Autophagy and disease: always two sides to a problem

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Abstract

Autophagy is a process traditionally known to contribute to cellular cleaning through the removal of intracellular components in lysosomes. In recent years, intensive scrutiny at the molecular level to which autophagy has been subjected has also contributed to expanding our understanding of the physiological role of this pathway. Added to the well-characterized role in quality control, autophagy has proved to be important in the maintenance of cellular homeostasis and of the energetic balance, in cellular and tissue remodelling, and cellular defence against extracellular insults and pathogens. It is not a surprise that, in light of this growing number of physiological functions, connections between autophagic malfunction and human pathologies have also been strengthened. In this review, we focus on several pathological conditions associated with primary or secondary defects in autophagy and comment on a recurring theme for many of them, ie the fact that autophagy can often exert both beneficial and aggravating effects on the progression of disease. Elucidating the factors that determine the switch between these dual functions of autophagy in disease has become a priority when considering the potential therapeutic implications of the pharmacological modulation of autophagy in many of these pathological conditions.

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Introduction to autophagy

The Greek term ‘autophagy’ (‘self-eating’) was coined almost half a century ago by Christian DeDuve [1]. Today, this intensely investigated pathway has come to be recognized as an evolutionarily conserved intracellular process through which cytosolic components, ranging from proteins, lipids, sugars and nucleotides to whole organelles and invading pathogens, are targeted for lysosomal degradation [2,3]. Autophagy contributes to both the removal of damaged long-lived proteins and organelles and the normal turnover of these intracellular components as part of its role in cellular quality control. In addition, autophagy also serves as a cellular adaptive response to compromised conditions, such as metabolic stress, when degradation of intracellular materials through this pathway becomes an alternative source of energy [4].

In recent years, a growing number of functions have been added to these two basic cellular functions of the autophagic process—quality control and maintenance of the cellular energetic balance—helping to shape the physiological relevance of this pathway. Furthermore, a better understanding of the molecular mechanisms that mediate the autophagic process has helped to establish connections between autophagy and the pathogenesis of different disorders and of ageing [3,5,6]. Findings in recent years also support the existence of more than one type of autophagy and the fact that different autophagic pathways co-exist in most cells. In this review, we briefly describe the main characteristics and molecular components of the different autophagic variants and then provide a general view of the multiple physiological functions of this cellular process. In the second part, we comment on specific autophagy-related pathologies, which either result from a primary defect in the autophagic process, or for which changes in autophagy have been described to exert a modulatory effect on the course of the disease.

The molecular dissection of the different autophagic pathways

To date, three basic forms of autophagy have been described, namely, macroautophagy, microautophagy and chaperone-mediated autophagy (CMA), which primarily differ in the way in which cytosolic components (cargo) are delivered to lysosomes [2,7,8] (Figure 1). Macroautophagy and microautophagy were both first...
Figure 1. Autophagic pathways. Three different general mechanisms of lysosomal delivery of cargo set the basis for the different types of autophagy described in mammalian cells. (A) Macroautophagy: different extra- and intracellular signals activate the recruitment of the macroautophagy initiation complex to the sites of autophagosome formation. Shuttling of proteins and lipids to these regions and posttranslational modifications of the lipids initiate the formation of a limiting membrane, which grows through the assembly of proteins conjugated to proteins or lipids while it sequesters components of the cytosol. Once the membrane seals to form the autophagosome, this double membrane vesicle is delivered to lysosomes where, upon membrane fusion, lysosomal hydrolases gain access to cargo. (B) Microautophagy: through stimuli that remain poorly identified, cytosolic soluble proteins and organelles are directly sequestered by invaginations in the boundary membrane of lysosomes and late endosomes. Cargo internalized in the small luminal vesicles is degraded after the vesicles pinch off from the limiting membrane. Although most microautophagy probably occurs in bulk, selective targeting by hsc70 of cytosolic proteins to microvesicles as they form in late endosomes has also been described. (C) Chaperone-mediated autophagy (CMA) is induced by stimuli such as prolonged starvation, oxidative stress and other conditions resulting in protein damage, but the signalling mechanism activated by these stimuli remains unknown. When CMA is activated, selective cytosolic proteins bearing a targeting motif are recruited by hsc70 and co-chaperones to the surface of lysosomes. Upon binding to the receptor protein LAMP-2A, substrates cross the membrane through the LAMP-2A-dependent translocation complex and are then rapidly degraded in the lumen.
Rapamycin, a well-characterized negative regulator of this pathway, prevents the recruitment and interaction of specific Atgs of the nucleation complex to the site of autophagosome formation [19–21]. Although less is known about the regulation of later steps of the autophagic process, recent studies have identified the transcription factor EB as a global regulator of Atgs that can up-regulate initiation, elongation and fusion, as well as lysosomal biogenesis in response to nutritional stress [22]. Homologues for most yeast Atgs have been identified in many other species (Drosophila [23], Caenorhabditis elegans [24], Dictyostelium [25], Arabidopsis [26], Trypanosoma [27]) and in mammals, where these genes often have several variants with diverse functions [28].

The number of yeast genes shown to participate in microautophagy does not exceed a dozen, but this process also partially depends on Atgs shared with macroautophagy [29]. Microautophagy-specific proteins contribute to the formation and sealing of the vacuolar membrane (equivalent of the lysosome in yeast) [30]. Failure to identify mammalian homologues of the yeast microautophagy genes initially led to the proposal that microautophagy was not evolutionarily conserved. However, recent studies show otherwise, as a microautophagy-like process has been described to occur in mammalian late endosomes. This process, known as endosomal microautophagy, has adopted the machinery involved in biogenesis of multivesicular bodies to form the invaginating vesicles that sequester cytosolic material for degradation [31].

