Review

At the end of the autophagic road: an emerging understanding of lysosomal functions in autophagy

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In the past decade, autophagy studies have largely focused on the early stage of autophagy: the molecular mechanisms leading to autophagosome formation. Recently, however, we have observed significant progress in understanding the role of lysosomes, the specific cellular organelle that degrades cellular components delivered via autophagy. The discoveries include connections between autophagy and lysosomal biogenesis, activation, reformation, and turnover, as well as the identification of an autophagosomal SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein in control of autophagosome–lysosome fusion. We illustrate these findings in the context of the underlying molecular mechanisms and the relevance to human health and disease.

Lysosomes and autophagy

Autophagy is an evolutionarily conserved process in which cellular proteins and organelles are engulfed by autophagosomes and eventually delivered to lysosomes for degradation [1–3]. In mammalian cells, there are three forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Among them, macroautophagy (referred to hereafter as autophagy in this review) is the most well studied and consists of two consecutive stages: the early stage is characterised by the formation of autophagosomes, which starts with the formation of a phagophore, or isolation membrane, followed by nucleation and elongation; whereas the late stage of autophagy, also referred to as the maturation or degradation stage, involves fusion between autophagosomes and endosomes–lysosomes, leading to the formation of amphisomes, in which the contents of the autophagosomes are degraded by the lysosomal hydrolases. By contrast, in microautophagy and CMA, the targeted cellular components are delivered to lysosomes independent of autophagosomes [4,5].

The lysosome, first discovered by the Nobel laureate Christian de Duve in the 1950s, is the major digestive organelle present in almost all eukaryotic cells. The lysosome is the terminal component of the endocytic pathway, which possesses a series of biological functions including endocytosis, exocytosis, macropinocytosis, plasma membrane repair, defence against pathogens, cell death, signal transduction, and autophagy [6–9]. The most important biochemical feature of the lysosome is its acidic lumen (pH 4.5–5.0), which contains more than 50 acid hydrolases, including proteases, peptidases, phosphatases, nucleases, glycosidases, sulfatases, and lipases designated for all types of macromolecules [10]. The acidification of the lysosome is maintained by the lysosomal membrane, which contains more than 20 lysosomal membrane proteins, such as lysosome-associated membrane protein (LAMP)1 and 2, and more importantly, the vacuolar-type H(+)-ATPases (V-ATPases) [8,11].

In the past decade, studies on the molecular mechanisms controlling autophagy have largely focused on the early stage of autophagy, because most of the autophagy-related genes (ATGs) identified so far work in the processes leading to the formation of autophagosomes. Relatively, the molecular mechanisms governing the function of lysosomes in the autophagic process are much less well studied. Recently, we have observed significant progress in understanding the role of lysosomes in autophagy. In this review, we summarise the main advances in understanding the involvement of the lysosome in autophagy, focusing on: (i) transcription factor (TF)EB-mediated lysosome biogenesis in autophagy; (ii) the autophagic lysosomal reformation (ALR); (iii) turnover of damaged lysosomes by autophagy (lysophagy); (iv) identification of novel autophagosomal SNARE protein that mediate autophagosome–lysosome fusion; and (v) activation of lysosomal function in the course of autophagy (Figure 1). Here, we illustrate the importance of such findings in the context of the molecular mechanisms, mainly in mammalian cells, and the relevance to human health and disease. Understanding the regulatory mechanisms of lysosomal function in autophagy opens up a new horizon in autophagy study and will eventually provide new opportunities for

