

Autophagy and cancer

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Core tip: The differential expression of selective autophagic receptors in cancers of different origin and stage might induce the selective removal or preservation of certain cellular components and contribute to either tumor suppression or cancer cell survival.

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Abstract

Autophagy is a homeostatic and evolutionarily conserved mechanism of self-digestion by which the cells degrade and recycle long-lived proteins and excess or damaged organelles. Autophagy is activated in response to both physiological and pathological stimuli including growth factor depletion, energy deficiency or the upregulation of Bcl-2 protein expression. A novel role of autophagy in various cancers has been proposed. Interestingly, evidence that supports both a positive and negative role of autophagy in the pathogenesis of cancer has been reported. As a tumor suppression mechanism, autophagy maintains genome stability, induces senescence and possibly autophagic cell death. On the other hand, autophagy participates in tumor growth and maintenance by supplying metabolic substrate, limiting oxidative stress, and maintaining cancer stem cell population. It has been proposed that the differential roles of autophagy in cancer are disease type and stage specific. In addition, substrate selectivity might be involved in carrying out the specific effect of autophagy in cancer, and represents one of the potential directions for future studies.

INTRODUCTION

Autophagy is an evolutionarily conserved catabolic pathway which delivers long-lived proteins and excess or damaged organelles into the lysosome for degradation and recycling^[1,2]. Three mechanistically distinguished subtypes including macroautophagy, microautophagy and chaperon-mediated autophagy exist, of which, macroautophagy (hereafter referred to as autophagy) is most studied. Traditionally known as a mechanism to maintain homeostasis and degrade cellular components in response to starvation, further functions have been identified as our understanding of autophagy has progressed. A novel role of autophagy in cancer has also been proposed in recent years. In the current review, we attempt to provide a brief evaluation of the current literature and discuss the potential mechanisms of how autophagy is involved in the pathogenesis of cancer.

AUTOPHAGY MACHINERY AND REGULATION

The basic machinery and regulation of autophagy has

been described in numerous excellent reviews^[1,3-6] and will not be discussed in detail here. We will briefly introduce the autophagy process and key players to facilitate our further discussion. Autophagy process is divided into four stages: nucleation, elongation, autophagosome formation and fusion. The nucleation is initiated by the dephosphorylation (*i.e.*, activation) of the unc-51-like kinase (ULK) complex. ULK complex is otherwise kept inactive by the mammalian target of rapamycin (mTOR), a highly conserved serine/threonine protein kinase. mTOR integrates the signal of growth factor and nutrition availability and serves as the pivotal inhibitory regulator of autophagy. In other words, limited growth factor and nutrient inactivates mTOR and release ULK complex from its inhibition. Upon activation, ULK complex induces the re-localization of a phosphatidylinositol-3-kinase-class III (PtdIns3K) complex, which is composed of vacuolar protein sorting 34 (Vps34), p150, mAtg14 and Beclin1, to the nucleation site. Beclin1 mediates the cross-talk between autophagy and apoptosis in that it is a binding partner of anti-apoptotic Bcl-2 family proteins (*e.g.*, Bcl-2, Bcl-xl and Mcl-1). Beclin1 can be sequestered by these Bcl-2 proteins, which will prevent the formation of PtdIns3K complex and thereby block the nucleation process. The pro-apoptotic BH-3 only Bcl-2 proteins (*e.g.*, Bnip-3, Bad and Puma) compete with Beclin1 for the binding to anti-apoptotic Bcl-2 proteins and hence promote autophagy. Once formed, PtdIns3K complex catalyzes the production of phosphatidylinositol (3)-phosphate [PtdIns(3)P], which further recruits autophagy related (Atg) proteins. The two interrelated ubiquitin-like conjugation systems, Atg12-Atg5-Atg16 and microtubule-associated protein light chain 3 (LC3)-phosphatidylethanolamine (PE) play a major role in the elongation of the phagophore. The subsequent step, autophagosome formation, is accomplished by the invagination of phagophore membrane and the sequestration of cytosolic contents. In order for its contents to be degraded, the autophagosomes will form autolysosomes by fusing with lysosomes or late inner body.