In recent years, in addition to this ‘in bulk’ non-selective degradation of cytosolic components, specific sequestration of cargo has also been described for both macro- and microautophagy. In the case of selective macroautophagy, the distinctive characteristic is the presence of a single type of cytosolic material in autophagosomes, in clear contrast to the ‘in-bulk’ process, where heterogeneous cargo occupies the lumen of the autophagosome. Selective forms of macroautophagy are named depending on the sequestered material, giving rise to terms such as mitophagy [32,33], pexophagy [34,35], reticulophagy [36,37], lipophagy [38], ribophagy [39] and argrophy [40]. Similar criteria apply to selective forms of microautophagy, such as micropexophagy [34,35] micronucleophagy [41,42] and microglycophagy [43]. All these selective forms of autophagy utilize the same essential components of the autophagic machinery described for their ‘in-bulk’ variants, but they add an extra step related to cargo recognition. A specific subset of proteins, known as cargo-recognition proteins, have been involved in this step and include, among others, p62, neighbour of BRCA1 (NBR1), Nix and the PINK1/Parkin pair [44,45]. These adaptor proteins connect the degradation machinery with particular components—often ubiquitin moieties—on the surface of the cytosolic organelle to be sequestered [44,46,47]. Cytosolic chaperones, in particular the heat shock cognate protein of 70 kDa, or hsc70, and BAG-3, have also been recently described to contribute to selective recognition of aggregates by macroautophagy in chaperone-assisted selective autophagy (CASA) [48].

Selectivity is the distinctive feature of CMA, the third type of mammalian autophagy, wherein particular soluble proteins bearing in their sequence a pentapeptide targeting motif biochemically related to KFERQ, are recognized by hsc70, which mediates their delivery to the lysosomes for direct translocation across the lysosomal membrane [8,49] (Figure 1). Although yeast genetic approaches could not be applied to the molecular dissection of CMA, since this pathway has so far been described only in mammals, about eight different proteins have been identified to participate in this pathway using biochemical procedures. Most of the identified CMA components have promiscuous functions and are shared with other intracellular processes. Such is the case for the cytosolic chaperones hsc70 and hsp90 [50,51] and the novel pair of regulators, glial fibrillary acidic protein and elongation factor 1α [52]. Interestingly, lysosome-specific variants have been identified for each of these proteins, suggesting that post-translational modifications of the cytosolic proteins determine their association with this degradative compartment and their commitment to CMA. So far, the only CMA-exclusive component is the lysosome-associated membrane protein type 2A (LAMP-2A), which acts as a receptor for substrates of this pathway [53]. Once the cytosolic proteins destined for CMA degradation bind to this single transmembrane protein, they promote its assembly into a higher-order molecular complex required for translocation of the CMA substrates into the lysosomal lumen [51].

Interested readers are referred to more focused reviews [2,3,8,29,54] for a comprehensive description of the molecular apparatus executing these different autophagic pathways. For the purposes of this review, the term ‘autophagy’ will represent macroautophagy, unless otherwise mentioned.

Physiological relevance of autophagy

As described in the Introduction, a growing number of cellular functions have been attributed to autophagy. We highlight here some of the best-characterized functions that have contributed to expanding the physiological relevance of this catabolic process. The role of autophagy in cellular defence, by participating in innate and adaptive immunity, is discussed in detail in the section dedicated to autophagy and the immune function.

Autophagy in the cellular energetic balance

Both macroautophagy and CMA are induced as an acute adaptive response to a variety of metabolic stressors, including, among others, nutrient starvation, growth factor withdrawal, high lipid content challenges or hypoxia [4,55–57]. Under these conditions,
free amino acids (especially branched-chain amino acids), released by autophagic proteolysis of intracellular proteins and organelles, are recycled to maintain protein synthesis even when nutrients are scarce. Autophagy in the liver converts this organ into a main source of amino acids that are then delivered to other organs through the blood stream during starvation [5,56,58,59]. In fact, the low plasma concentrations of essential amino acids and decreased ATP levels in most tissues observed in autophagy defective Atg5−/− and Atg7−/− neonates confirm the importance of this pathway during nutritional deprivation, in this case as a result of the cessation in transplacental nutrient supply at birth [60,61]. Amino acids resulting from autophagic breakdown could also be utilized for the production of cellular ATP through direct oxidation, or by fuelling the TCA cycle and gluconeogenesis with intermediates such as oxaloacetate. This contribution of amino acids to energy homeostasis has been confirmed in specific conditions, such as in the case of interleukin-3-deprived haematopoietic cells, where the addition of TCA cycle substrates, such as methylpyruvate, is enough to preserve cellular viability [62]. However, amino acids are not an efficient source of energy. Instead, the array of energy stores mobilized by autophagy is now recognized to include more energetically efficient molecules, such as lipids, glyco- gen and nucleic acids. The hydrolytic products of these molecules, free fatty acids, glucose and nucleotides, can be funnelled into the TCA cycle, gluconeogenesis or glycolysis to produce ATP [4,43].

This capability of autophagy to maintain ATP production and support macromolecular synthesis makes it a pro-survival pathway of particular importance in organs with high energetic requirements, such as the heart or skeletal muscles. Alterations of this specific autophagic function also constitute the basis of some common metabolic disorders.

Autophagy and cellular quality control

The proteome and cellular organelles are susceptible to different toxic insults that can lead to the generation of misfolded or modified soluble proteins, protein cross-linking and oligomerization into high-order irreversible structures or aggregates, and accumulation of defective, no longer functional, organelles that could become harmful for the cells [63–65]. Basal autophagy, often referred to as ‘quality control autophagy’, is integral to the cellular surveillance machinery responsible for recognition and removal of these aberrant structures [66,67]. Basal autophagy is particularly important in non-dividing post-mitotic cells that cannot dilute the cellular damage through division. For example, conditional knock-out of the essential autophagy genes atg7 or atg5 in hepatocytes, neurons and cardiomyocytes leads to marked accumulation of ubiquitin-positive protein aggregates and damaged organelles, even in the absence of an added toxic challenge [60,68,69].

Exposure to stressors, such as oxidative stress, ER stress or other conditions resulting in massive amounts of unfolded proteins and organelle damage, elicits activation of inducible forms of autophagy [70–73]. In this context, activation of CMA contributes to the selective removal of soluble (aggregate-prone) proteins, whereas macroautophagy facilitates the clearance of protein aggregates [40,63,74,75] and whole organelles [33,36,37]. However, it is possible that macroau- tophagy ameliorates proteotoxicity not only through aggregate removal but also by the in-bulk sequestration of ‘still-soluble’ forms of the pathogenic proteins before they aggregate [68,76,77]. Autophagy is also important to restore organelle homeostasis [36,72,78], to eliminate damaged organelles after stress [37,79] and to assist cells to adapt their organelle content to the changing environmental conditions (eg it controls peroxisome number during nutrient auxotrophy or total mitochondria mass for energetic balance) [80].

