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REVIEW

Autophagy: for better or for worse

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Autophagy is a lysosomal degradation pathway that degrades damaged or superfluous cell components into basic biomolecules, which are then recycled back into the cytosol. In this respect, autophagy drives a flow of biomolecules in a continuous degradation-regeneration cycle. Autophagy is generally considered a pro-survival mechanism protecting cells under stress or poor nutrient conditions. Current research clearly shows that autophagy fulfills numerous functions in vital biological processes. It is implicated in development, differentiation, innate and adaptive immunity, ageing and cell death. In addition, accumulating evidence demonstrates interesting links between autophagy and several human diseases and tumor development. Therefore, autophagy seems to be an important player in the life and death of cells and organisms. Despite the mounting knowledge about autophagy, the mechanisms through which the autophagic machinery regulates these diverse processes are not entirely understood. In this review, we give a comprehensive overview of the autophagic signaling pathway, its role in general cellular processes and its connection to cell death. In addition, we present a brief overview of the possible contribution of defective autophagic signaling to disease.

***Keywords*:** autophagy; autophagosome; vesicle; degradation; cell death

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Introduction

Cells have mechanisms that clear out superfluous, misfolded, damaged and harmful proteins: the ubiquitin-proteasome degradation pathway and autophagy. In contrast to the proteasome, autophagy has a nearly unlimited degradative capacity. It can target large protein aggregates and entire organelles. It is not restricted to protein degradation but breaks down lipids, DNA and RNA. In this way, autophagy provides new pools of amino acids, fatty acids and nucleosides for anabolic processes and drives a continuous flow of materials in the cell in a degradation-regeneration cycle [1]. The executors of autophagic degradation are the lysosomes, which enclose acidic hydrolases, such as peptidases, lipases and nucleases, which degrade large molecules into their basic units. Although all autophagic routes end up in the lysosomal compartment (vacuole in yeast), many ways are used to reach the lysosomes.

Three types of autophagy mechanisms have been recognized in mammalian cells: chaperone-mediated autophagy, microautophagy and macroautophagy [2] (Figure 1). Chaperone-mediated autophagy sequesters proteins that expose a KFERQ-like motif. Mediated by heat shock cognate 70 and its co-chaperones, these proteins are directly targeted to the lysosomes. Degradation then occurs upon interaction with and internalization through the lysosomal receptor lysosomal-associated membrane protein 2 (LAMP-2A) [3]. During microautophagy, the lysosomal membrane invaginates to engulf portions of the cytoplasm,which are broken down once they are entirely enclosed. Macroautophagy differs from the other types of autophagy because it involves specialized vacuoles for cargo transportation. These vacuoles, called autophagosomes, result from *de novo* synthesis of autophagic membranes (phagophores), which upon closure form vesicles with a double membrane. Macroautophagy is evolutionarily well conserved and occurs in all eukaryotes. Because mouse models only exist

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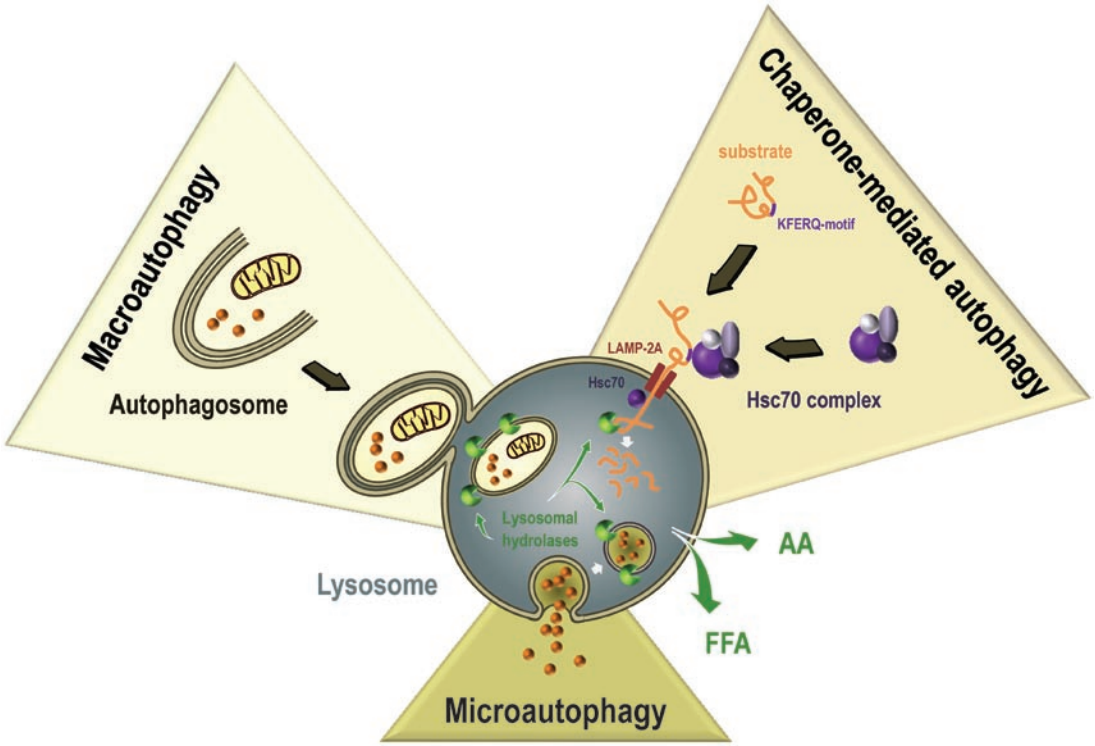

Figure 1 Schematic representation of the different types of autophagy. Chaperone-mediated autophagy sequesters proteins harboring a KFERQ-like motif that, mediated by the Hsc70 complex, are directly targeted to the lysosomes for degradation. During microautophagy the lysosomal membrane invaginates to engulf portions of the cytoplasm, which are consequently broken down once entirely enclosed. During macroautophagy specialized vacuoles are formed for cargo transportation. These vacuoles, called autophagosomes, are double membrane bound and deliver proteins, lipids and organelles to the lysosome.

for macroautophagy so far, extensive research has been dedicated to the understanding of this type of autophagy. This research has brought to light the clear relevance of macroautophagy to human disease. Therefore, in this review, we will focus on macroautophagy, and for the sake of simplicity, we will refer to it as autophagy.

Autophagy is primarily a non-selective, bulk degradation pathway, but the importance of more selective forms of autophagy is becoming increasingly apparent. Mitophagy, pexophagy, reticulophagy, nucleophagy, lipophagy and xenophagy refer to the selective removal of mitochondria, peroxisomes, endoplasmic reticulum (ER), nuclei, lipids and intruding microorganisms, respectively. Moreover, autophagy can sequester selective protein targets, such as ubiquitinated protein aggregates or key effectors of important signaling pathways [4-6]. The importance of autophagic signaling to homeostasis has been shown by the study of autophagy-defective systems. Autophagy primarily fulfills a pro-survival role during adaptation to unfavorable growth conditions or following cellular stress. Accumulating data also demonstrate its involvement in general processes such as development, differentiation, immune homeostasis, defense against pathogens, ageing and cell death. Therefore, interest in autophagy has experienced exponential growth during the last decade. Yet many questions concerning its specific role in these diverse cellular and (patho)physiological processes remain unanswered, and our knowledge about its molecular signaling is far from complete.