SUBSTRATE SPECIFICITY OF AUTOPHAGY

In recent years, the concept of substrate selectivity in autophagy has gained further recognition. This is quite different from the initial understanding of autophagy, which was regarded as a non-specific self-eating process. However, recent studies have indicated that a specificity for substrate in autophagy is conveyed through different receptor proteins. More importantly, a correlation between the targeted removal of cellular components by autophagy and human diseases has been established^[7]. The autophagy receptors, which play a key role in the substrate selectivity^[8], tether the substrate of interest to the autophagic machinery (LC3) through a specific sequence called LC3-interacting region (LIT) motif^[9-12]. For example, p62/SQSTM1 (p62) participates in aggrephagy

(protein aggregate autophagy) and p62 binds ubiquitinated protein aggregates through an ubiquitin-associated (UBA) domain. On the other hand, BCL2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3), which is a mitochondrial localized BH-3 only Bcl-2 family protein, is involved in mitophagy (mitochondrial autophagy). Both p62 and BNIP3 induce degradation of their specific target by autophagy via their LIT motifs^[10-13].

AUTOPHAGY AND CANCER

As a pro-survival pathway, the role of autophagy in cancer has long been speculated. However, significant evidence suggests that autophagy might participate in both tumor suppression and tumor maintenance. Furthermore, the resistance to chemotherapy, which is one of the major obstacles in the treatment of cancers, has been linked to autophagy, as supported by the latest studies. This multiplicity function of autophagy in cancer is discussed in detail below.

Autophagy as a tumor suppression mechanism

The first evidence of a tumor suppressive role of autophagy in cancer originated from the observation that heterozygous loss of the Beclin1 encoding gene (*Becn1*) was detected in breast, ovarian and prostate cancer^[14]. Subsequent studies with mouse models further established the role of autophagy in tumor suppression. *Becn1* heterozygous knockout mice developed tumors of both benign and malignant nature in various tissues^[15,16], suggesting that *Becn1* is a haploinsufficient tumor suppressor gene^[16]. Similarly, a mouse model with systemic mosaic deletion of Atg5 and the liver-specific homozygous deletion of Atg7 both developed benign liver adenomas^[17]. Vice versa, reintroducing beclin-1 into human breast carcinoma cells decreased both the proliferation *in vitro* and tumorigenesis capacity *in vivo*^[14]. Apart from the experimental evidence, the tumor suppressive role of autophagy is also supported by the observation that other tumor suppressor genes, such as ULK3, UV irradiation resistance-associated gene and Bif-1, frequently participate in autophagy signaling^[2,18]. On the other hand, the overexpression of oncogenes usually imposes a negative effect on autophagic activity. For instance, PI3K/AKT pathway, which is activated in various cancers, suppresses autophagy through mTOR phosphorylation (*i.e.*, activation)^[19]. The up-regulation of anti-apoptotic Bcl-2 proteins in cancer also suppresses autophagy via Beclin1, as described above.

The knowledge regarding the mechanisms underlying the role of autophagy in tumor suppression is still limited. However, an interesting study by Mathew *et al.*^[20] have reported that the allelic loss of Beclin1 results in increased chromosomal instability. They further showed that the altered regulation of nuclear factor κ B, which resulted from p62/SQSTM1 (p62) and reactive oxygen species (ROS) accumulation, is responsible for the damage induced by autophagy deficiency^[20].

Senescence is also a potential mechanism by which

autophagy can exert a tumor suppressive role. Senescence is the status of cell cycle arrest with active metabolism^[21]. Autophagy has been shown to activate senescence in cultured human lung fibroblast cells^[21]. Similarly, autophagy has been suggested to mediate senescence in primary biliary cirrhosis^[22]. By inducing senescence in transformed cells, autophagy can induce cell cycle arrest in transformed cells and prevent tumorigenesis.

Another plausible route of tumor suppression is through autophagy-mediated cell death^[23]. Although the definition and mechanisms by which autophagy induces cell death is still under debate, several studies strongly support a role for autophagic cell death in tumor suppression. Gurpinar *et al.*^[24] have shown the involvement of autophagy in cell death induced by the treatment of lung adenocarcinoma cells with sulindac sulfide amide. Interestingly, cell death in this system occurred in the absence of caspase activation^[24]. In addition, Lamy *et al.*^[25] have shown that myeloma cells can avoid cell death by restricting the autophagic activity through the cleavage of autophagic inducer, BCL2-interacting protein BCLAF1, by caspase-10.