Autophagy-mediated control of protein and organelle quality, as well as organelle number, is essential for the maintenance of cellular homeostasis and to guarantee cellular survival during stress.

Cellular remodelling and autophagy

Cellular differentiation often requires the elimination of large subsets of proteins, nucleic acids or organelles in order to switch into the next developmental stage or final differentiated state. Furthermore, cellular remodelling is an energy-demanding process often associated with nutrient-deprived developmental phases. It is no surprise, therefore, that autophagy has emerged through evolution as one of the favoured cellular tools to accomplish these developmental remodelling tasks. The capability to eliminate whole regions of the cytosol, unique to some of the autophagy variants, and its recycling properties underlie the important role that this process plays during development. Autophagy has been shown to be necessary in yeast during sporulation [81], in C. elegans during dauer formation [24], in Drosophila during the transition from the larval to the pupal stage [82] and in Dictyostelium for switching from amoeba to fruiting body [25]. Examples of autophagy-mediated differentiation events in mammalian cells include, among others, the removal of maternal macromolecules during early embryogenesis [83] and the clearance of mitochondria during erythrocyte [84–86], lymphocyte [87,88] and adipocyte [89,90] differentiation.

Autophagy in cellular death and cell survival

As indicated in the previous sections, the ability of autophagy to assist cells to adapt to the changing environment, to protect them against the damage caused by toxic insults and to defend them from pathogens has led to the classification of this pathway as a cell-survival mechanism. However, interplay between autophagy and mechanisms related to cell death have also been described. The best-accepted example of autophagy as
a cell death effector is the degradation of the salivary glands during pupal transition in Drosophila [82,91]. In contrast, direct evidence of a similar role for autophagy in mammals is sparse [92]. In fact, many of the original claims of ‘cell death by autophagy’ have currently been revised and reformulated as ‘cell death with autophagy’, since the main support for autophagic involvement in many of these cases was the observation of autophagic vacuoles in the dying cell [93]. Although an increase in the number of autophagic vacuoles could result from their increased formation, the large capability of the lysosomal system often prevents their accumulation by facilitating their rapid clearance. It is more frequent that the increase in autophagosome content results from a compromise in their clearance by lysosomes and, consequently, from impaired rather than enhanced macroautophagy. Systematic analysis of autophagic flux, rather than autophagic markers, should help to clarify these issues. However, independent of this initial controversy, it has become clear that, as described in the following sections, once in a disease context interactions between autophagy and apoptosis (or type I cell death) are complex and need to be analysed in the specific disorder or cell type [94–96]. In fact, autophagy and apoptosis share some molecular components and appear to be in a delicate balance with each other in most systems examined [97,98].

Pathology of autophagy

The broad array of physiological functions attributed to autophagy justifies why alterations in this catabolic process lead to cellular malfunctioning and often cell death, and has set the basis for its contribution to the pathogenesis of severe human disorders. Rather than providing an exhaustive list of disease conditions in which autophagy has been shown to be altered, in the following sections we have selected four common pathological conditions, cancer, neurodegeneration, heart dysfunction and immune and infectious diseases, to comment on their interplay with the autophagic system. The reason behind our selections is the fact that in all of these pathologies growing evidence supports a dual role of autophagy in their pathogenesis and disease progression, which leads to the question, ‘Should we inhibit or activate autophagy in these pathologies?’.

Autophagy and cancer

Cancer was the first human pathology connected to autophagy through the discovery that Beclin1 (Atg6/VPS30 homologue), a core component of the autophagosome nucleation complex, was monoallelically deleted in 40–75% of sporadic human breast, ovarian and prostate cancers [99]. Independent studies verified that heterozygous Beclin1+/− mice develop spontaneous tumours, including lymphomas, lung carcinomas, hepatocellular carcinomas and mammary pre-cancerous lesions [100,101]. Analogous generation of spontaneous hyperproliferating tumours was also reported later upon deletion of other autophagy-related genes, such as the ultraviolet resistance-associated gene (UVRAG), the Bax interacting factor-1 (Bif-1) and the LC3 (Atg8 homologue) processing protease Atg4c [102–104]. Furthermore, the products of common oncogenes, such as class I PI3K, PKB, TOR and Bcl-2, have been shown to act as autophagy repres- sors, whereas tumour suppressor gene products such as p53, PTEN, DAPK and TSC1/TSC2 exert a stimulatory effect on autophagy (reviewed in [3,98,105]). Although, in light of this opposite effect of oncogenes and tumour suppressors on autophagy, this process was initially classified as an anti-oncogenic mechanism, this conclusion has been challenged by experimental evidence supporting that under certain conditions autophagy can also be pro-oncogenic (Figure 2).

From the perspective of cellular energetics, autophagy is likely a pro-survival mechanism in both early and late stages of cancer development, as it supplies cells with the energy and essential macromolecules to sustain normal cellular functions. However, it has been proposed that in the early stages of cancer development, quality control by autophagy, particularly over genome maintenance, inhibits tumourigenesis, conferring anti-oncogenic functions upon this pathway. In addition, autophagy could also play a role in the maintenance or entry of cells into the G0 phase and consequently, prevent spontaneous hyperproliferation of cells [106,107]. In contrast, in the late stages of oncogenesis, autophagy is necessary for cancer survival, as it contributes both energy for the rapidly dividing cancer cells and quality control functions to eliminate intracellular damage caused by the aggressive tumour microenvironment and by anti-oncogenic interventions (Figure 2). In fact, consistent with the pro-survival function of autophagy, compromise of this pathway (ie Beclin1 interference) in apoptosis-deficient tumour cells impairs their survival in metabolic stress conditions in vitro and in vivo [108]. In general, blockage of autophagy sensitizes cells to metabolic stress, often leading to necrotic cell death accompanied by inflammation, a condition shown to promote tumourigenesis [109–111]. Metabolic stress is intrinsic to rapidly growing tumours, in which poor vascularization results in lack of nutrients and oxygen for long periods of time [112–116]. Activation of macroautophagy under these conditions has the potential to accommodate the acute energy demands by providing cancer cells with amino acids, free fatty acids and glucose, required to generate energy through the TCA cycle and via β-oxidation (Figure 2). Macroautophagy has also been recently implicated in facilitating the unique utilization of glu- cose by cancer cells, known as the ‘Warburg effect’ [117,118], by which cells favour glycolysis to accelerate ATP production and generate glycolysis intermediates required for the transformed cells [119,120]. Blockage of autophagy has been shown to prevent Ras-mediated oncogenic transformation by reducing glu- cose uptake and its utilization toward glycolysis [121].
Figure 2. Autophagy and cancer. Autophagy may play opposite roles in the oncogenic process. Anti-tumoural effect (left): active maintenance of cellular quality control for cytosolic pro-oncogenic proteins such as p62 prevents malignant transformation of non-tumoural cells. In addition, the supply of energy provided through macroautophagy activation reduces the dependence on glycolysis, while assuring the energy required for maintenance of a stable genome, further preventing oncogenesis. Pro-oncogenic effect (right): the reduction in macroautophagic activity in early stages of the oncogenic process favours malignant transformation, as the accumulation of molecules such as p62 activates signalling mechanisms that promote necrosis and inflammation. Poor quality control as a result of diminished macroautophagy can also result in accumulation of defective mitochondria, with the subsequent release of harmful molecules (cytochrome c and reactive oxygen species) that contribute to further altering genome maintenance. However, as the tumour progresses, activation of macroautophagy is observed in many oncogenic processes, in part to compensate for the poor nutritional supply associated with rapidly growing tumours and to defend cancer cells against damage induced by anti-oncogenic therapies. In addition, enhanced mitochondrial degradation in this stage may contribute to the up-regulation of glycolysis to maintain the energetic balance (Warburg effect) characteristic of malignant cells.

