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Review: Spotlight on Cancer

Autophagy Signaling and the Cogwheels of Cancer

Joëlle Botti

Moïgan Djavaheri-Mergny

Yannick Pilatte

Patrice Codogno*

INSERM U756; Faculté de Pharmacie; Université Paris-Sud; 92296 Châtenay-Malabry, France

*Correspondence to: Patrice Codogno; INSERM U756; Faculté de Pharmacie 92296; Châtenay-Malabry, France; Tel.: +33.1.46835528; Fax: +33.1.46835844; Email: codogno@vjf.inserm.fr

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KEY WORDS

macroautophagy, signal transduction, proliferation, cell growth, cell survival, cell death, tumor immunity

ABBREVIATIONS

Atg	Autophagy-related
DAPk	Death associated protein kinase
eIF2 α	eukaryotic initiation factor-2- α
Erk	Extracellular signal-regulated kinase
IL	Interleukin
INF	Interferon
MAP kinase	Mitogen Activated Protein kinase
MHC	Major Histocompatibility Complex
(m)TOR	(mammalian)Target Of Rapamycin
PI3K	phosphatidylinositol 3-kinase
PTEN	Phosphatase and tensin homologue deleted from chromosome 10
TNF	tumor necrosis factor

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ABSTRACT

The downregulation of macroautophagy observed in cancer cells is associated with tumor progression. The regulation of macroautophagy by signaling pathways overlaps with the control of cell growth, proliferation, cell survival and death. Several tumor suppressor genes (*PTEN*, *TSC2* and *p53*) involved in the mTOR signaling network have been shown to stimulate autophagy. In contrast, the oncoproteins involved in this network have the opposite effect. These findings, together with the discovery that haploinsufficiency of the tumor suppressor *beclin 1* promotes tumorigenesis in various tissues in transgenic mice, give credibility to the idea that autophagy is a tumor suppressor mechanism. The induction of macroautophagy by cancer treatments may also contribute to cell eradication. However, cancer cells sometimes mobilize autophagic capacities in response to various stimuli without a fatal outcome, suggesting that they can also exploit macroautophagy for their own benefit.

INTRODUCTION

The recent elucidation of the molecular control of macroautophagy (referred to below as "autophagy")¹ has provided support for the long known seminal observation that tumor and transformed cells display reduced autophagic capacities.^{2,3} Recent findings in transgenic animal models,^{4,5} and in cultured cells after the knockdown of autophagy genes^{6,7} in response to cancer therapy⁸ (see accompanying review by Kondo and Kondo, this issue) strongly suggest that autophagy is indeed a tumor suppressor mechanism. Nevertheless one can wonder whether the role of autophagy is unequivocal in a complex pathology such as cancer. In other words, is autophagy a failsafe mechanism that cells have to overcome to become cancerous, or can cancer cells sometimes exploit their autophagic capacities for their own benefit? If so, does this depend on the tissue origin of the cancer, the stage of cancer development, the stromal and nutritional environment, or the degree of differentiation of tumors and cancer cells.^{9,10}

Autophagy is a multistep process that is controlled by molecules that fall into two categories: sensors and effectors. Effectors can be further divided into type-I and type-II effectors. Type-I effectors are involved in the steps (nucleation, expansion, uncoating and completion) leading to the formation of autophagosomes from the preautophagosomal membrane or phagophore.¹¹ Type-I effectors are represented by the generally evolutionarily conserved Atg proteins and class III phosphatidylinositol 3-kinase (PI3K), the ortholog of yeast Vps34. The activity of type-I effectors is regulated by the formation of a class III PI3K-Atg6 (Beclin 1) complex,^{12,13} and ubiquitin-like conjugation systems (the Atg8/LC3 and Atg12 systems).¹⁴⁻¹⁷ One type-I effector, Beclin 1, is a tumor suppressor gene product. Monoallelic deletion of this effector is observed in 40 to 75% of sporadic ovarian and breast cancers.^{18,19} Recently, transgenic mouse models have shown that monoallelic deletion of *beclin 1* promotes tumor development in various tissues.^{4,5} Type-II effectors are involved in the maturation of autophagosomes (e.g., Lamp-2, Rab proteins, SNAREs, SKD1).²⁰⁻²⁵

Sensors comprise the diverse signaling pathways, second messengers and protein kinase complexes that respond to environmental changes, and regulate the steps in autophagosome formation that occur upstream of the effectors. Many sensors involved in autophagy signaling are tumor suppressor gene products [e.g., phosphatase and tensin homologue deleted from chromosome 10 (PTEN), TSC1-TSC2, p53, death associated protein kinase (DAPk)] and oncogenes (e.g., Akt, Ras). These sensors are not specific for autophagy, but are also implicated in pathways that control a variety of cell functions. Thus, the signaling pathways that regulate autophagy form part of a cell program that is altered in cancer cells.