Molecular signaling of autophagy

Autophagy induction is tightly controlled by complex regulatory mechanisms involving diverse input signals, including nutrients, growth factors, hormones, intracellular Ca^{2+}-concentrations, adenosine triphosphate (ATP) levels, hypoxia, accumulation of misfolded proteins and many more (Figure 2). Many signals converge at the level of the mammalian target of rapamycin complex 1 (mTORC1). mTORC1 consists of mTOR, regulatory associated protein of mTOR (raptor), DEP-domain-containing mTOR-interacting protein (Deptor), proline-

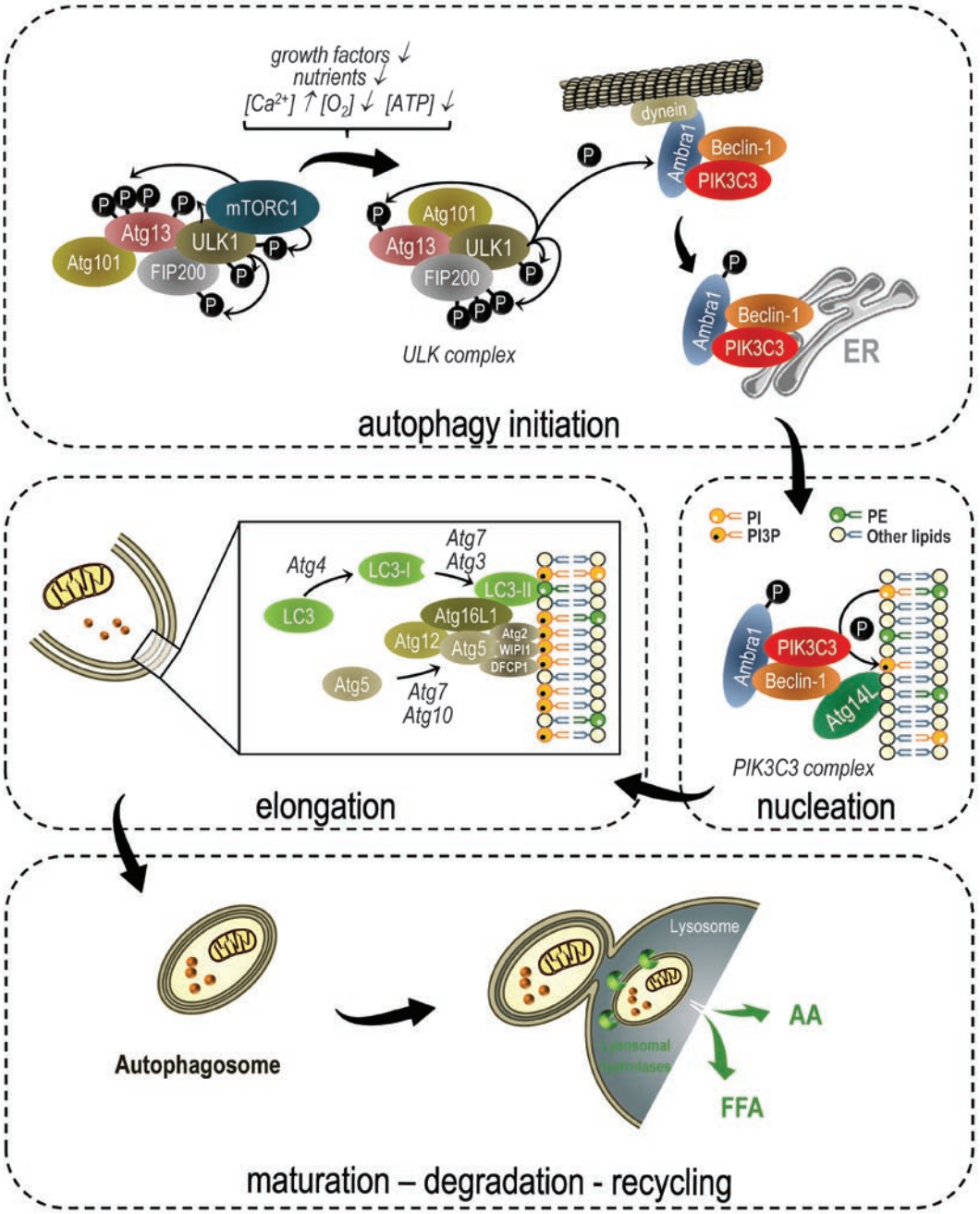

Figure 2 Molecular signaling of macroautophagy. During cellular and metabolic stress the mTOR is inactivated, which allows ULK complex activation. ULK complex activation involves ULK1-dependent phosphorylation of Atg13, FIP200 and ULK1 itself. These phosphorylation events are required for autophagy initiation. ULK1 also phosphorylates Ambra1, which interacts with the PIK3C3 complex at the microtubule. Phosphorylation of Ambra1 results in the release of Ambra1 and the PIK3C3 complex from the microtubule and their translocation to the ER, the major site of autophagosome formation. During autophagosome nucleation, the PIK3C3 complex generates PI3P, which interacts with DFCP1, Atg2 and WIPI1 and recruits other Atg proteins involved in membrane elongation. To this end, two ubiquitin-like processes are carried out. Atg7 and Atg10 mediate Atg5-Atg12 complex formation. This Atg5-Atg12 complex subsequently binds to Atg16L1, generating the Atg16L1 complex. LC3 is cleaved by the cysteine protease, Atg4, to LC3-I. Mediated by Atg7, Atg10 and the Atg16L1 complex, LC3-I is conjugated to phosphatidylethanolamine (PE), generating LC3-II. After the completion of autophagosome formation, the outer autophagosomal membrane fuses with the lysosome, releasing the autophagic cargo into the lysosomal lumen. The inner autophagosomal membrane and its content become degraded and the resulting amino acids (AA), free fatty acids (FFA) etc. are released back into the cytosol. Figures were produced using Servier Medical Art (http://www.servier.com).

rich AKT substrate 40 kDa (PRAS40) and G-protein β-subunit-like protein (GβL) [7]. mTORC1 regulates a variety of cellular responses, such as cell growth, proliferation, protein synthesis and autophagy. When amino acids and growth factors are present, class I phosphatidylinositol-3-kinase (PIK3C1) activates mTORC1, which suppresses autophagic signaling. Active mTORC1 inhibits autophagy by binding and phosphorylating uncoordinated-51 (unc-51)-like kinase 1 or 2 (ULK1 or ULK2) and Atg13 within the ULK complex [8-10]. This complex is composed of ULK1 or ULK2, Atg13, focal adhesion kinase family interacting protein of 200 kDa (FIP200), and Atg101 [10-12]. Consequently, repression of mTORC1 by nutrient deprivation or rapamycin treatment is commonly used to activate autophagy. When mTORC1 is inactivated, it dissociates from the ULK complex, promoting ULK activity and FIP200 hyperphosphorylation [10]. The exact role of the ULK complex has long been elusive. However, recent data demonstrate its involvement in the proper localization of another crucial autophagy-inducing complex, the phosphatidylinositol-3-kinase class-III (PIK3C3) complex [13]. In nutrient-rich conditions, the PIK3C3 complex connects to the cytoskeleton. This interaction is mediated by the activating molecule in Beclin-1-regulated autophagy 1 (Ambra1), which binds both the PIK3C3 complex and the microtubule-associated dynein motor complex [13]. During starvation, ULK1 phosphorylates Ambra1, which releases Ambra1 and the PIK3C3 complex from the microtubules and enables the PIK3C3 complex to relocalize to the ER, which is regarded as the major organelle contributing to autophagosome formation [13]. Also in yeast, the Atg1 (ortholog of ULK1) complex is implicated in regulating the localization of the vacuolar protein sorting 34 (homolog of PIK3C3) complex, as Atg13 targets the latter to the site of autophagosome formation, called the phagophore assembly site (PAS) or pre-autophagosomal structure [14]. In addition, ULK1 has an important role in regulating the trafficking of mAtg9 (Atg9), a multispanning transmembrane protein that cycles between the trans Golgi network (TGN) and endosomal compartment in basal conditions [15]. During starvation, mAtg9 is recruited to the growing autophagosome, where it might supply the lipids needed for membrane elongation [16]. This mAtg9 trafficking was found to involve ULK1-mediated activation of the myosin II motor protein [17].