Although it is clear that interrupting glycolysis eliminates the energetic advantage of the cancer cells and compromises proliferation, the mechanism by which autophagy up-regulates glycolysis is not known. It has been proposed that this effect could in part be mediated by activation of the removal of mitochondria via autophagy (mitophagy) to force energy dependence on glycolysis [119,120,122] (Figure 2). Autophagy could impact oncogenic transformation in multiple ways; in addition to the proposed effect on glycolysis, a better maintenance of highly efficient mitochondria through mitophagy has been recently proposed to also contribute to meeting the energetic requirements of the transforming cells [123]. Furthermore, an additional factor in this already complex scenario is the possible impact that metabolic changes in the surrounding stroma cells could have on the cancer cells. In fact, an autophagy-dependent metabolic switch in the cancer stroma fibroblasts has been recently proposed. This switch in the stroma could provide cancer cells with glycolytic intermediates necessary for efficient ATP production by oxidative phosphorylation [122].

Reduction in cellular energy upon autophagic compromise also decreases the fidelity of cellular processes such as DNA replication and mitosis, resulting in genetic aberrations [124]. The occurrence of these aberrations in tumour cells with defective quality control of organelles and toxic proteins due to the autophagic compromise can precipitate death of the cancer cells. In this respect, accumulation of the protein p62 (normally degraded by macroautophagy) when this pathway is compromised leads to the formation of p62 aggregates capable of stimulating inflammation by inhibition of nuclear factor-κB (NF-κB) and activation of NF-E2 related factor 2 (Nrf-2) [109,125,126] (Figure 2).

Little is known to date about the contributions of other forms of autophagy (CMA and microautophagy) to cancer biology. Recent studies have revealed that CMA contributes to the turnover of inactive forms of the M2 isoform of pyruvate kinase, a key enzyme in the maintenance of glycolysis in cancer cells [127]. A number of additional glycolytic enzymes have been recognized as CMA substrates, including glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, aspartate aminotransferase and aldolase B, strengthening the possible link between CMA and the energetic balance of cancer cells [49,128].

Collectively, the relationship between autophagy and cancer has proved to be a very complex one. This dual capability of autophagy to, on the one hand facilitate
tumour progression and, on the other hand diminish malignant transformation, has reinforced the need to critically evaluate autophagy in a disease-context and stage-specific manner, especially during the design of targeted therapy, as autophagy function is tightly related to these factors [78,94].

Effectors and modulators of macroautophagy have become attractive targets in the treatment of cancer. Autophagy up-regulation has been described in many different types of cancers in response to antioncogenic drugs, when it often exerts a protective effect [129,130]. Based on these findings, down-regulation of autophagy should be an effective way of enhancing the cytotoxic effect of different chemotherapy protocols. In fact, inhibition of macroautophagy has proved to be effective in enhancing the toxicity of drugs commonly used in cancer therapy, such as oxaloplatin [129] and the kinase inhibitors sorafenib [131], epirubicin [132] or 5-fluorouracil [133].

Different reports have also documented that the cytotoxic effect of some common anti-cancer agents is in part due to their ability to up-regulate autophagy [134–138]. These findings now raise a note of caution in the universal applicability of autophagy inhibitors as anti-cancer drugs. However, additional studies are required to clarify the contribution of the observed increase in autophagy in cancer cell death under these conditions. Often, the proposed increase in autophagy is based on the fact that levels of autophagy-related proteins are higher during the treatment. Up-regulation of autophagy markers does not necessarily mean increased autophagic flux. In fact, if autophagy is functioning properly, accumulation of autophagic compartments rarely occurs. It is thus possible that the observed increase in autophagic markers in some of these studies is actually a reflection of a compromise in the clearance of autophagic compartments, rather than in their formation. Accumulation of autophagolysosomes can contribute to cell toxicity and be detrimental to the affected cells. Interestingly, this toxic effect of the ‘congestion’ of the autophagic pathway is currently being explored for therapeutic purposes. Although some success has been obtained by directly targeting the final steps of autophagy, the lysosomal degradation, the characteristic plasticity of the lysosomal compartment makes a very prolonged inhibition necessary before this intervention becomes detrimental. One way around this problem, which is currently utilized in different clinical trials, is to combine the lysosomal blockage with enhanced formation of autophagosomes (eg by inhibition of the mTOR complex with rapamycin) [139–141]. The higher content of autophagic vacuoles, as a result of both increased formation and reduced clearance, can compromise cell viability by different mechanisms. Accumulation of these compartments in the cytoplasm interferes with intracellular trafficking and prevents recycling of essential cellular components (amino acids, free fatty acids, etc.). In addition, as autophagolysosomes persist inside cells longer than usual, their membranes often become leaky, facilitating the release of potent lysosomal enzymes in the cytosol, further enhancing cellular damage and toxicity.