This can be represented by an integrative model symbolized by cogwheels moving in a concerted manner to influence autophagy and other cell properties in a reciprocal manner (see Fig. 1). The aim of the present review is to examine autophagy signaling in the context of the essential changes in cell physiology that are observed during malignancy,²⁶ such as a loss of balance between growth and proliferation, between cell survival and death, and its potential role in the regulation of angiogenesis and the immune response. Readers interested in a detailed analysis of the signaling pathways involved in the regulation of autophagy should consult recent reviews of this topic.²⁷⁻²⁹

OVERVIEW OF THE REGULATION OF AUTOPHAGY

The kinase TOR³⁰⁻³² is a major sensor in the autophagy signaling pathway that is conserved in eukaryotes. Once activated, TOR promotes protein synthesis, and has an inhibitory effect on autophagy. In metazoans, this kinase is activated by a growth factor signal via the class I PI3K/Akt (also known as Protein Kinase B; PKB) pathway, and inhibited by AMP-dependent kinase and p53, which integrate the energy status of the cell and genotoxic stresses, respectively (reviewed in Refs. 33-36).

The mammalian TOR kinase, mTOR, is also activated by amino acids;³⁷ however, the mechanism by which amino acids control mTOR is still a matter of debate. It has been proposed that the GTPase Rheb, which has GTPase activity controlled by the GTPase-activating protein TSC2, may integrate amino acid signaling upstream of mTOR.³⁸ Amino acids may also act at the level of the rapamycin-sensitive mTOR complex by controlling the stability of the mTOR/raptor complex.³⁹ The stability of this complex is increased in cells starved of amino acids, and is correlated with the inhibition of mTOR-dependent signaling. However, the role of amino acids in controlling the stability of the mTOR/raptor complex has not been confirmed by other studies.⁴⁰ For example, amino acids have recently been shown to mediate mTOR/raptor-dependent signaling by activating class III PI3K.^{41,42} Moreover, in one of these studies, the Beclin 1-associated class III PI3K was inhibited by amino acid starvation. These latter findings are difficult to reconcile with the role of the class III PI3K-Beclin 1 complex in autophagy,^{12,43} because this complex is generally considered to be stimulatory and required for autophagy. However, in these studies, autophagy was not investigated directly. It is possible that an autophagy-related, class III PI3K-Beclin 1 pool might be regulated differently. The existence of different pools of class III PI3K-Beclin 1 is an intriguing possibility that remains to be investigated. A further degree of complexity in the role of amino acid signaling is suggested by studies that report mTOR-independent control of autophagy during