Following autophagy induction, autophagosomes are formed. Autophagosome biosynthesis can be divided into three main processes: vesicle nucleation, elongation and maturation (Figure 2) [18]. PIK3C3, p150, Ambra1 and Bcl-2-interacting protein (Beclin-1) form the core of the PIK3C3 complex, which can further bind either UV-radiation resistance associated gene (UVRAG) or Atg14L [19]. Within this complex, Beclin-1 (Atg6) constitutes a platform for the binding of several interactors regulating the kinase activity of PIK3C3 [20]. PIK3C3 generates phosphatidyl-inositol-3-phosphate (PI3P), which recruits additional autophagy-related (Atg) proteins to the site of autophagosome formation, a process called autophagosome nucleation. Some of these PI3P-binding autophagic proteins have recently been identified: WD repeat domain phosphoinositide interacting 1 (WIPI1) and 2 (human orthologs of Atg18), mAtg2 (Atg2) and double-FYVE containing protein-1 (DFCP1) [21]. However, further studies are needed to clarify their precise functions in autophagosome biogenesis. Two ubiquitin-like conjugation systems are implicated in autophagosome membrane expansion, shaping and sealing [18]. First, Atg12 is activated by the E1-like enzyme, Atg7. Atg12 is then transferred to Atg10, an E2-like enzyme, and then covalently linked to Atg5. This Atg12-Atg5 conjugate further interacts with Atg16L1 to form a trimer, which consequently homodimerizes to form a large multimeric complex [22, 23]. This Atg16L1 complex is transiently associated with the outer autophagosomal membrane of the growing autophagosome, and has been suggested to determine its curvature. In addition, the Atg16L1 complex acts as an E3 ligase, allowing the second conjugation reaction to be completed [24]. This second ubiquitin-like conjugation pathway involves LC3 (microtubule-associated light chain-3) lipidation. LC3 (homolog of Atg8) is cleaved by Atg4, a cysteine protease, and this exposes a glycine at the C-terminal end [25]. Following the combined action of Atg7, Atg3 (E2-like enzyme) and the Atg16L1 complex (E3-ligase), LC3 is finally conjugated to phosphatidylethanolamine (PE) to produce LC3-PE (also called LC3-II). In contrast to LC3 (or LC3-I), LC3-II specifically localizes to the autophagosomal membranes and so it is suited to serving as an autophagy-specific marker [26]. Upon vesicle completion, Atg4 removes LC3-II from the outer autophagosomal membrane in a process called deconjugation. However, LC3-II remains associated with the inner autophagosomal membrane and thus becomes partially degraded after fusion with the lysosome.

Once autophagosome formation is completed, autophagosomes can fuse with early or late endosomes to become amphisomes. Fusion with early or late endosomes delivers cargo and components of the membrane fusion machinery and lowers the pH before delivery of the lysosomal hydrolases. Autophagosomes can also be directly targeted to the lysosomes along the microtubules [27]. The outer autophagosomal membrane fuses with the lysosomes, releasing the inner autophagosomal membrane and its contents (the autophagic body) into

the lysosomal lumen. Several proteins are involved in these fusion events, including LAMP2, the UVRAG-C-Vps tethering complex, Rab (Ras-related GTP-binding protein), HOPS (homotypic fusion and protein sorting), SNAREs (soluble N-ethylmalemide-sensitive factor attachment protein receptor), AAA ATPases, LC3, FYCO1 and the ESCRT (endosomal sorting complex required for transport) machinery [28]. Also "kiss-and-run" fusions often take place, during which the autophagic cargo is transferred from the autophagosome to the lysosome before both vesicles segregate [29]. Once the autophagic body is taken up by the lysosome, it disintegrates and its cargo is degraded by lysosomal hydrolases and lipases. Subsequently, lysosomal efflux transporters, e.g., Atg22 in yeast (mammalian ortholog not yet identified), mediate the release of the resulting amino acids, fatty acids and nucleosides back into the cytosol [30].

Although much progress has been made in the research on autophagosome biogenesis, several key questions remain on the cellular topology of autophagosome formation and on the origin of the lipids that constitute the autophagic membrane. In yeast, autophagosome formation starts at the PAS, which is located near the vacuole but is still of unknown origin. In mammals, autophagosome formation occurs at dispersed locations in the cytosol. Most studies designate the ER as the main source of autophagic membranes [31]. However, several others also demonstrate the emergence of autophagic vesicles from mitochondria, TGN, plasma membrane and nucleus [16, 31-34]. Whether all these organelles collectively participate in autophagosome formation in the same autophagy process, or whether regulation mechanisms determine which membrane source is used, needs further investigation.

Autophagy in quality control

Although autophagy is especially known for its pro-survival function during metabolic stress, it is constitutively active in virtually all tissues of eukaryotic organisms and exerts an important housekeeping role. At basal levels, autophagy maintains cellular homeostasis and provides an intracellular quality control mechanism. To this end, autophagy targets misfolded proteins and damaged organelles for degradation, preventing the accumulation of aberrant cellular components, which can disturb cellular function. Accordingly, protein aggregates and inclusion bodies are found in autophagy-deficient hepatocytes ($Atg7^{-/-}$), cardiomyocytes ($Atg5^{-/-}$) and neural cells ($Atg5^{-/-}$ or $Atg7^{-/-}$) [35-38]. Moreover, liver-specific Atg7-knockout mice develop hepatomegaly and neuron-specific Atg5 or Atg7 deficient mice suffer from neurodegeneration [35, 37, 38].

One of the key events in the removal of misfolded proteins is ubiquitination [6]. Misfolded proteins are recognized by molecular chaperones of the heat shock protein family and are targeted for ubiquitination by E3-ligases, such as CHIP (carboxyl terminus of the heat shock cognate protein 70-interacting protein) and Parkin [39, 40]. Often these ubiquitinated misfolded proteins polymerize and form protein aggregates and inclusion bodies, which cannot be removed by the proteasome, but are cleared by autophagy. Two ubiquitin-binding proteins, p62/SQSTM1 (sequestosome 1) and NBR1 (neighbor of BRCA1 gene 1), are involved in the formation of these inclusion bodies and have been implicated in the selective removal of aggregation-prone proteins [41, 42]. In addition to an ubiquitin-binding domain, both proteins contain an LC3-interacting region (WxxL), which enables them to target their substrates to the growing autophagosome [42, 43]. Interestingly, when the rate of formation exceeds that of clearance, protein aggregates are delivered to microtubule-organizing center (MTOC)-associated aggresomes, a process requiring histone deacetylase 6 (HDAC6) [44]. HDAC6 binds ubiquitin and directly interacts with dynein motor proteins to transport aggregated proteins to the MTOC along the microtubules [45]. At the MTOC, lysosomes are enriched, which allows for efficient autophagic clearance of protein aggregates.