### Autophagy and neurodegeneration

Maintenance of cellular homeostasis is essential in neurons, a typical example of non-dividing differentiated cells. The importance of autophagy in neuronal homeostasis has now been well supported, despite original disagreement on whether autophagy was active in neurons. The reason for this contention was the fact that autophagosomes, the morphological feature of macroautophagy, were rarely observed in neurons. However, recent studies support that this pathway is constitutively active and that it can be further up-regulated in neurons in response to very different stressors [142–144]. Perhaps the most conclusive support for the existence of basal autophagy in neurons and for its contribution to the maintenance of neuronal survival came from studies in transgenic mouse models conditionally knocked out for essential autophagy genes in the central nervous system [68,69]. These animals, even in the absence of any added stressor, displayed marked neurodegeneration associated with the accumulation of intracellular protein inclusions and neuronal loss. The efficient capability of the lysosomal system to remove newly formed autophagosomes could be behind the low occurrence of detectable autophagosomes in neurons in a given moment.

As with many other cells, neurons up-regulate autophagy in response to common stressors but also in defence against neuron-specific injuries, such as axotomy, neuronal ischaemia or excitatory toxicity [145–147] (Figure 3). Often, failure to up-regulate autophagy under these conditions or primary compromise of autophagic activity during the stress precipitates neuronal death post-injury [148]. However, activation of autophagy in neurons may have a relatively narrow window of opportunity, because recent reports have revealed that genetic or chemical inhibition of autophagy becomes beneficial at some specific states post-injury [105]. These findings suggest that either excessive up-regulation of autophagy, or up-regulation of this pathway under conditions in which autophagosome formation or clearance cannot be guaranteed, may be harmful to neurons [149]. In further support of the possible detrimental effect of massive up-regulation of autophagy, recent studies have shown that, under specific conditions, autophagosomes can become a source of reactive oxygen species and thus aggravate neurotoxicity [150].

Changes in autophagic activity have been described in many protein conformational disorders, and among them neurodegenerative diseases have received particular attention. The feature shared by all these conditions is the presence of pathogenic proteins that accumulate inside neurons, organized in the form of oligomeric or multimeric structures. The disease originates from...
both the loss of function of the pathogenic protein and the toxicity associated with the presence of the abnormal protein structures, which often leads to neuronal death. The first connection between neurodegeneration and autophagy originated from the observation that protein aggregates can be eliminated by autophagy [40, 74] (Figure 3). In fact, pharmacological activation of this catabolic process in experimental animal models of Huntington’s disease reduced cellular toxicity and slowed down progression of the disease [151]. After these initial reports, the degradation of many other pathogenic proteins by autophagy and the beneficial effect of up-regulation of this pathway have proved to be true in experimental models of many other neurodegenerative conditions [152]. In fact, not only the acute pharmacological activation of autophagy but even chronic conditions that lead to a progressive maintained up-regulation of this process, such as under conditions when ER stress is maintained, have been shown to have a positive effect [153]. These findings have now opened the possibility of utilizing modulators of autophagy as the basis for therapeutic approaches for these types of pathology and, in fact, ongoing chemical screenings aim at identifying chemical modulators of this pathway with higher selectivity and potency [154].

However, there are certain limitations to the universal use of up-regulation of macroautophagy for anti-neurodegenerative purposes. In fact, it has been reported that not all protein aggregates are recognized by the macroautophagic machinery. Despite the current dissection of molecules that participate in autophagic recognition of aggregates (p62, NBR1 and several cytosolic chaperones, including hsc70 and BAG-3), the presence of these proteins in an aggregate, although necessary, does not seem to be sufficient for autophagic recognition. Thus, some protein inclusions positive for these cargo-recognition proteins remain unnoticed by the autophagic machinery, making superfluous any attempt to enhance autophagic formation as a way to eliminate these toxic protein products [155]. Further studies are needed to address whether expression of other cellular components or specific post-translational modifications in the

Figure 3. Autophagy and Neurodegeneration. (A) Protective autophagy: macroautophagy and CMA both contribute to the maintenance of neuronal homeostasis and are necessary in the defence of neurons against injury and stressors. (B) Defective macroautophagy: defects in macroautophagy have been described to occur at very different levels in neurodegenerative diseases. Some of the possible steps affected in this process are highlighted in the model, and the conditions in which they have been observed are described in the text. (C) Defective CMA: primary defects in CMA have been described both in Parkinson's disease and in certain tauopathies. While in the former condition, pathogenic proteins such as α-synuclein can block access of other cytosolic proteins to lysosomes via CMA by abnormally binding to the translocation machinery, in tauopathies the accumulation at the surface of lysosomes of oligomeric forms of pathogenic tau targeted via CMA destabilizes the lysosomal membrane and results in leakage of lysosomal enzymes into the cytosol, which often triggers cellular death.
aggregated proteins could enhance their recognition by the autophagic systems.

A second scenario, in which chemical up-regulation of macroautophagy may not be effective against neurodegeneration, is in those conditions in which macroautophagy itself is compromised. Understanding the macroautophagy step or steps defective in specific neurodegenerative disorders is a priority if autophagy enhancers are to be moved forward in the treatment of these conditions. Examples of defects in almost all of the steps of macroautophagy have been described in different neurodegenerative disorders [66] (Figure 3). For example, initiation of autophagy may be compromised because of altered signalling through the insulin or mTOR pathways, which is tightly bound to activation of autophagy. Also, conditions resulting in lower content of available Atgs will reduce neuronal autophagic capability, such as the recently described depletion of LC3-II through abnormal interaction and aggregation with p62 in the presence of dopaminergic neurotoxin [156]. Along the same lines, abnormal interaction between mutant α-synuclein (the protein that accumulates in protein inclusions in Parkinson’s disease) and Rab1 has been shown to misplace Atg9, a protein required for the formation of autophagosomes, out of the ER membrane [157]. Under other conditions, problems arise at the level of cargo recognition, either because of alterations in the organelle-specific markers for degradation or in the autophagic machinery. A primary defect at the level of the limiting membrane that seems to prevent selective recognition of cargo has been recently described in cellular and animal models of Huntington’s disease [158]. In these cases, mutant huntingtin binds tightly to the inner surface of the forming autophagosome, resulting in an abnormally high interaction with p62 in this compartment that affects its ability to bind cargo. In other conditions, autophagosomes form and sequester the pertinent cargo but they fail to be cleared from the cytosol. Problems with clearance could result from alterations at very different levels. For example, problems with vesicular trafficking could indirectly interfere with the mobilization of autophagosomes toward the lysosomal compartment [159]. Pathogenic proteins can also interfere with the fusion step, which, although still not completely elucidated at the molecular level, is known to depend on different SNARE proteins, the actin cytoskeleton and the histone deacetylase 6 (HDAC6) [160]. Consequently, changes in any of these components could lead to intracellular accumulation of autophagosomes. Lastly, primary defects in the lysosomal compartment also have a negative impact on clearance of autophagosomes in different neurological disorders. The reasons for lysosomal failure could be multiple. In principle, most lysosomal storage disorders could compromise autophagosome clearance because often the accumulation of the undegraded product inside lysosomes limits their degradative capacity [161]. In addition, other conditions that alter lysosomal membrane stability, decrease lysosomal biogenesis or change lysosomal pH could also alter autophagosome clearance. In fact, defective acidification of lysosomes, because of compromised targeting of a component of the proton pump from the ER to the lysosomal membrane, has been already identified as causative of the reduced rates of autophagy in some models of Alzheimer’s disease [162]. As functional analysis of autophagy becomes more broadly utilized in the study of neurodegenerative diseases, it is likely that new connections between these pathologies and autophagy will become evident.

