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Mini Review

# Cell death pathology: Cross-talk with autophagy and its clinical implications

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ABSTRACT

Autophagy is a self-digesting mechanism that cells adopt to respond to stressful stimuli. Morphologically, cells dying by autophagy show multiple cytoplasmic double-membraned vacuoles, and, if prolonged, autophagy can lead to cell death, "autophagic cell death". Thus, autophagy can act both as a temporary protective mechanism during a brief stressful episode and be a mode of cell death in its own right. In this mini-review we focus on recent knowledge concerning the connection between autophagy and programmed cell death, evaluating their possible implications for therapy in pathologies like cancer and neurodegeneration.



## 1. Introduction

Autophagy is a physiological self digestive process which is involved in the degradation of damaged proteins and intracellular organelles [1]. Under specific stress circumstances, however, autophagy contributes to the regulation of proliferation, differentiation and cell death. During autophagy, cytoplasmic proteins, organelles and other cellular components are surrounded by autophagosomes, which form autolysosomes by fusing with lysosomes, resulting in the degradation of these components by resident hydrolases. The link between autophagy and cell death is demonstrated at the molecular level, for example, by the physical interaction between Bcl2 and Beclin-1 [2], however at the functional level it is still somewhat controversial. Autophagy undoubtedly enhances cell survival in response to nutrient deprivation, but dying cells often display accumulation of autophagosomes, and sustained autophagy can lead to cell death.

The molecular basis of autophagy was initially characterised in yeast in which at least 15 autophagy related genes (ATG) have been identified; subsequently their mammalian counterparts have also been characterised (Fig. 1) [3]. While other reviews describe the molecular pathways of autophagy in detail, here we would like to draw attention to the fact that knockout and knockdown of Atg proteins enhances cell death induced by starvation and growth factor withdrawal (Fig. 1), but in other situations inhibition of autophagy maintains cellular viability [4–11]. The finding of vesicular accumulation in dying cells has led to the concept of autophagic cell death (ACD). ACD is defined morphologically as a type of cell death that occurs in the absence of chromatin condensation and with a large degree of vacuolization of the cytoplasm. ACD does not necessarily imply that the cell death occurs by autophagy, but that death occurs concurrently with morphological features of autophagy. Thus, if cell death occurs only in parallel with autophagic features, inhibition of autophagy does not alter cell fate, while when autophagy is the crucial effector mechanism of cell death, its inhibition determines cell fate. Therefore the current definition of ACD has an important limitation in that it fails to establish the necessary role of autophagy in the cell death process, and thus contributes to the confusion in the literature regarding the role of autophagy in cell death and cell survival.

## 2. Cross-talk between autophagy and cell death

Increasing evidence is now accumulating on the crosstalk between apoptotic and autophagy pathways. A key regulator of autophagy initiation is the mammalian orthologue of the yeast Atg6, Beclin 1 (Bec1), that forms part of the class III phosphatidylinositol 3-kinase (PI3K) complex [12]. The anti-apoptotic protein Bcl-2 was the first to be identified of an increasing number of Bec1-interacting proteins. The dissociation of Bec1 from Bcl-2 is essential for its autophagic activity, and Bcl-2 only inhibits autophagy when it is present in the endoplasmic reticulum (ER) (Fig. 2) [2,13–15]. A similar interaction has also been described for the Bcl-2 homologue, Bcl-XL [16]. Bec1 has also been shown to be one direct caspase substrate among the large number of caspase targets [17,18]. Caspase-mediated cleavage of Bec1 results in the loss of its

*Abbreviations:* ATGS, autophagy related genes; ACD, autophagic cell death.

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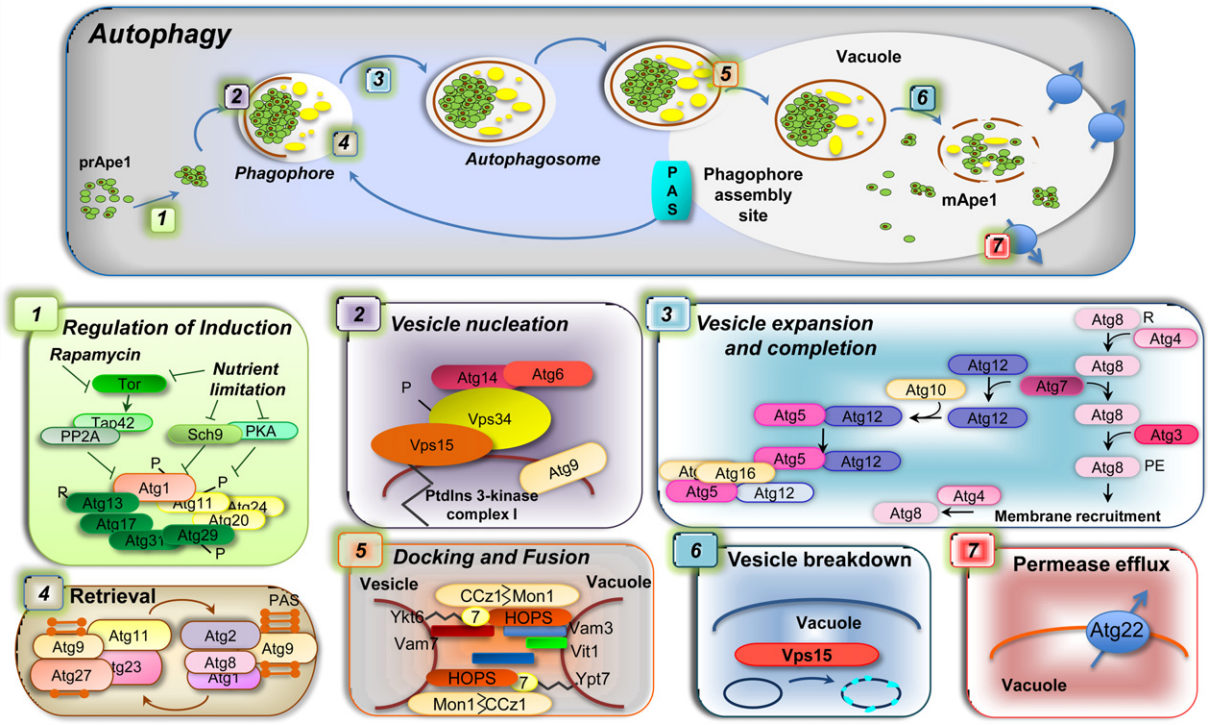