Autophagy and tumor maintenance

Interestingly, a role in promoting and maintaining tumors has also been suggested for autophagy regarding cancer development. The conditions which induce autophagy, such as nutrient deprivation, hypoxia and reactive oxygen species, are also present in the tumor microenvironment, especially in tumors with limited blood supply. Yang *et al.*^[26] have shown that the basal level of autophagy is elevated in pancreatic cancers. Blocking autophagy by chemical inhibitors or RNAi methodology inhibits the tumorigenic potential of the cancer cells, as determined by both *in vitro* and *in vivo* assays^[26]. Autophagy inhibition is also correlated with a decrease in oxidative phosphorylation and ATP production. Similar findings were also reported with Ras-transformed immortal, nontumorigenic mouse kidney epithelial cells isolated from baby mice^[27]. In addition, the requirement for a functional autophagy machinery for Ras-induced cellular transformation has also been confirmed in other cell models^[28,29].

The understanding of the mechanisms by which autophagy supports oncogenic growth is still in its infancy. One possibility is that autophagy process might be used by cancer cells to meet their energy requirements. As discussed in earlier sections, there is a connection between autophagy inhibition and the depletion of intracellular ATP stores and oxidative phosphorylation^[26,27]. However, the requirement of oxidative phosphorylation by cancer cells is unclear because cancer cells have been suggested to be dependent more on glycolysis to fuel their growth (aka Warburg effect) even in the presence of oxygen (*i.e.*, aerobic glycolysis)^[30]. Nevertheless, some studies point to the intriguing possibility that cancer cells can stimulate autophagy in the adjacent stromal cells, which in turn provide cancer cells with metabolic substrates^[31]. Another potential mechanism may be linked to the organelle qual-

ity control function of the autophagy process. Damaged organelles, such as mitochondria, can be targeted for autophagy by the BH-3 only Bcl-2 family members including Pink3, BNIP3 and Nix proteins^[12,13,32]. Of note, any damage to mitochondria will induce ROS production and may lead to genomic instability^[33,34]. Autophagy has also been shown to be directly involved in the degradation and elimination of oxidized proteins. Despite playing a positive role in the initial stages of tumorigenesis, oxidative stress and genomic instability are detrimental to tumor growth in the later stages^[2]. It is therefore feasible that autophagy can mitigate these damages and thereby sustain oncogenic growth^[35].

Autophagy and tumor therapy resistant tumors

An association between autophagy and the effectiveness of treatment has also been suggested by recent studies. For example, autophagy has been reported to be elevated in pancreatic cancer cells treated with chemotherapeutic drugs^[36-38]. It should however be noted that there was no consensus as to whether the increased autophagic activity contributes to cell death^[36,37] or facilitates cancer cell survival under stress conditions in pancreatic cancer^[38]. On the other hand, in a different model using Myc-induced lymphoma, Amaravadi *et al.*^[39] have reported that chemotherapy induces autophagy and that the inhibition of autophagy enhances apoptosis induced by chemotherapy drugs. Furthermore, autophagy inhibitors, such as chloroquine and hydroxychloroquine have been shown to exhibit a synergistic effect with chemotherapy and radiotherapy^[39-43]. Autophagy inhibitors are currently being tested in clinical trials as part of the combined therapy approach for various cancers^[6].

Tumor maintenance function (see above) of autophagy may also alleviate the stress induced by cancer therapy and thereby induce therapy resistance. Besides, the new role ascribed to autophagy in the regulation of cancer stem cell (CSC) phenotype^[44,45] might serve as a potential mechanism for autophagy to promote therapy resistance. The so-called "cancer stem cell theory" has generated lively discussion in recent years. CSCs are a small (< 5%) subpopulation of heterogeneous cancer cells, which are capable of self-renewing and differentiating into the whole spectrum of tumor cell population. CSCs have also been suggested to be resistant to treatment^[46-50]. Despite the controversy, which still exists regarding the characteristics of CSC in various solid tumors, a correlation between autophagy and CSC population has been suggested. Autophagy has been shown to be involved in the maintenance of CSCs in breast cancer^[51]. Accordingly, inhibiting ATG12 and LC-3 by siRNA methodology or with the pharmacological inhibitors of autophagy altered the phenotype of breast CSCs^[51]. Similarly, Rausch *et al.*^[52] reported that the autophagic markers co-localize with CSC markers in tumors which were surgically removed from pancreatic cancer patients. The pancreatic cancer cell line, MIA-PaCa2 has been shown to exhibit more prominent stem-like properties (as determined by