As mentioned above, autophagy also removes damaged organelles, including mitochondria and ER. For example, loss of mitochondrial potential can lead to the selective degradation of mitochondria in a process called mitophagy [46]. Although the mechanisms involved in selective organelle removal are not well understood, specific autophagy-receptors seem to play a role. In yeast, two mitochondria-associated proteins, Uth1p and Aup1, mark mitochondria for sequestration in autophagosomes [47, 48]. Similarly, in mammals, BNIP3L (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like)/Nix is indispensable for mitochondria elimination during reticulocyte maturation [49]. Recent data also suggest the importance of ubiquitination for organelle removal by autophagy [50]. Parkin, an E3 ubiquitin ligase, was found to localize at depolarized mitochondria, to ubiquitinate outer mitochondrial membrane proteins and to induce mitophagy [51, 52]. Parkin requires PINK1 (PTEN-induced putative kinase 1)-dependent phosphorylation for its recruitment to damaged mitochondria [53]. Interestingly, PINK1 is stabilized by its interaction with Parkin and enhances basal and starvation-induced autophagy through binding with Beclin-1 [54, 55].

Autophagy in development and differentiation

Studies using autophagy-defective yeast, ameba, fungi, worms, flies and mice have demonstrated an important role for autophagy during development [56]. In lower eukaryotes, autophagy is often associated with tissue remodeling or metamorphosis, while in mammals it is mainly activated during pre-implantation development. Unicellular organisms, such as yeast (*S. cerevisiae* and *S. pombe*) and ameba (*D. discoideum*) activate adaptive processes in nutrient poor conditions that result in sporulation or the generation of fruiting bodies, respectively. During these processes, autophagy recycles amino acids needed for *de novo* protein synthesis and increases the organism's chance of survival [57, 58]. Also *C. elegans* uses autophagy to adapt to stressful conditions [59]. In times of nutrient starvation, increased temperature or high population density, *C. elegans* enters dauer diapause, a third larval stage that can survive unfavorable conditions. Several key autophagy proteins, e.g., unc-51 (homolog of yeast Atg1), Atg7, LGG-1 (homolog of Atg8), Atg18 and Bec-1, are required for normal dauer morphogenesis, and their silencing causes various defects [59, 60]. Furthermore, during metamorphosis of *D. melanogaster*, autophagy contributes to degradation of larval midgut and salivary gland cells so that they can be replaced by adult tissues, although the role of autophagy in midgut cell death is debated [61-63]. In addition, autophagy in *D. melanogaster* is involved in synaptic development and regulates neuromuscular junction formation [64].

In mammals, autophagy is strongly activated during the early stages of embryogenesis and seems to be crucial for the pre-implantation development of mouse embryos [65]. After fertilization, autophagy is triggered to enable degradation of maternal mRNAs, proteins and sperm mitochondria in the oocyte. Autophagy-defective oocytes derived from oocyte-specific Atg5-knockout mice fail to develop beyond the four- and eight-cell stages when fertilized by Atg5-null sperm [65]. Furthermore, protein synthesis rates are reduced in autophagy-deficient embryos. How autophagy governs later stages of development is not completely clear. However, the importance of Atg proteins during mammalian development is emphasized by the early embryonic death of mice deficient in Beclin-1, FIP200 or Ambra1 [66-70]. These mice suffer from early developmental abnormalities such as defective pro-amniotic cavity formation, neuronal tube formation and heart and liver development. Yet, many other knock-out mice (Atg3, Atg4C, Atg5, Atg7, Atg9, Atg16L1 and ULK1) do not exhibit severe embryonic defects and survive the entire embryonic period [37, 38, 71-75]. This disparity of phenotypes between different Atg proteins might be explained by the pleiotropy of some autophagic proteins that also have essential non-autophagic functions. Alternatively, redundancy in functions or pathways could compensate for the loss of certain Atg proteins, which is probably the case for ULK1 [75]. Nevertheless, except for ULK1 and Atg4C, all other Atg-knockout mice that are born die within 1 day due to nutrient deficit and energy depletion following disruption of the trans-placental nutrient supply [71, 75, 76].

Autophagy is activated during different stages of embryogenesis, and thereafter it is involved in differentiation of specialized tissues. Many studies were performed using conditional knock-out mice to investigate the consequences of autophagy deficiency on cellular and tissue differentiation. These studies clearly demonstrate the importance of autophagy during differentiation of multiple cell types. Cellular differentiation often requires extensive morphological changes and intracellular remodeling. Maturation of erythrocytes and T-lymphocytes, for example, requires a reduction in mitochondrial content, which is made possible by autophagy [77, 78]. Lack of key autophagy components (e.g., ULK1 or Atg7) results in mitochondrial accumulation and, in the case of Atg7, severely alters blood cell counts [75, 77, 78]. Similarly, autophagy is involved in lipid droplet formation during adipogenesis, the efficiency of which is drastically reduced when autophagy is perturbed, for example by Atg5 or Atg7 deficiency [79-81]. Further, autophagy impairment inhibits neuronal differentiation of neuroblastoma cells and affects glioma stem/progenitor cell differentiation [82, 83].

Autophagy in immunity

Increasing data show that autophagy plays a considerable role in the control of immunity and inflammation. At distinct levels, autophagy contributes to both innate and adaptive immunity as a regulator and effector [84]. One autophagy effector function in innate immunity is xenophagy, which refers to the autophagy-dependent elimination of intracellular pathogens, including bacteria (*S. pyogenes* [85], *S. flexneri* [86], *M. tuberculosis* [87], *S. typhimurium* [88] and *L. monocytogenes* [89]), parasites (*T. gondii* [90]) and viruses (Herpes simplex virus [91]). In this way, autophagy restricts pathogen replication and survival, and inhibits pathogen-induced cell death in both plants and mice [92, 93]. Interestingly, NDP52 (nuclear dot protein 52 kDa) and p62 (also called /SQSTM1), both of which contain LC3- and ubiquitin-binding domains, have been identified as autophagy receptors targeting intracellular ubiquitin-coated pathogens to the

autophagic pathway [94, 95]. The importance of p62 in innate immunity has been emphasized by the recent finding that p62 is a downstream target of innate defense regulator-1, an antimicrobial peptide that suppresses infection and inflammation [96]. Some pathogens, however, have evolved mechanisms to escape autophagy-mediated capture or degradation [84]. The existence of virulence factors that counter autophagy indicates a fundamental role for autophagy in innate immunity. ICP34.5, which is expressed by Herpes simplex virus, effectively inhibits autophagy induction by binding host Beclin-1 [97]. Also, human immunodeficiency virus (HIV) protein Nef interacts with Beclin-1 and thereby inhibits autophagosome maturation to protect HIV from degradation [98]. Other viruses, such as gamma-herpes virus and human cytomegalovirus, possess B-cell lymphoma-2 (Bcl-2)-like proteins or activate the mTOR signaling pathway to render the cells less sensitive to autophagy induction [99-101].

Interestingly, when Toll-like (TLR) and NOD-like (NLR) receptors recognize pathogen-associated molecular patterns, they not only regulate pro-inflammatory cytokine production but can also stimulate autophagy. Following activation of TLR-1, -3, -4, -5, -6 or -7, myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF) recruit Beclin-1 into the TLR signaling complex and thereby reduce the autophagy-inhibiting interaction between Beclin-1 and Bcl-2 [102]. In addition, TNF receptor-associated factor 6 (TRAF6)-mediated ubiquitination of Beclin-1 was shown to be required for TLR4-triggered autophagy in macrophages [103]. The NLRs (NOD1 (nucleotide-binding oligomerization domain-containing protein 1) and NOD2) direct Atg16L1 to the site of bacterial entry at the plasma membrane, a process critical for elimination of bacteria by autophagy [104]. Autophagy is also induced by many immune signals, such as the T helper (Th1) cytokines interferon (IFN)-γ and tumor necrosis factor-α, and by CD40 signaling. In contrast, Th2 cytokines, such as interleukin (IL)-4 and IL-13, inhibit autophagy [105]. Conversely, cytokine production can be regulated by autophagy, as shown by the increased production of potent anti-viral factors upon RIG-I receptor activation in the absence of autophagy [106], enhanced IFN-β production in dsDNA-stimulated Atg9a-deleted mouse embryonic fibroblast cells [73], and increased IL-1β and IL-18 production in autophagy-deficient macrophages ($Atg16^{-/-}$, $LC3B^{-/-}$ and $Beclin\text{-}1^{+/-}$) [74, 107]. In relation to this, it has been reported that inhibition of autophagy by Beclin-1 or Atg5 knockdown results in reactive oxygen species (ROS) accumulation and sensitization of NLRP3 inflammasome activation [108]. In addition, autophagy has been reported to target pro-IL-1β for lysosomal degradation [109].

