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Review article

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Autophagy and autophagy-related pathways in cancer

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Supplementary Box 1: LC3 conjugation as a mediator of autophagy-related processes

LC3-associated processes are defined here as those requiring LC3 lipidation on pre-formed single membranes in the absence of de novo formation of autophagosomes. Similar to autophagy, this form of LC3 lipidation invariably requires the conjugation machinery, including ATG3, ATG4, ATG7, ATG5–ATG12, and ATG16L1 (Ref.[1]). Interestingly, specific domains within the C-terminus of ATG16L1, namely the WD40 domain[2] and C-terminal lipid binding domain (VRV motif)[3], are required for conjugation of ATG8 to single membranes (CASM) but not autophagosome formation. In addition, all types of CASM occur independently of ULK complex components (including ULK1, FIP200, and ATG13) and the autophagosomal VPS34 complex component, ATG14 (Ref.[1,4]). Additional requirements for upstream components depend on the specific CASM cargo or inducer. In the case of LC3-associated phagocytosis (LAP) induced by *Aspergillus fumigatus* infection, a Rubicon-containing VPS34 complex (including Beclin-1 and VPS15) as well as NAPDH oxidase 2 (NOX2) are required[1]. Rubicon was shown to suppress autophagy while its role in regulating the endolysosomal system remains unclear[5,6]. The specific requirements of protein complexes for different CASM processes are summarised in the table below. The ultimate consequences of these LC3-related processes are variable. During LAP, LC3 lipidation facilitates the recruitment of lysosomal hydrolases resulting in the clearance of immunogenic signals thereby suppressing pro-inflammatory signals[6]. CASM also has non-degradative functions whereby LC3-associated endocytosis (LANDO) affect surface receptor recycling[7], while LC3-dependent EV loading and secretion (LDELS) is required for specific cargo loading on EVs[8]. The relevance of LAP-like LC3 conjugation on endosomal vesicles induced by treatment with drugs (such as chloroquine, CQ) remains unclear[9].

Requirement of protein complexes in autophagy and LC3-associated processes.

Protein/complex	**Autophagy**	**LAP**	**LDELS**	**LANDO**	**Drug induced LAP-like**
Requirement of protein complexes					
ULK complex	✓	X	X	X	X
ATG14-containing VPS34 complex	✓	X	X	X	X
Rubicon-containing VPS34 complex	X	✓	NR	✓	X
ATG4	✓	✓	NR	✓	NR
Conjugation machinery	✓	✓	✓	✓	✓
ATG16L1 WD40	X	✓	NR	NR	✓
ATG16L1 VRV	X	NR	NR	NR	✓
Consequence of activation					
Overall outcome of	Cargo	Clearance of	Specific loading of	Recycling of cell	NR

pathway activation	degradation and recycling	immunogenic fragments	cargo on EVs	receptors	
References	10	6	8	7	9

Table footnotes: NR, not reported; LAP, LC3-associated phagocytosis; LANDO, LC3-associated endocytosis; LDELS, LC3-dependent extracellular vesicle loading and secretion.

Supplementary Box 2: Autophagy Cargo Receptors Mediate Selective Autophagy

Autophagy cargo receptors (ACRs) mediate the selective autophagic degradation of proteins and organelles. In mammalian cells, this growing family of proteins includes p62 (also called SQSTM1 for sequestosome 1), NBR1, OPTN, NDP52, BNIP3, TAX1BP1, and TOLLIP (Ref.[11]). These proteins typically interact with the autophagosomal membrane via an LC3-interacting region (LIR), which mediates binding to LC3 and other ATG8 family proteins. In addition, many ACRs possess a C-terminal ubiquitin binding domain (UBD), which allows these proteins to bind to a large array of ubiquitinated proteins[12]. This has motivated the working hypothesis that ACRs act as a bridge to selectively capture cargo during autophagy via UBD binding to ubiquitinated cargo, which is coupled through a LIR-dependent interaction of the ACR with LC3 on the autophagosomal membrane[11]. In addition to ubiquitin, other post-translational modifications and oligomerization of ACRs are proposed to play important roles in facilititating selective autophagy. For example, the phosphorylation of Ser403 and Ser409 on the p62-UBD enhances its avidity for ubiquitin chains, which facilitates both autophagic cargo recognition and clearance[13,14]. Similarly, phosphorylation of LIR motifs promotes binding affinity for LC3 (Ref.[15]). More recent work has uncovered that ACRs form phase-separated condensates in cells, which requires ubiquitin chains[16,17]. Remarkably, p62 oligomerizes via PB1 domains which, along with ubiquitin binding, may promote the formation of these phase-separated condensates[17]. Moreover, NBR1 facilitates p62-positive condensate formation and the overexpression of NBR1 prevents the autophagic clearance of p62 condensates[18]. How phase separation of ACRs influences oncogenesis and cancer progression, both in the setting of autophagy competence and deficiency, remains an important unanswered question.

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