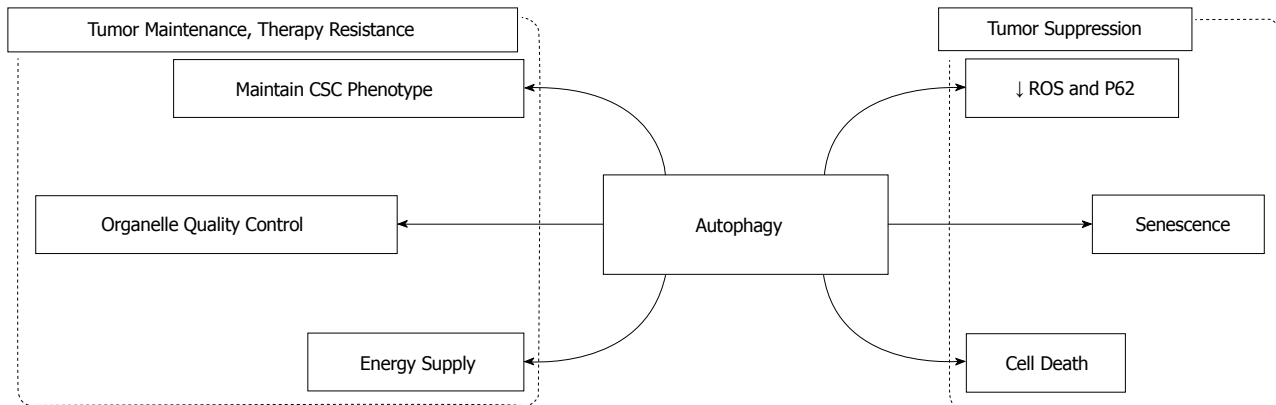


Figure 1 Schematic summary of the role of autophagy in cancer. Autophagy contributes to tumor suppression as well as tumor maintenance and therapy resistance. The mechanisms by which autophagy is involved in tumor suppression include limiting the accumulation of ROS and P62, and inducing senescence and cell death. On the other hand, autophagy facilitates tumor maintenance and therapy resistance by providing the tumor with metabolic substrates and maintaining intracellular homeostasis (organelle quality control), and by possibly contributing to the maintenance of CSC phenotype. CSC: Cancer stem cell; ROS: Reactive oxygen species.

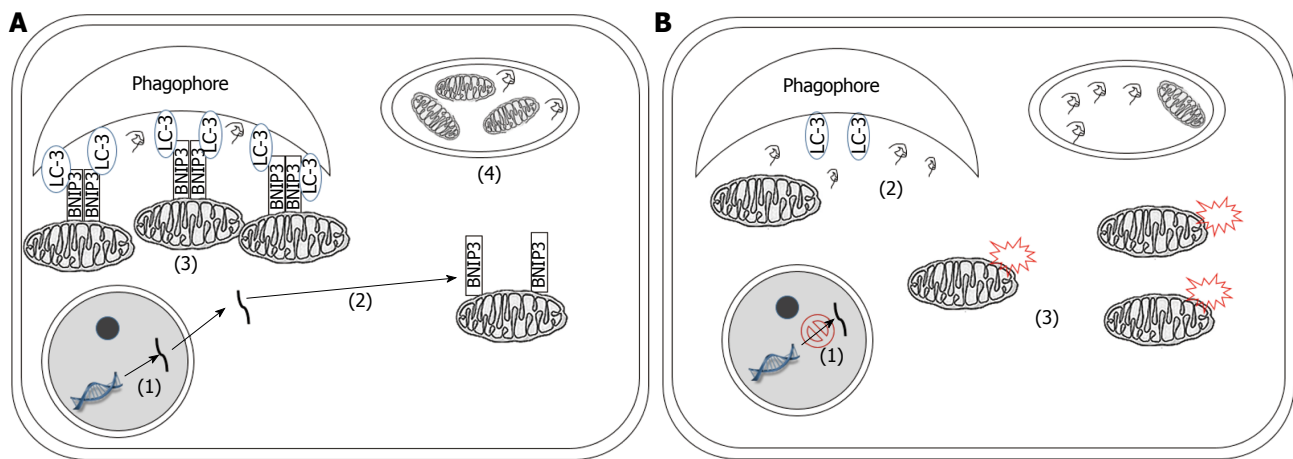


Figure 2 Schematic illustration of differential regulation of mitophagy participating in the progression of pancreatic cancer. Numbers in parentheses indicate successive mitophagy processes that are interrelated. A: During the early stages of pancreatic cancer: (1) *BNIP3* is transcribed; (2) translated and inserted into mitochondria membrane; (3) Active *BNIP3* tethers mitochondria to the phagophore through its interaction with LC3; (4) Mitochondria are therefore selectively engulfed in the autophagosome and degraded by the lysosome. In this way, mitochondria-induced ROS production is limited and genome stability is preserved; B: In the later stages of pancreatic cancer: (1) *BNIP3* gene is silenced; (2) The absence of *BNIP3* on the mitochondrial outer membrane will prevent the process of selective targeting of mitochondria to the autophagy machinery; (3) Accumulation of damaged mitochondria will result in elevated production of ROS and increased genome instability, which further contributes to the progression of cancer.