Autophagy also participates in adaptive immunity. First, it is important in the regulation of T- and B-cell survival and differentiation and in Paneth cell homeostasis [78, 110-113]. Second, autophagy contributes to major histocompatibility complex (MHC) class I and II antigen presentation. Molecules captured by autophagosomes are delivered to the endosomal compartment, where antigens are proteolytically processed and loaded onto the MHC class I and II molecules for presentation to $CD8^+$ and $CD4^+$ T-cells, respectively [114]. For example, during herpes simplex virus (HSV-1) infection, autophagy facilitates presentation of HSV-1 antigens on MHC class I molecules, and mice with Atg5-deficient dendritic cells show impaired $CD4^+$ T-cell priming upon HSV-1 infection [115, 116]. Also, immunization with cells undergoing autophagy enhances the efficiency of cross-priming of antigen-specific $CD8^+$ T-cells [117]. Furthermore, autophagy functions in delivery of cytoplasmic viral nucleic acids to endosomal TLRs, which results in type I IFN production and IFN-dependent immune responses [118]. Finally, autophagy modulates self-tolerance and controls T-cell selection in the mouse thymus [119]. Consequently, Atg5 deficiency in thymic epithelium causes colitis and multi-organ lymphoid infiltration.

Autophagy in cell death processes

Autophagy is a cytoprotective mechanism that enables the cell to survive unfavorable growth conditions. It can prevent cell death by apoptosis, necrosis, necroptosis or pyroptosis [120]. In unfavorable settings, autophagy is crucial for reducing cellular stress, whether this implies energy shortage, ROS production, genomic instability, accumulation of misfolded proteins, organelle damage or microbial infection. However, accumulating evidence also demonstrates a tight interconnection of autophagy with several cell death pathways and reveals an active contribution of autophagy to cell death. When autophagy has a functional role in the death process, the cell death process is designated "autophagic cell death" (ACD) [121]. Although many studies failed to demonstrate a causal link between autophagy and cell death, an increasing number of reports suggest a contribution of Atg proteins to the execution of cell death. The interplay between autophagy and cell death is complex and diverse. Depending on the cell type, environment and stimulus, autophagy and cell death mechanisms can have inhibitory, additive or even synergistic effects.

Apoptotic cell death

As mentioned above, autophagy can prevent apoptotic

cell death. The crucial role for autophagy in cell survival is apparent from studies using Atg-knockout mice. Mice deficient in Atg3, Atg5, Atg7, Atg9 or Atg16L1 fail to induce autophagy and die on the day of birth due to starvation following disruption of the trans-placental nutrient supply [122]. Also, mice with neuron-specific Atg5 or Atg7 knockout suffer from neurodegeneration and apoptotic death of neurons, and T-cell-specific Atg5 deficiency results in increased apoptosis in peripheral T-cells upon T-cell activation [35, 37, 111]. Furthermore, autophagy promotes epithelial cell survival during anoikis [123]. Apparently, autophagy prevents apoptotic cell death in several ways. During starvation for example, autophagy inhibits apoptosis by breakdown of cellular components to recycle essential metabolites and to replenish the energy reserves [124]. Moreover, autophagy degrades protein aggregates and targets damaged organelles, such as mitochondria, preventing ROS accumulation and cell death. In addition, recent reports demonstrate selective removal of apoptotic effectors by autophagosomes. In this regard, TRAIL-induced autophagy continuously targets active caspase-8 for lysosomal degradation, thereby inhibiting cell death by apoptosis [4].

Conversely, autophagy can also contribute to apoptosis. In this case, depletion of key autophagy regulators and pharmacological interference with autophagy prevent apoptosis. For example, autophagy precedes apoptotic cell death of salivary glands in *D. melanogaster* and inhibition of apoptosis only partially prevents cell loss [62, 125]. Also, in a model for HIV infection in which T cell expressing cluster of differentiation 4 (CD4)/CXCR4 interact with cells expressing HIV-1-encoded envelope glycoproteins, autophagy precedes apoptosis [126]. When autophagy is inhibited using 3-methyladenine (3-MA) or by knockdown of Beclin-1 or Atg7, caspase activity is reduced and apoptosis is attenuated. Moreover, autophagy contributes to damage-induced apoptosis as Atg5 knockdown causes a dramatic decrease in cell death [127]. The mechanisms of how the autophagy machinery drives apoptotic cell death are still under intense investigation. Interestingly, during late *D. melanogaster* oogenesis, autophagy controls DNA fragmentation and apoptosis in nurse cells by removal of dBruce, the *D. melanogaster* inhibitor of apoptosis [128]. Furthermore, several Atg proteins were shown to trigger cell death and to engage the apoptotic pathway (Table 1). Atg5 was shown to directly bind Fas-associated protein with death domain (FADD), thereby triggering IFN-γ-induced cell death [129]; Atg7 mediates lysosome dysfunction-induced apoptosis in neural cells [130]; and Beclin-1 stimulates apoptosis in response to the Bcl-2 inhibitor, obatoclax [131]. Moreover, a recent study uncovered the existence of an Atg12-Atg3 conjugation, which sensitizes cells to death downstream of mitochondrial pathways

Table 1 List of Atg proteins exerting a pro-apoptotic function

Protein	Prot event	Role in apoptosis	Reference
Atg4D	Casp-3 (Atg4D-C)	Targets mitochondria	[136]
		Induces apoptosis	
Atg5	Uncleaved	Triggers IFN-γ-induced cell death	[129]
		Binds FADD	
	Calp-I, -II (Atg5-N)	Induces apoptosis	[133]
		Binds Bcl-x_L	
		Induces cyt c release	
Beclin-1	Uncleaved	Stimulates obatoclax-induced apoptosis	[131]
		Triggers caspase-3 activation	
	Casp-3, -8, -9 (Beclin-1-C)	Enhances IL-3 withdrawal-induced cell death	[135]
		Targets mitochondria	
		Induces Omi and cyt c release	
Atg7	Uncleaved	Mediates bafilomycin-induced apoptosis	[130]
		Mediates chloroquine-induced apoptosis	
		Triggers p53 activation	
		Triggers caspase-3 activation	
Atg12-Atg3	Uncleaved	Enhances mitochondrial apoptosis	[132]
		Decreases Bcl-x_L levels	

Prot event: proteolytic event, -C: C-terminal fragment, -N: N-terminal fragment

[132]. Also fragments resulting from proteolytic cleavage of typical Atg proteins can enhance apoptotic cell death. Truncated Atg5, Atg4D and Beclin-1 localize at the mitochondria and trigger apoptosis, for example by release of pro-apoptotic factors from mitochondria [133-136]. Finally, autophagy can also assist in the clearance of apoptotic cells. It has been suggested that autophagy maintains the ATP levels needed for exposure of phosphatidylserine ("eat-me" signal) and secretion of lysophosphatidylcholine ("come-and-get-me" signals) [68].