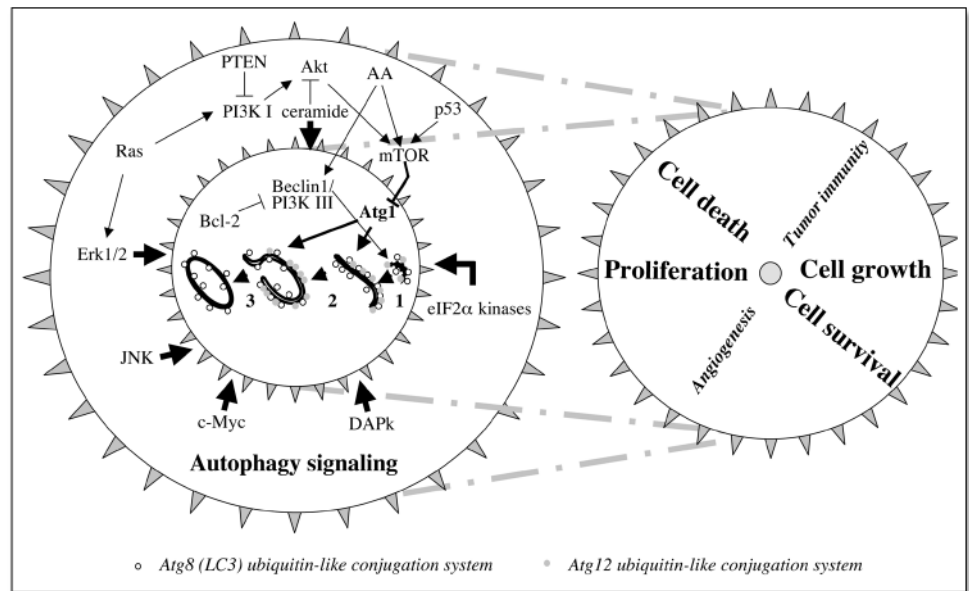


Figure 1. Potential implications of autophagy and signaling in malignant cell physiology. This figure is not intended to be exhaustive, but attempts to show the close interconnections between autophagy and cell functions that are often disrupted in cancer cells, i.e., growth, proliferation, survival, death and processes that play important roles in tumor progression, such as tumor immunity and angiogenesis. Autophagy signaling, autophagy machinery and cell functions are symbolized by cogwheels connected by chains. The movement of one cogwheel drives the other two. Key elements in the autophagy machinery, including Beclin 1 and its partners, class III PI3K, Bcl-2 and Atg proteins, and diagrams depicting the early stages of autophagosome formation are shown in the inner cogwheel on the left. Atg proteins function during the different steps (1: nucleation, 2: expansion and 3: completion) of autophagosome formation from the preautophagosomal membrane. The Beclin-1-class III PI3K complex is required for nucleation. Ubiquitin-like conjugation systems (Atg8/LC3 and Atg12) are implicated during expansion and completion. The Atg1 complex is thought to be involved in various steps of autophagosome formation downstream of mTOR. Signaling pathways (including the mTOR network, MAP-Erk1/2 and other molecules) that regulate autophagy, cancer cell functions and tumorigenesis are depicted in the outer cogwheel on the left. Bold black arrows and bold black bars indicate the stimulation and inhibition of autophagy, respectively.

leucine restriction.^{44,45}

In yeast, the Atg1 Ser/Thr protein kinase complex functions downstream of TOR to regulate various steps of autophagosome formation.⁴⁶⁻⁴⁸ However, the role of Atg1 kinase activity in autophagy is not clear even in yeast.^{11,49} Furthermore, Atg1 may act at more than one step of the process, during regulation as well as vesicle formation. This suggests that the Atg1 complex can act either as a sensor or as a type-I effector, depending on where it intervenes in the formation of the autophagosome. Homologs of Atg1 have been shown to be involved during autophagy in multicellular organisms.^{32,50,51} However, the role of Atg1 during the formation of the autophagosome in metazoans is not known, in part because several components of the Atg1 complex are not evolutionarily conserved (Atg11, Atg13, Atg17), and in part because the physiological target of the kinase activity of Atg1 remains to be identified.¹¹ Despite unresolved questions about the regulation of autophagy, the tumor suppressor genes (PTEN, TSC1/TSC2, p53) acting in the mTOR signaling network are known to stimulate autophagy^{32,52,53} and an increase in the function of oncogenes acting in the mTOR signaling network (Akt, Ras) has an inhibitory effect on autophagy.^{52,54} This implies that there must be a relationship between cellular autophagic capacity and the activity of tumor suppressors and oncogenes that are active in the mTOR signaling network.

Starvation-induced autophagy also depends on the activity of GCN2,⁵⁵ a member of the eukaryotic initiation factor-2- α (eIF2 α)

kinase family, which downregulates translation by phosphorylating eIF2 α at Ser.^{51,56} PKR, another member of the eIF2 α kinase family, which is stimulated by viral infection, is essential for virus-induced autophagy.⁵⁵ Deciphering the time sequence and interplay of eIF2 α kinases and mTOR signaling would probably help to elucidate how autophagy is initiated. Other protein kinases and signaling pathways, such as the MAP kinases Erk1/2 kinases^{57,58} and JNK,⁶ DAPk⁵⁹ (see accompanying review by Gozuacik and Kimchi, this issue) and the TNF- α receptor family signaling pathway⁶⁰ have been shown to control autophagy in cancer cells, and will be discussed where appropriate in the following sections.

AUTOPHAGY, CELL GROWTH AND PROLIFERATION

Several lines of evidence suggest that the downregulation of autophagy could play a role in the active proliferation of malignant cells, which is one of the major hallmarks of cancer. In proliferating cells, including cancer cells, cell size control relies on the coordinated regulation of cell growth and cell division⁶¹ so that on average each cell division is accompanied by a doubling in cell mass. Autophagy is the main degradation pathway of long-lived proteins, and has a major impact on cell size modulation. In many cell types, cell cycle progression depends on growth to such an extent that blocking cell growth by nutrient or growth-factor deprivation (treatments known to stimulate autophagy) results in cell cycle arrest.⁶¹ It was also shown recently that mitotic cells shut down their autophagic protein pathway while their chromosomes and organelles are dividing, and that the autophagic response reemerges during the late telophase/G₁ phase.⁶² These observations have been supported by mechanistic studies showing that several regulatory proteins exerting pivotal functions in autophagy are also involved in regulating cell proliferation.