Alterations of CMA have also been described in some neurodegenerative disorders (Figure 3). Although in many of them, this pathway becomes up-regulated as a consequence of the failure in macroautophagy, different pathogenic proteins have been shown to directly interfere with CMA activity [163]. Mutant forms of α-synuclein can be targeted to lysosomes via CMA but, in contrast to the wild-type protein that binds to the lysosomal receptor and rapidly reaches the lumen for degradation, the mutant variants fail to translocate. Persistence of mutant α-synuclein tightly bound to the CMA receptor at the lysosomal membrane inhibits lysosomal degradation of other cytosolic proteins via this pathway [164]. A similar effect was later described for wild-type α-synuclein upon modification by dopamine, which makes CMA blockage relevant for sporadic forms of Parkinson’s disease, more common than the familial forms [165]. Later studies have also proposed interference of CMA by other Parkinson’s disease-related proteins, such as mutant forms of UCH-L1 [166]. A higher degree of lysosomal compromise as a result of lysosomal targeting of pathogenic proteins via CMA has been described in certain tauopathies, where the mutant forms of the protein not only interact abnormally with CMA components blocking this pathway, but also disrupt the lysosomal membrane as they organize into toxic oligomeric species on their surface [167].

To date, systematic studies analysing the contribution of alterations in microautophagy to neurodegeneration have not been performed. However, the fact that protein unfolding is not a prerequisite for microautophagy makes attractive the possibility that micro-aggregates can be delivered to this pathway for degradation.

Growing evidence supports that neurons utilize diverse ways to eliminate pathogenic proteins and that multiple steps of the autophagic process are often affected in a given neurodegenerative disorder. Thus, for example, in the case of Alzheimer’s disease (AD), macroautophagy contributes to the elimination of the toxic product of the amyloid precursor protein (APP) A-β. However, clearance of A-β is compromised as the disease progresses, due to the failure in this autophagic pathway [168]. In most forms of AD, the autophagic defect occurs in the late steps of macroautophagy. Thus, autophagosomes form properly sequestering the usual cargo but they are not eliminated through the
lysosomal system [168]. As described above, deficient acidification of the lysosomal lumen, due to the inability to target to lysosomes one of the subunits of the proton pump that usually acidifies this compartment, has been recently described in some types of AD [169]. Persistence of autophagosomes in AD neurons is particularly toxic because the enzymes responsible for processing of APP into A-β as well as APP accumulate in these compartments. Autophagosomes become in this case a new site of intracellular production of toxic A-β [168]. Failure of the autophagic system compromises the elimination of aggregate forms of tau, a protein that also accumulates in AD neurons. In fact, for certain types of tau mutations, this pathogenic protein could contribute to the failure of macroautophagy, due to the toxic effect that the still soluble forms of the protein exert in the membrane of lysosomes when they are delivered to this compartment by CMA [167].

Similarly, in the case of Parkinson’s disease (PD) the most common pathogenic protein, α-synuclein, is usually degraded by different autophagic and non-autophagic pathways. Soluble forms of the protein are substrates of both the ubiquitin proteasome system and of CMA [164,170]. However, macroautophagy is the only plausible way for the elimination of the pathogenic variants of this protein once they aggregate [171]. As in the other disorders, pathogenic forms of α-synuclein interfere with the normal functioning of the ubiquitin proteasome pathway [170], CMA [164,165] and even macroautophagy at the level of autophagosome formation [157]. The recently identified physiological role for Parkin, a protein also related to PD, in mitophagy, suggests that alterations of this selective form of autophagy could also contribute to the pathogenesis of this disease [172].

Macroautophagy has proved to be an effective mechanism for the degradation of aggregates of huntingtin, the protein mutated in Huntington’s disease (HD) and main component of the inclusions observed in the affected neurons [151,173]. Huntington is a very dynamic protein that undergoes physiological cleavage into different functional fragments that likely are turned over by different proteolytic systems. Mutant huntingtin, even before aggregation, seems to be primarily a target for macroautophagic degradation [174], although specific posttranslational modifications can also favour its elimination by CMA [175]. As in the other neurodegenerative disorders, growing evidence supports a direct toxic effect of the mutant protein on the autophagic system. For example, mutant huntingtin has been described to accumulate on the luminal side of the limiting membrane as the autophagosome forms and thus interfere with the recognition of cytosolic cargo, including huntingtin protein aggregates [158]. Alterations at the level of autophagosome trafficking and clearance have also been described in some experimental models of HD [176]. Although huntingtin bears several CMA-targeting motifs in its amino acid sequence, the contribution of this pathway to the removal of the pathogenic protein is, overall, modest. However, recent studies support a beneficial effect of the artificial targeting of mutant huntingtin for CMA degradation that could be explored for therapeutic purposes [177].

It is anticipated that a similar dual interaction of different pathogenic proteins with the autophagic systems—by which they can be eliminated through these pathways but also interfere with their normal functioning—could also contribute to pathogenesis in other neurodegenerative disorders.