The interplay between apoptosis and autophagy is also evident from the extensive molecular crosstalk between autophagy-related and apoptosis-related proteins. Several apoptotic proteins regulate the autophagic process. Conversely, typical autophagy-related proteins can modify apoptotic signaling. One of the most obvious interactions between apoptosis and autophagy effectors occurs within the Bcl-2-Beclin-1 complex. Bcl-2 is a central apoptosis inhibitor that also effectively impedes autophagy [137]. Bcl-2 directly binds Beclin-1 *via* its BH1 and Bcl-2 homology domain 3 (BH3) domains, and this inhibits the autophagic activity of Beclin-1 [92]. B-cell lymphoma extra long (Bcl-x_L) and myeloid cell leukemia sequence-1 (Mcl-1), two other anti-apoptotic Bcl-2 family members, also inhibit autophagy by binding Beclin-1. In fact, Beclin-1 has been identified as a new BH3-only protein [138]. However, binding of Beclin-1 to Bcl-2 does not seem to affect the anti-apoptotic role of Bcl-2 [139]. Bcl-2 has also been shown to inhibit autophagy induction through its interaction with Ambra1 at the mitochondria. Bcl-2-Ambra1 complex formation prevents Ambra1 from promoting Beclin-1 activity [140]. Further, FLICE-inhibitory protein (FLIP) can inhibit autophagy by interacting directly with Atg3. By binding and inactivating Atg3, FLIP abrogates LC3 lipidation, which is essential for autophagosome formation [141]. In contrast, other typical apoptosis proteins stimulate autophagy induction. For example, several BH3-only proteins, such as Bcl-2-associated death promoter protein (Bad), tBid (truncated BH3 interacting domain death agonist) and BNIP3, abrogate the Bcl-2-Beclin-1 interaction by competing with Beclin-1 for Bcl-2 binding. Furthermore, other apoptotic modulators (death-associated protein kinase, c-jun N-terminal kinase, TRAF6 and A20) mediate Beclin-1 or Bcl-2 post-translational modifications that enable dissociation of Beclin-1 from Bcl-2 [103, 142, 143]. Surprisingly, caspases were also implicated in autophagy-mediated survival. Indeed, caspase-9 promotes autophagic flux probably by regulating lysosomal pH and cathepsin activity [144]. Another interesting finding is the involvement of p53, a well-known pro-apoptotic tumor suppressor, in autophagy regulation. Depending on its subcellular localization, p53 either decreases or stimulates autophagic signaling [145]. When located in the cytoplasm, p53 can repress autophagy. p53 inactivation probably induces ER stress, a potent activator of autophagy, as IRE1α (inositol requiring 1) deficiency prevents induction of autophagy following p53 neutralization [146]. Conversely, nuclear p53 signals to autophagy *via* transactivation of DRAM (Damage-regulated autophagy modulator), a lysosomal protein that induces autophagy upon DNA damage [127]. In addition, p53 can inhibit mTOR through activation of AMP-activated protein kinase (AMPK) [147]. p62 also constitutes a molecular link between autophagic and apoptotic signaling. During autophagy, p62 binds ubiquitinated targets and is involved in their aggregation and delivery to the autophagosome through its interaction with LC3 [42]. p62 is also implicated in the activation of caspase-8 when cell death is triggered [148]. Following cullin-3-dependent ubiquitination of caspase-8, interaction between p62 and caspase-8 results in caspase-8 aggregation, which enhances caspase-8 activity and enables the autoproteolytic release of caspase-8 into the cytosol to trigger apoptosis [148].

Non-apoptotic cell death

Also non-apoptotic modes of cell death, such as necrosis, necroptosis, pyroptosis and NETosis, are linked to autophagy. In this regard, autophagy can protect apoptosis-deficient cells from necrosis following metabolic stress caused by ischemia [149]. Accordingly, autophagy-deficiency in apoptosis-defective tumor cells activates necrotic cell death and promotes necrosis-related inflammation and tumor growth [149]. Autophagy also protects macrophages from caspase-1-dependent cell death and against pyroptosis induced by *Shigella* infection [150]. Similarly, malfunction of autophagy due to absence of Atg7 or Atg16L1 increases pyroptosis upon LPS treatment through enhancement of caspase-1 activity and IL-1β and IL-18 production [74]. In contrast, autophagy activity can also provoke non-apoptotic cell death. For example, in *C. elegans*, autophagy is required for necrotic neuron destruction, and both 3-MA and knockdown of key Atg proteins (Bec-1, Igg-1, Atg18) reduce necrotic cell death [151, 152]. Often, autophagy-associated necrotic cell death occurs when apoptosis is defective. In L929 cells, zVAD-fmk-induced necrotic cell death is triggered by ROS-mediated membrane lipid peroxidation resulting from autophagy-dependent degradation of catalase, one of the major ROS scavenging enzymes [153, 154]. Similarly, triggers of cell death induce cytotoxic autophagy in Bax/Bak double knockout cells [155-157]. Also, although autophagy is required for T cell homeostasis, in proliferating T cells lacking FADD or caspase-8

it is strongly increased and triggers receptor-interacting protein 1 (RIP1)-dependent necroptotic cell death [112]. Interestingly, this mechanism of cell death can be used to re-sensitize glucocorticoid-resistant leukemia cells to dexamethasone after treatment with obatoclax (GX15-070), a Bcl-2 antagonist (see below) [158]. Of note, p62 was shown to interact with RIP1, which regulates nuclear factor κB activation in a TRADD/RIP/p62/aPKCs/IKK-beta signaling cascade [159]. Finally, autophagy enables the neutrophil extracellular trap cell death (NETosis) induced by phorbol-myristate acetate (PMA). NETosis is a specialized form of neutrophil cell death in which microbes are trapped and killed within neutrophil extracellular traps (NETs); these traps are chromatin structures loaded with concentrated anti-microbial molecules [160]. During PMA-induced NETosis, autophagy prevents caspase activation and triggers chromatin decondensation, which is required for NET formation and concurrent cell death [160].

Autophagy and ageing

Aged cells are characterized by the accumulation of altered or damaged DNA, proteins, lipids and organelles, increase in oxidative stress and defects in several biological processes. One of the most intriguing properties of autophagy is its ability to counteract the ageing process and to promote longevity in different animal species. Indeed, environmental conditions or use of agents that promote the induction of autophagy have been shown to improve cellular fitness and survival [161]. In *C. elegans* and *D. melanogaster*, for example, caloric restriction (not involving malnutrition) enhances autophagic activity and increases the mean lifespan [162, 163]. In addition, other autophagy-promoting treatments, e.g., rapamycin, resveratrol and spermidine, enhance cell survival, fitness and/or lifespan. Conversely, knockdown or deficiency of key autophagy components reverses these effects [164-166]. How resveratrol and spermidine regulate autophagy needs further investigation, but it seems that hypoacetylation might be involved. Both caloric restriction and resveratrol require sirtuin-1, a deacetylase, to promote lifespan extension [167, 168]. Interestingly, sirtuin-1, apart from targeting histones, also targets key Atg proteins, such as Atg5, Atg7 and Atg8, and its overexpression induces autophagy [169, 170]. Similarly, spermidine triggers epigenetic deacetylation through inhibition of acetyltransferases and, in addition, prevents oxidative stress and necrosis [171]. Strikingly, the altered epigenetic acetylation status did not affect Atg7, which points to a regulatory mechanism that protects Atg genes from hypoacetylation [171]. Resveratrol-induced autophagy might also involve the induction of ER stress as resveratrol has been shown to induce several ER stress markers, e.g., eukaryotic translation initiation factor 2α (eIF2α) phosphorylation, X-box-binding protein 1 splicing and CHOP (C/EBP homologous protein) expression [172]. In line with the notion that autophagy is important in slowing down the ageing process, a decline in formation and elimination of autophagosomes is often observed in ageing cells [173]. Thus, diminished autophagy increases the accumulation of harmful protein aggregates and damaged organelles. This results in increased ROS, higher risk of mutations in DNA and defects in biological processes, which eventually affect lysosomal and autophagic functionality and give rise to a vicious pro-aging cycle [174].