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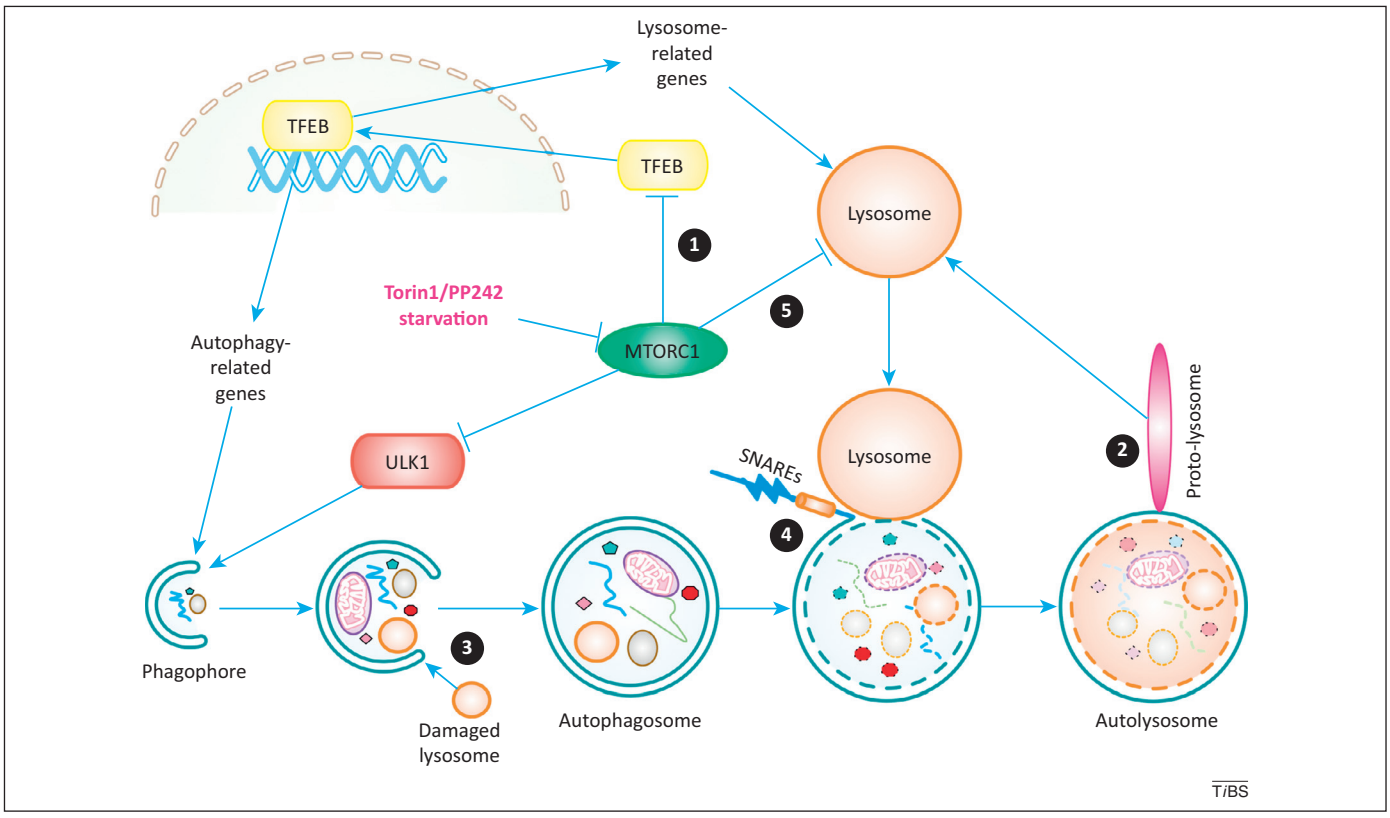

Figure 1. Regulation of lysosomes in the course of autophagy. 1 TFEB-mediated lysosomal biogenesis initiated by suppression of MTORC1, 2 autophagic lysosomal reformation, 3 turnover of damaged lysosomes by autophagy (lysophagy), 4 autophagosome–lysosome fusion mediated by a newly identified autophagosome-specific SNARE protein Stx17, and 5 activation of lysosomal function following MTORC1 suppression and autophagosome–lysosome fusion. Abbreviations: MTORC1, mechanistic target of rapamycin complex 1; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; Stx, syntaxin; TFEB, transcription factor EB; ULK1, UNC51-like kinase 1.

development of novel interventional strategies for autophagy–lysosome-related diseases.

Lysosome biogenesis mediated by TFEB in the course of autophagy

One significant development in recent years is the discovery of a specific gene network, named coordinated lysosomal expression and regulation (CLEAR) [12]. Among this network, the basic helix–loop–helix leucine zipper TFEB is considered to be a master regulator of lysosomal biogenesis, function and autophagy, via transcriptional control of gene expression. It coordinates the cellular responses to various stresses, including nutrient starvation, metabolic stress, and lysosomal stress, to maintain cellular homeostasis [9,13].

Whether the autophagic process requires *de novo* protein synthesis has been an unresolved question on which the recent TFEB data shed light. In resting cells, TFEB is sequestered in the cytoplasm, and upon activation, TFEB translocates to the nucleus and binds to the CLEAR consensus sequence to activate *de novo* gene transcription. So far, more than 400 direct TFEB target genes have been identified [12,14]. Among them, many genes are directly related to the lysosome and autophagy (Figure 2), including lysosomal hydrolases and accessory proteins (23 genes), lysosomal membrane proteins (nine genes), lysosomal V-ATPase pumps (14 genes), lysosomal biogenesis regulators (10 genes) and autophagy regulators (17 genes) [14]. The discovery of TFEB as a positive regulator of autophagy strengthens the argument that autophagy requires *de novo* gene transcription and protein synthesis, at least for sustained autophagy. In addition, the regulatory function of TFEB in autophagy and lysosomal function is evolutionally well conserved, from *Caenorhabditis elegans* to *Drosophila* and mammalian cells [15–17].

In understanding the molecular mechanisms governing the function of TFEB, the mechanistic target of rapamycin complex (MTORC)1 has emerged as the key negative regulator, thus forming a critical signalling axis linking TFEB, the lysosome, and control of autophagy. Interestingly, only starvation or the catalytic inhibitors of MTOR (Torin1 and PP242), but not an allosteric inhibitor of MTOR (rapamycin), were found to be effective in causing TFEB activation and enhancing lysosomal function [18–21]. Rapamycin is known to be incapable of fully blocking MTORC1 function [22,23], thus, it is believed that the inhibitory effect of MTORC1 on TFEB is mediated via the rapamycin-resistant component of MTORC1. At present, the lysosome is well established as a key player in the amino-acid-mediated MTORC1 signalling pathway. In the presence of amino acids, MTORC1 localises to the lysosomal cytoplasmic surface via Rag GTPases and interacts with a protein complex at the lysosome called Ragulator, which is required for MTORC1 activation [24,25]. However, the lysosome is not merely a docking station for the MTORC1 signalling complex: the lysosomal V–ATPase complex is required for sensing the amino acids inside the lysosomal lumen, which is required for MTORC1 activation [26]. Therefore, it appears