**Autophagy and the failing heart**

The heart is comprised of long-lived, post-mitotic cells with little regenerative capacity, which are continually subjected to stress, such as ischaemia, pressure overload and ischaemia–reperfusion injury. Adaptation to these conditions is attained through remodelling (myocytes elongate and undergo hypertrophy) and failure to do so frequently constitutes the basis of coronary artery disease, hypertension and congestive heart failure [178,179]. Evidence for the role of basal autophagy in the quality control and housekeeping of cardiomyocytes was first obtained through genetic models of LAMP-2 knock-out mice (defective for autophagosome–lysosome fusion) that demonstrated cardiomyopathy and abnormal accumulation of autophagic vacuoles, similar to that observed in Danon’s disease patients [180,181]. In fact, mutations in the LAMP-2 gene were subsequently described in these patients [182]. Studies in cardiomyocyte-specific Atg5 and Atg7 knock-out models have reiterated the need for functional autophagy to preserve normal cardiac function, both under basal conditions and in response to stress [183]. In fact, pronounced loss of autophagy in cardiomyocytes with age seems to be behind different forms of age-related cardiomyopathy [184].

Added to the key role of basal macroautophagy in cardiomyocyte homeostasis, growing evidence also supports an important contribution of this pathway in the heart in response to stressors. As described in the brain, macroautophagy has also proved to be essential for the prevention of cardiomyocyte proteinopathies, such as those arising from desmin and α-β-crystallin accumulation [185,186]. Autophagy induced in response to cardiac stress (especially during ischaemia–reperfusion injury) also plays a cytoprotective role in the heart. In fact, in support of this prosurvival function, different studies have demonstrated that pharmacological inhibition of autophagy during mild ischaemic stress enhances cardiomyocyte death [187,188], whereas macroautophagy activation is cardioprotective [189,190]. The beneficial effect of macroautophagy up-regulation under these conditions could result, in part, from an efficient removal of the mitochondrial population compromised under these conditions. Thus, mitochondrial membrane depolarization and increased production of reactive oxygen species by mitochondria are common features associated to ischaemia–reperfusion. Degradation of the altered mitochondria by macroautophagy under these conditions could lead to a cytoprotective response.
conditions has been shown to efficiently ameliorate cellular damage by reducing the activation of pro-apoptotic cascades [191–193].

However, induction of macroautophagy in the stressed heart can also be detrimental and contribute to heart failure, judging by the fact that pharmacological or genetic blockade of macroautophagy enhances cell survival post-ischaemia–reperfusion injury in specific settings [194]. The timing and the nature of the condition that induced cardiac stress may be essential for the switch in the effect of macroautophagy. Thus, some studies suggest that macroautophagy is cardioprotective during ischaemic recovery but maladaptive during reperfusion recovery [195]. It is possible that, during reperfusion, the need for removal of dysfunctional mitochondria and oxidatively damaged cellular structures that accumulated during the ischaemic period could drive the autophagic response to be more aggressive than desired, resulting in cell death. In fact, genetic up-regulation of autophagy in response to the haemodynamic stress-induced hypertrophic growth response has also proved to be maladaptive and to result in pathological cardiac hypertrophy [196].

In summary, as in the case of tumour biology and neurodegeneration, the role of autophagy in heart pathology is also context-dependent and excessive or insufficient macroautophagy is often associated with disease. However, when maintained under strict control, up-regulation of macroautophagy by compounds such as resveratrol has proved to be a useful cardioprotective strategy during ischaemia–reperfusion injury [197–199].

Although the heart is one of the organs in which CMA is up-regulated most rapidly in response to starvation [200], the contribution of CMA to cardiomyocyte homeostasis and to heart pathology has not been explored in depth. As mentioned above, mutations in \textit{LAMP-2} occur in patients with Danon’s cardiomyopathy, but the phenotype seems, for the most part, to be related to the presence of large autophagic vacuoles in muscle, reflective of compromised macroautophagy. This could be explained by the fact that the \textit{lamp2} gene undergoes splicing, giving rise to three protein variants, A, B and C [201] and that mutations in only the B variant, tightly related to macroautophagy, are enough to reproduce the full vacuolar phenotype [182], whereas changes in \textit{LAMP-2A}, the variant required for CMA, do not affect macroautophagy [202].

\section*{Autophagy, infectious disorders and autoimmunity}

The autophagy machinery can act as a cell-autonomous defence against invading pathogens through a process known as xenophagy [203]. Activation of this process has been extensively reported, for example, in response to Group A \textit{Streptococcus} infection in epithelial cells (Figure 4). The fact that Atg5 deficiency allows bacteria to survive and multiply robustly supports the normal contribution of autophagy in the elimination of this pathogen [204]. Autophagic surveillance is not limited to bacteria as, in fact, a protective role for autophagy has been also demonstrated for the vesicular stomatitis virus, herpes simplex virus 1 or HIV-1, among others [205–207].

Cytosolic autophagy often becomes a second surveillance point for pathogens such as \textit{Listeria monocytogenes} that can escape phagosomes by puncturing their membrane to replicate in the cytoplasm. However, once in the cytoplasm, the autophagic surveillance mechanism is activated to entrap the escaped bacteria and deliver them to endocytic and lysosomal compartments for degradation [208]. Pathogens that survive inside vesicular compartments can also be controlled by macroautophagy. For example, \textit{Mycobacterium tuberculosis} resides in phagosomes by avoiding their fusion to lysosomes. Interestingly, this blockage can be overcome by induction of autophagy, leading to the degradation of this pathogen [209].

Autophagy proteins may also help to accelerate phagosome maturation through a process that has been termed ‘LC3-associated phagocytosis’. Engagement of toll-like receptors has been shown to induce recruitment of LC3 to the phagosomal membrane and favour fusion with the lysosome [210].

Despite this active involvement of autophagy in pathogen elimination, some pathogens have evolved to take advantage of the different compartments of the autophagy pathway to establish replicative niches [211,212] (Figure 4). Evidence that the autophagosome compartment is required for the replication of certain pathogens, such as \textit{Porphyromonas gingivalis} or \textit{Coxiella burnetti} was provided by demonstrating that treatment with 3-methyladenine, a well-characterized inhibitor of autophagosome formation, was efficient in decreasing the survival of these pathogens [213,214]. In fact, some of these microorganisms that utilize compartments of the autophagic system have developed mechanisms to expand the size and number of these compartments. For example, \textit{Staphylococcus aureus} is capable of secreting a factor that activates autophagy [215]. Seclusion inside the autophagosomes may be beneficial for pathogens as a way to escape the cytosolic surveillance mechanisms, but these compartments may also contain cytosolic materials that can be utilized as an energy source by the microorganisms.