Autophagy in tumorigenesis and cancer treatment

Because of its major role in cell survival during unfavorable conditions, it is not surprising that autophagy protects tumor cells from cellular and metabolic stress. This implies that autophagy has an oncogenic function. However, much data also favor a tumor suppressive role for autophagy. Seemingly, autophagy plays a dual role in cancer [175]. One current idea is that, depending on cell type, context or stage of tumor development, autophagy exerts one of the two opposing functions: autophagy, as an intracellular quality control mechanism, prevents malignant transformation and cancer progression, while its pro-survival role during non-optimal growth conditions provides the established tumor with an adaptation mechanism that promotes its survival [175]. Taking into account this apparent dual role, the design of cancer treatments that modulate autophagy becomes quite complex [176]. Recent data suggest that therapeutic interventions enhancing or decreasing autophagy, depending on the context, can be beneficial to cancer patients, as described in more detail in the paragraphs below [176-179].

Autophagy has tumor-promoting properties and most likely provides growth advantages to tumor cells residing in a nutrient poor and hypoxic environment [175]. Indeed, autophagy increases the viability of cells exposed to deprivation of serum, amino acids or growth factors [124, 180, 181]. In this respect, recent reports nicely demonstrate the autophagy dependency of tumors for growth and survival [181, 182]. Autophagy supports Ras-mediated tumorigenesis by maintaining mitochondrial metabolic function and energy levels [181] and is required for tumorigenic growth of pancreatic cancers probably by constraining oxidative damage and preserving metabolic homeostasis [182]. In addition, autophagy can protect cells from anoikis upon detachment from the extracellular matrix, which suggests a role for autophagy

in metastasis [123]. In line with a tumor-protecting role for autophagy, tumor cells induce autophagy in response to most anticancer therapies and utilize autophagy to overcome the therapeutically induced stress and cellular damage [176, 177, 183]. In these cases, autophagy inhibition might enhance the efficacy of anticancer treatments. Inhibition of autophagy by use of chloroquine or Atg5 knockdown has been shown to enhance tumor death induced by p53 or alkylating drugs [184]. In addition, use of 3-MA or Atg7 knockdown in 5-fluorouracil-treated cancer cells enhances apoptosis induction [185]. These data demonstrate a potential role for autophagy inhibitors in combination with conventional therapies in sensitizing apoptosis induction in human cancers.

However, there is generally a strong association between molecules that are implicated in autophagy induction, e.g., PTEN (phosphatase and tensin homolog), p53, tuberous sclerosis protein 1 (TSC1), TSC2 and Bax-interacting factor-1, and tumor suppression [186]. More importantly, several Atg proteins, including Beclin-1, UVRAG, Atg5 and Atg4C, have tumor-suppressing effects [67, 71, 133, 187] (Table 2). Indeed, monoallelic deletions of Beclin-1 and UVRAG are frequently found in human cancers, and both Beclin-1 and UVRAG overexpression inhibit proliferation and tumorigenesis of tumor cell lines [187-189]. Similarly, forced expression of Atg5 effectively reduces tumor growth of breast cancer cells, and Atg4C$^{-/-}$ mice are more susceptible to fibrosarcoma development following treatment with chemical carcinogens [71, 133]. The mechanisms by which autophagy protects against tumorigenesis are not entirely clear. In favor of a tumor-suppressing role, it has been shown that dysfunctional autophagy in apoptosis-defective tumors stimulates necrotic cell death and inflammation, which promotes tumor growth [149]. Additionally, autophagy plays an important role in limiting the accumulation of genomic damage, which favors oncogene activation and tumor initiation [190, 191]. In this regard, autophagy can eliminate damaged or harmful organelles, such as mitochondria, that produce ROS and cause oxidative stress that would otherwise result in a higher rate of DNA damage. Moreover, protein aggregates, imbalanced energy homeostasis and enhanced proliferation could contribute to genomic instability when autophagy is defective [67, 192, 193]. In addition, a recent report demonstrates enhanced proliferation of epithelial cells through Ras-dependent degradation of Beclin-1 following detachment from the extracellular matrix [193]. As autophagy is able to contribute to cell death in apoptosis-deficient cells, pharmacological intervention aiming at enhancing autophagic function could be of high therapeutic interest. For example, in acute lymphoblastic leukemia cells,

Table 2 List of Atg protein mutations found in distinct cancer types

Protein	Alteration	Cancer type	Reference
Atg2B	Frameshift mutation	Gastric cancer	[215]
		Colorectal cancer	
Atg5	Frameshift mutation	Gastric cancer	[215]
		Colorectal cancer	
Atg9b	Frameshift mutation	Gastric cancer	[215]
		Colorectal cancer	
Beclin-1	Mono-allelic deletion	Breast cancer	[187, 189]
		Ovarian cancer	
		Prostate cancer	
	CpG hypermethylation	Breast cancer	[216]
	Loss-of-heterozygosity	Breast cancer	[216]
	Missense mutations	Gastric cancer	[217]
		Colorectal cancer	
		Breast cancer	
FIP200	Compound heterozygous deletions	Breast cancer	[218]
	Loss-of-heterozyosity		
UVRAG	Mono-allelic deletion	Colon cancer	[219]
	Frameshift mutation	Colon cancer	[220, 221]
		Gastric cancer	

the BH3 mimetic, obatoclax, abrogates glucocorticoid resistance by promoting Beclin-1-mediated autophagy and inducing necroptotic cell death [158]. In addition, knockdown of transglutaminase 2 (TG2) or Bcl-2, both of which are important autophagy suppressors strongly expressed in many tumors, was shown to induce ACD in cancer cells [194, 195].

Autophagy in other diseases

As autophagy plays a role in many important biological processes, defects in autophagy signaling could be associated with many diseases. Autophagy has been linked to neuronal disorders, liver and heart diseases, infectious diseases, cancer, diabetes type II, cystic fibrosis and many more [186]. A considerable part of these diseases are associated with accumulation of proteins that are prone to aggregation due to mutation or misfolding. Protein aggregates can be sequestered and subsequently degraded by autophagosomes [196]. When their removal is perturbed, e.g., when autophagy is defective, they can be detrimental [197]. Therefore, autophagy can prevent the emergence of neurodegenerative diseases. Indeed, autophagy protects against aggregation-prone mutant proteins in spinocerebellar ataxia, mutated forms of α-synuclein in Parkinson's disease, mutant Huntingtin in Huntington's disease, tau mutants that cause frontotemporal dementia, pathogenic intraneuronal amyloid beta in Alzheimer disease brain and polyglucosan inclusion bodies in Lafora disease [162, 196, 198-202]. Interestingly, most of these neurodegenerative diseases are associated with decreased Beclin-1 levels, which might account for the impaired autophagic clearance. In this respect, Beclin-1 expression in human brain was found to decrease with age [203]. Moreover, caspase-mediated cleavage of Beclin-1 was observed in brain tissues of Alzheimer's patients [204].

