drive nucleation of isolation membranes. Notably, endophilin B1, also known as Bax-interacting factor 1 (Bif-1) [44] or SH3GLB1 [45], is hitherto the only N-terminal Bin-Amphiphysin-Rvs (N-BAR) domain-containing protein identified in this Beclin 1/PI3K complex, and is believed to generate membrane curvature during vesicle nucleation [46]. The function of endophilin B1 in macroautophagy regulation requires both its N-BAR domain and Src-homology 3 domain, with the latter shown to mediate its interaction with UVRAG to recruit Beclin 1 and facilitate PI3K activation [47]. Endophilin B1 was also reported to have a role in coordinating the intracellular trafficking of another macroautophagy-related membrane protein Atg9 [48]. Additional proteins such as PTEN-induced putative kinase 1 (PINK1) [49], death-associated protein kinase (DAPK) [50], IP_3 receptor [32] and high mobility group box 1 [51] have also been identified as Beclin 1-binding protein and marcoautophagy regulator. How they work with other partners in the Beclin 1/PI3K complex to direct membrane nucleation are to be further examined.

Following the nucleation step, other Atg proteins are recruited to the membrane of the pre-autophagosomes to promote elongation and expansion, and eventually the completion of autophagosome formation [2,38] (Fig. 1). During the elongation and expansion steps, Atg7 and Atg10 facilitate the formation of a covalently-linked Atg5–Atg12 complex [52], which also interacts with Atg16L1 [35]. Together with Atg3, Atg4 and Atg7, the Atg5–Atg12–Atg16L1 complex mediates the conjugation of phosphatidylethanolamine (PE) to the microtubule-associated protein 1 light chain 3 (LC3)-I to form LC3-II, leading to the translocation of LC3 from cytoplasm to the membrane of the pre-autophagosomes [34–36,53]. Once autophagosome formation is completed, the Atg proteins are released back to the cytoplasm by a yet uncharacterized mechanism.

2.3. Autophagosome maturation and degradation

Subsequent to the elongation step, autophagosomes are fused with lysosomes for degradation of their contents, a process known as autophagosome maturation [38,54,55]. Autophagosomes fuse with several types of vesicles from the endosomal/lysosomal pathways including late endosomes and lysosomes [54]. Consistently, autophagosome maturation and degradation require the action of late endosome marker protein Rab7 and lysosomal membrane protein LAMP-2 [56,57]. The molecular mechanisms governing autophagosome maturation remain unclear, but recent studies have identified new regulators of autophagosome maturation and degradation such as UVRAG [58], Rubicon [59,60], presenilin-1 [14], valosin containing protein (VCP) [61], and syntaxin-5 SNARE complex proteins [62]. UVRAG interacts with the class C vacuolar protein sorting complex, a key machinery for endosomal fusion, and enhances Rab7 activity for promoting autophagosome fusion with late endosomes and lysosomes [58]. Interestingly, interaction of UVRAG with Rubicon negatively regulates autophagosome maturation [59,60]. On the other hand, inhibition of presenilin-1 [14], VCP [61] or syntaxin-5 SNARE complex [62] impairs lysosomal degradation of autophagosomes. Knowing how these molecules function and work together would provide a clearer picture of the maturation and degradation process.

2.4. Post-translational control of macroautophagy regulators

Regulation of macroautophagy further involves post-translational modifications of macroautophagy-related proteins (Fig. 1). In particular, phosphorylation often modulates the function of macroautophagy regulators that are implicated in the initiation of macroautophagy and