functional assays) compared to another pancreatic cancer cell line, BxPc-3^[53]. In accordance with their stronger stem-like features, MIA-PaCa2 cells also displayed higher autophagic activity^[54] and resistance to cell death induced by chemotherapeutic drug gemcitabine^[54,55] than that observed with BxPc-3 cells. It is therefore possible that autophagy is associated with the maintenance of the stem cell phenotype of pancreatic CSCs and thereby contributes to the resistance observed with therapy. Nevertheless, the underlying mechanisms by which autophagy modulates CSC phenotype and contributes to drug resistance requires further research.

AUTOPHAGY-A DOUBLE-EDGED SWORD

In summary, based on current knowledge, autophagy can act both as a positive and negative regulator of tumor growth in various cancers (Figure 1). Several hypoth-

eses have been proposed to reconcile these seemingly contradictory observations, which can be summarized as follows: (1) The differential effects of autophagy in cancer might be attributed to the tissue specificity. This is supported by the fact that the highest correlation between tumor growth and elevated autophagy is observed in Ras-induced oncogenesis^[2]; (2) A dynamic role for autophagy has been proposed in the development of cancer. Namely, autophagy might play a suppressive role in the initiation stages of cancer but support the maintenance of tumor growth in the later stages of tumorigenesis. This hypothesis is supported by the observation that homozygous *Atg5* and *Atg7* KO mice, which display more significant autophagic inhibition, developed only benign tumors^[17]. In contrast, *Becn1* KO mice, which exhibit relatively higher level of autophagy, displayed both benign and malignant tumors^[2,15,16]; and (3) The substrate selectivity of autophagy has recently emerged as a poten-

tial mechanism responsible for the differential roles of autophagy in cancer. Mitophagy has been shown to be activated in Ras transformed cells. Autophagy deficiency results in accumulation of abnormal mitochondria when cells are challenged with starvation^[27]. In contrast, in pancreatic cancer cells, initial attempts have failed to detect any significant mitophagic activity^[26]. Interestingly, the specific receptor for mitophagy, BNIP3, has been found to be silenced in various pancreatic cancer cell lines^[56,57]. Since damaged mitochondria are the major source of ROS which promote tumorigenesis and malignant transformation, it is feasible that mitophagy might serve as a protective mechanism in the initial stage of tumorigenesis (Figure 2A). The loss of this protective role, resulting from the silencing of mitophagic receptor, may promote the tumor to a more advanced stage (Figure 2B). Indeed, immunohistochemical staining of BNIP3 in pancreatic tissues indicated that BNIP3 silencing is a late event in pancreatic cancer pathogenesis^[56]. In the early stage pancreatic cancer tissues, BNIP3 exhibits a perinuclear distribution pattern^[56]. These findings strongly suggest that mitophagy is activated in the early stages of pancreatic cancer^[58-60]. In contrast, this distinct pattern of BNIP3 expression is missing in late stages of pancreatic adenocarcinoma. In addition, Takahashi *et al.*^[61] have found that haploinsufficiency of a tumor suppressor gene, Bif-1, attenuates mitophagy and subsequently promotes chromosomal instability in a mouse model of B-cell lymphoma. Similarly, mitochondrial content has been shown to be elevated in breast cancer^[62], colorectal cancer^[63], and ovarian cancer^[64]. Although direct evidence is still lacking for the substrate specificity of autophagy in cancer, further studies are required to understand the importance of this mechanism in various cancers.

CONCLUSION

Studies so far support both a tumor suppressive and an initiative role for autophagy in cancer. These differential effects of autophagy could be due to several reasons including the tissue specificity of tumors and the different stages of tumorigenesis. The role of substrate specificity of autophagy (*e.g.*, mitophagy) and other potential mechanisms warrant further research. It is of great importance that we improve our understanding of the roles which autophagy plays in cancer. Notably, this will enable the development of individualized treatments for cancer patients according to their cancer type and its progression. In fact, autophagy inhibitors are already being tested in clinical trials and hold promise for combined cancer therapies.

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