Cystic fibrosis (CF), which is caused by mutations in cystic fibrosis transmembrane conductance regulator, is associated with impaired autophagy and the accumulation of protein aggregates in CF lung epithelia. In this condition, ROS accumulation causes TG2-dependent crosslinking of Beclin-1 and the sequestration of the PIK3C3 complex in perinuclear aggregates, which prevents the PIK3C3 complex from fulfilling its autophagic function at its proper locations [205].

Also certain myopathies are associated with impaired autophagic clearance of aggregation-prone proteins or damaged organelles. Muscular dystrophies linked to collagen VI deficiency, for example, are characterized by the accumulation of dysfunctional organelles and spontaneous apoptosis of muscle fibers caused by defective autophagy induction [206]. Reduced levels of Beclin-1 and BNIP3 were observed in muscle biopsies from subjects with Bethlem myopathy or Ullrich congenital muscular dystrophy [206]. Strikingly, forced activation of autophagy by genetic, dietary and pharmacological approaches restored myofiber survival and ameliorated the dystrophic phenotype of collagen VI-knockout mice [206]. Pompe disease and Danon disease involve primary defects in the lysosomal proteins, LAMP-2 and acid alpha-glucosidase, respectively, and they are associated with extensive accumulation of autophagosomes in muscles. In addition, the skeletal muscles of patients with X-linked myopathy show excessive autophagy [207]. Autophagy has also been implicated in certain heart diseases [208]. LAMP-2-deficient mice suffer from cardiomyopathy, and cardiac-specific Atg5 deficiency results in increased accumulation of polyubiquitinated proteins, ER stress and apoptosis [36, 209]. Moreover, autophagic activity protects from hemodynamic stress such as pressure overload and hypertrophy in failing hearts [36]. However, autophagy seems detrimental for the heart during ischemia/reperfusion, possibly due to excessive activation of autophagy and consequent cell death [210, 211].

As mentioned above, autophagy also protects against human liver diseases. α1 anti-trypsin deficiency is a liver disorder associated with chronic inflammation and carcinogenesis. The Z mutation in α1 anti-trypsin causes protein misfolding and polymerization and the formation of intrahepatic inclusions, which are degraded by autophagy. Carbamazepine, an anti-epileptic drug, induces autophagy, which clears out inclusions of α1 anti-trypsin in mice and reduces hepatic fibrosis [212, 213]. In addition, autophagy might be involved in the development of type II diabetes [214]. Pancreatic β-cells of mice with β-cell-specific Atg7 knockout accumulated large ubiquitinated inclusion bodies and dysfunctional mitochondria and exhibited hallmarks of ER stress, possibly due to impaired autophagy. Moreover, these mice showed increased β-cell death, hyperglycemia, reduced insulin production and higher insulin resistance [214]. Finally, recent studies have implicated autophagy in the predisposition to Crohn's disease (CD), a major form of chronic inflammatory bowel disease. Both *Atg16L1* and *IRGM1* (immunity-related GTPase family M member 1), two autophagy-related genes, have been identified as susceptibility loci for CD. The CD-associated Atg16L1*300A polymorphism results in impaired autophagic activity, reduced autophagic clearance of bacteria and Paneth cell granule abnormalities [113]. Interestingly, the CD-related frameshift mutation in NOD2 (L1007insC) encodes a mutant NOD2 protein incapable of recruiting Atg16L1 to

the site of bacterial entry, which leads to inefficient clearance of bacteria by autophagy [104].

Conclusions and perspectives

Over recent years, our knowledge about the autophagic process has greatly expanded. Many autophagy-related proteins have been identified and functionally characterized in yeast and mammals. In addition, we are starting to unveil the physiological roles of autophagy. But despite these recent advances, many questions remain largely unanswered. How is the specificity in the many types of autophagy regulated? Which organelles contribute to autophagosome biogenesis and what determines the origin of the membranes? What is the redundancy between the Atg proteins and which pleiotropic functions do they fulfill? Does the effect of caloric restriction on lifespan occur mainly through autophagy induction and how can this be therapeutically exploited to treat obesity? What are the downstream targets of autophagy induction during dietary restriction? How do micro-autophagy and chaperone-mediated autophagy contribute to human disease? Which parameters determine whether autophagy is pro-survival or pro-death, tumor promoting or tumor suppressive? Hopefully, future studies will increase our understanding of the mechanisms underlying autophagy, its functions in multiple biological processes and the regulatory signaling pathways that control its activation. This knowledge will be of great importance in validating autophagy as an effective target for the treatment of various diseases, including cancer.

Abbreviations: 3-MA (3-methyladenine); ACD (autophagic cell death); Ambra1 (activating molecule in Beclin-1-reguated autophagy); AMPK (AMP-activated protein kinase); Atg (autophagy related); ATP (adenosine triphosphate); Bad (Bcl-2-associated death promoter protein); Bcl-2 (B-cell lymphoma-2); Bcl-x_L (B-cell lymphoma extra long); Beclin-1 (Bcl-2-interacting protein); BH3 (Bcl-2 homology domain 3); BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3); CD (Crohn's disease); CD4 (cluster of differentiation 4); Deptor (DEP-domain-containing mTOR-interacting protein); DFCP1 (double FYVE-containing protein-1); eIF2α (eukaryotic translation initiation factor 2α); DRAM (damage-regulated autophagy modulator); ER (endoplasmic reticulum); ESCRT (endosomal sorting complex required for transport); FADD (Fas-associated protein with death domain); FIP200 (focal adhesion kinase family interacting protein of 200 kDa); FLIP (FLICE-inhibitory protein); FYCO1 (FYVE and coiled-coil domain containing 1); GβL (G-protein β-subunit-like protein); HDAC6 (histone deacetylase 6); HIV (human immunodeficiency virus); HOPS (homotypic fusion and protein sorting); HSV (herpes simplex virus); IL-1β (interleukin-1β); IRE1α (inositol requiring 1); IRGM1 (immunity-related GTPase family M member 1); IFN (interferon); LAMP-2 (lysosomal-associated membrane protein 2); LC3 (microtubule-associated light chain-3); Mcl-1 (myeloid cell leukemia sequence-1); MHC (major histocompatibility complex); MTOC (microtubule-organizing centre); mTORC1 (mammalian target of rapamycin complex 1); MyD88 (myeloid differentiation primary response gene 88); NBR1 (neighbor of BRCA1 gene 1); NDP52 (nuclear dot protein 52kDa); NLR (NOD-like receptor); NOD1 (nucleotide-binding oligomerization domain-containing protein 1); PAS (phagophore assembly site); PE (phosphatidylethanolamine); PIK3C3 (phosphatidyl-inositol-3-kinase class III); PINK1 (PTEN-induced putative kinase 1); PI3P (phosphatidyl-inositol-3-phosphate); PMA (phorbol-myristate acetate); PRAS40 (proline-rich AKT substrate 40 kDa); PTEN (phosphatase and tensin homolog); Rab (Ras-related GTP-binding protein); ROS (reactive oxygen species); RIP1 (receptor-interacting protein 1); SQSTM1 (sequestosome 1); SNARE (soluble N-ethylmalemide-sensitive factor attachment protein receptor); tBid (truncated BH3 interacting domain death agonist); TG2 (transglutaminase 2); TGN (trans Golgi network); Th1 (T helper); TLR (Toll-like receptor); TRAF6 (TNF receptor associated factor 6); TRIF (TIR-domain-containing adapter-inducing interferon-β); TSC1 (tuberous sclerosis protein 1); UCMD (Ullrich congenital muscular dystrophy); ULK (unc-51-like kinase); unc-51 (uncoordinated-51); UVRAG (UV-radiation resistance associated gene); WIPI1 (WD repeat domain phosphoinositide interacting 1)

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