**Fig. 1.** Schematic overview of autophagy. Autophagy can be divided into seven steps: (1) induction; (2) vesicle nucleation; (3) vesicle expansion and completion; (4) retrieval; (5) fusion; (6) breakdown; (7) efflux. (1) Regulation of induction. Activation of autophagy upon nutrient starvation or growth factor deprivation appears to be mainly mediated by inhibition of the Tor protein kinase. Formation of the Atg1–Atg13 protein kinase complex occurs downstream of Tor inhibition. (2) Vesicle nucleation. Formation of the Atg1–Atg13 complex induces acquisition and processing of membrane material for autophagosome formation. The specific components that act in vesicle nucleation are not completely clear. The PI 3-K complex I, consisting of Vps15, Vps34, Atg6/Vps30 and Atg14, is required for vesicle nucleation in autophagy. (3) Vesicle expansion and completion. Atg8 undergoes proteolytic cleavage of the C-terminal arginine residue (R), by the Atg4 cysteine protease. Atg8 and Atg12 are ubiquitin-like proteins that are activated by the E1-like enzyme Atg7. Atg8 and Atg12 are then transferred to the E2-like enzymes Atg3 and Atg10 and are then conjugated to phosphatidylethanolamine (PE) and Atg5, respectively. Atg8 conjugated to PE acquires the ability to be anchored in the membrane of the PAS (Phagosome Assembly Site) and acts there as a component of the nascent and mature autophagosome. Atg8 is released from the outer membrane of the completed vesicle by a second Atg4-dependent cleavage. (4) Retrieval. Retrieval involves several other Atg proteins. Most Atg proteins are soluble and can easily be released from the membrane surface while Atg9 and Atg27 are integral membrane proteins. The mechanism of targeting and release is unknown. (5) Docking and fusion. Vam3, Vam7, Vti1 and Ykt6, are members of the SNARE family, and, together with Ypt7 and HOPS, they play a role in membrane fusion in a variety of cellular contexts including autophagy. (6 and 7) Breakdown and efflux. The vesicle lysis step depends on the acidic pH of the vacuole lumen and some proteinases. Atg15 has also been implicated in this process. Atg15 has sequence similarity to a family of lipases, and seems likely to function directly in vesicle breakdown. Atg22 is an integral membrane protein located in the limiting membrane. Atg22 is not directly required for the breakdown of autophagic bodies within the lysosome/vacuole, but it acts to mediate the efflux of amino acids resulting from autophagic degradation. This figure was modified from figures previously published by D.J. Klionsky [80,81].

autophagy-inducing capacity, and the release of pro-apoptotic factors due to a direct interaction of a C-terminal fragment, Beclin-1-C, with mitochondria (Fig. 2) [19].

A further link between autophagy and apoptosis is the suppressor effect that the apoptosis inhibitor, cFLIP (Flice inhibitory protein), can exert on autophagy. FLIP competes with the Atg8 orthologue, LC3, for Atg3 binding, thereby preventing Atg3-mediated autophagosome elongation (Fig. 2) [20]. A further point of interconnection is mTOR. The PI3K/Akt/mTOR pathway has been implicated in promoting cell survival in several different tissues [21]. mTOR, along with AMPK, has been shown to phosphorylate the mammalian homologue of Atg1, Ulk1, and thus influence the early stages of autophagic initiation. Therefore regulation of mTOR may represent a crucial point in regulating the balance between cell death and autophagy as reported in different contexts [22–24].

The same cellular stress can in some cases activate both the apoptosis pathway and the autophagic mechanism. As an example, p73 [25–27] is able to both kill via a c-Abl-dependent activation [28] that leads to Puma activation [26,29,30] and also to activate the mTOR pathway [31]. While the former mechanism has been strongly linked to cancer, the latter still awaits a pathological link, and, important for this discussion, the two mechanisms are closely interrelated in cancerogenesis.

These examples highlight the role of factors known to regulate apoptosis in the regulation of autophagy and suggest potential mechanisms how the interrelationship between these processes may be coordinated.

## 3. Autophagy and cancer therapy

The interaction between apoptosis and autophagy has important implications for cancer therapy. Because one function of autophagy is to act as a survival response to unfavourable conditions, it is reasonable to postulate that it may play a negative role in cancer therapy outcome.

Many factors and mechanisms, implicated at different levels in the regulation of apoptosis, show features which can modulate or predict cytotoxic drug response, such as mitochondrial- and ER-dependent apoptosis pathways [32–34], death receptor pathways [35–37], microRNAs [38–41], and kinases and phosphatases involved in signal transduction [42,43]. Some of these have already been targeted therapeutically while others are potential new pharmacological targets [44,45]. In addition, components of these pathways may be useful as molecular biomarkers [46–48] to monitor and predict cancer therapy outcome.

From what has been discussed above, it is likely that autophagy may act as a protective mechanism to counteract the cellular stress induced by chemotherapy. This may be especially the case, since cells within the tumour core already have low nutrient supplemen-