This bivalent role of autophagy in pathogen defence suggests that, although it should be possible to pharmacologically manipulate the autophagic system to eliminate different pathogens, it is essential to first understand the characteristics of the pathogen–autophagy interaction in each of the individual instances [216,217].

Apart from its role in cell-autonomous immunity, autophagy plays a role in activation of adaptive immunity through its recently described involvement in antigen processing and presentation to lymphocytes [218–220] (Figure 4). In professional APCs, phagosome-processed extracellular antigens are presented through MHC class II molecules to CD4 T
Figure 4. Autophagy and the immune system. (A) **Macroautophagy against pathogens**: macroautophagy contributes to the elimination of different types of pathogens—bacteria and viruses—when they escape to the cytosol after internalization in the phagosome. Under certain conditions, fusion of autophagosomes with phagosomes is required before degradation can occur. (B) **Pathogens using macroautophagy**: growing evidence supports that certain pathogens have evolved to utilize autophagosomes as a site of replication and can actively prevent the fusion of this compartment, with lysosomes to guarantee their survival. (C) **Antigen presentation**: all three forms of autophagy, macroautophagy, CMA and microautophagy have been shown to contribute to antigen loading of MHC class II molecules for presentation of antigens to activate T cells.

cells. However, several cytosolic and nuclear proteins have been found associated with MHC II molecules [221,222]. In fact, pharmacological inhibition of macroautophagy has been shown to be efficient in reducing MHC class II intracellular antigen presentation [223–225], whereas activation of autophagy promotes this type of presentation [226]. Later studies have confirmed the presence of intracellular antigens in autophagosomes, and marked reduction of MHC II presentation in lymphoblastoid cell lines upon knock-down of essential Atgs, as well as in vivo in Atg5-deficient dendritic cells [227,228]. Interestingly, presentation of self-antigens by class II molecules is not limited to macroautophagy but may also involve other types of autophagy. In fact, over-expression of LAMP-2A, the receptor for CMA, has been shown to enhance MHC II presentation of intracellular antigens [229], and a new microautophagy-dependent process of antigen delivery into late endosomes in dendritic cells has also been recently characterized [31]. Furthermore, participation of autophagy in antigen presentation may not be limited to presentation through MHC II. Although it is traditionally accepted that MHC class I presents intracellular antigens processed through the proteasomal pathway, growing evidence supports that macroautophagy could also assist in MHC I presentation by collaborating with the proteasome in the processing of intracellular proteins [230,231].

Also of interest is the recently proposed contribution of autophagy in the establishment of tolerance to self antigens. Self-reactive T cells are usually actively eliminated through MHC class II presentation in epithelial cells of the thymus to allow T cell tolerance to self-antigens [232]. Proof that autophagy regulates central T cell tolerance has been provided by a recent study showing that transplantation into athymic nude hosts of Atg5-deficient thymus results in defects in positive and negative selection and the development of autoimmunity [233]. Interestingly, the antigen-loading compartments for MHC class II in immature dendritic cells, which are involved in peripheral tolerance, continuously receive input from autophagosomes [234]. Furthermore, genetic polymorphisms in two different autophagy genes (ATG16L1 and IRGM) have been linked to Crohn’s disease [235,236]. Compromised autophagic function may result in insufficient induction of tolerance against commensals or self-antigens in the gut, [235,236], although it may also affect the secretion of antimicrobial proteins by Paneth cells [7]. This novel role of autophagy in the establishment and maintenance of tolerance also suggests a possible role for autophagy in autoimmunity [237]. In this

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Conclusions and future perspectives

The better molecular characterizations of the different autophagic pathways, as well as the possibility of genetically manipulating these cellular processes, have helped to establish tight connections between autophagic malfunctioning and disease. The initial excitement about the possibility of modulating autophagy with therapeutic purposes in different disorders has been followed by some degree of confusion as to whether autophagy should be up- or down-regulate in these conditions. As described in this review, both up- and down-regulation of macroautophagy have been shown to be protective against cancer, neurodegeneration, infectious diseases and ischaemic insult in the heart. How to decide what to do? Further analysis in most of these conditions is required to fully understand the contribution of autophagic malfunctioning to the disease. Questions, such as when in the course of the disease autophagic function becomes compromised, what changes in each autophagic pathway and whether these changes are primary or compensatory to failure in other systems, need to be answered before autophagy modulators can be systematically used in the treatment of these conditions.

A considerable advance in this respect has been the introduction of functional read-outs of autophagy to complement the initial morphological analysis. For example, the presence of a higher number of autophagosomes in many disease conditions, initially interpreted as an increase in macroautophagy, is now more cautiously analysed, because blockage in the late steps of this pathway can also have a similar morphological signature. Thus, conditions initially labelled as having ‘too much autophagy’ are being currently revised as having ‘a blockage in autophagic clearance’. Blocking autophagosome formation may only be beneficial then, when excessive autophagosome content and autophagic clogging contribute to the pathology, but not when autophagy is up-regulated in the disease to compensate for failure in other systems. Along the same lines, the ultimate goal may not be to repress autophagosome formation in some of these cases, but instead to facilitate their clearance by directly repairing possible defects in the late steps of the autophagic process.

In addition to determining the right timing for modulating autophagy in many of these diseases, and whether this process should be up- or down-regulated, further expansion of the chemical options to manipulate autophagy is needed for it to become a routine therapeutic target. To date, most of the macroautophagy inhibitors used in clinical trials act during the late steps of this process, on the lysosomal compartment, which is also shared by other autophagic pathways. In contrast, for activators, most of the available drugs have an effect on initiation and autophagosome formation, but few compounds have been shown to be effective at increasing the overall autophagic flux. Ongoing chemical screenings are currently addressing the need for these types of modulators and should render usable molecules in a timely fashion [154].

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Author’s contributions

All authors contributed separate sections to this review and all read and edited the final submitted version.

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