The implication of *beclin 1* in tumor progression was suggested by the observation that this gene is monoallelically deleted in a high percentage of human cancers and tumor cell lines.¹⁹ Stable transfection of *beclin 1* into MCF-7 human breast carcinoma cells reduced their tumorigenicity in nude mice, and slowed their proliferation rate.¹⁹ Furthermore, *beclin 1*-haploinsufficient mice displayed an increased incidence of spontaneous tumors^{4,5} and the cell proliferative capacity was markedly increased in some of their tissues.⁴ How *beclin 1* modulates signaling pathways regulating cell proliferation is unclear, but it has recently been suggested that this protein might act as a regulator of the selective turnover of proteins involved in the control of cell growth and proliferation.⁶³

In mammalian cells, mTOR integrates signals from nutrients and growth factors to produce coordinated regulation of cell growth and cell-cycle progression.⁶⁴ mTOR regulates translation via its downstream targets: p70S6 kinase (which phosphorylates the S6 ribosomal protein) and 4E-BP1 (a binding protein for the translation initiation factor eIF4E).⁶⁵ In addition to these functions, mTOR also acts as a rheostat, adjusting the rate of autophagy in response to the levels of amino acids and ATP.²⁷

The class I PI3K/Akt pathway is an upstream nutrient- and growth factor-responsive regulator of mTOR that plays an evolutionarily conserved role in the regulation of autophagy.^{32,50,66,67} Akt activation phosphorylates mTOR, and this in turn inhibits autophagy. PTEN, a phosphatase that counteracts the lipid kinase activity of class I PI3K,^{68,69} has recently been shown to promote autophagy in HT-29 colon cancer cells.⁵² Since many cancers display aberrantly high class I PI3K-dependent signaling, either via the constitutive activation of class I PI3K or Akt or via inactivation of PTEN, the decreased autophagic activity of tumor cells observed in these cases

may result from activation of the mTOR pathway.

Several other tumor suppressors and oncogenes that were initially identified as regulators of cell growth and proliferation were recently shown to be capable of modulating autophagy. One of the most intriguing examples is p53, a tumor suppressor that plays an important role in preserving the integrity of the genome (see accompanying review by Jin, this issue). Genotoxic stresses activate p53 which in turn initiates tumor suppressor processes such as growth arrest.⁵³ Although p53 can affect cell growth and proliferation by activating cyclin-dependent kinase p21, recent observations indicate that once activated, p53 also inhibits mTOR activity and upregulates autophagy.⁵³ These findings indicate that p53 and mTOR pathways can crosstalk, regulate cell growth, proliferation and autophagy, and suggest that in cancers in which p53 is mutated, mTOR activation resulting from abnormal p53 activity may contribute to the lower autophagic capacities of the malignant cells.

A recent study has shown that *c-myc*, a proto-oncogene that controls cell division and cell growth, increases autophagic activity when overexpressed in rat 3Y1 fibroblasts.⁷⁰ Interestingly, a c-Myc mutant deleted in the Myc Box II region still induced autophagy without triggering apoptosis or inducing oncogenic transformation. This indicates that c-Myc-induced autophagy is not dependent on its apoptogenic or tumorigenic functions.⁷⁰ Ras is another oncogene involved in regulating cell proliferation and oncogenesis that could modulate autophagy. Ras activates pathways that produce conflicting effects on autophagy. Ras-dependent activation of the class I PI3K pathway has an inhibitory effect on autophagy,⁵⁴ whereas Ras-dependent activation of the MAP-Erk1/2 pathway has a stimulatory effect on starvation-induced autophagy in colon carcinoma cells.⁵⁷

The possibility of interplay between autophagy and cell proliferation has been further supported by a recent report showing that p27^{k1p1} is a potential regulator of autophagy.⁷¹ p27^{k1p1} belongs to the family of cyclin-dependent kinase inhibitors that downregulate cell cycle progression, and its expression is frequently reduced in human cancer. In this study, overexpression of p27^{k1p1} using recombinant adenoviral vectors induced autophagic cell death in human glioma cell lines, whereas the same treatment did not affect the viability of nonmalignant cultured astrocytes or induce autophagy.⁷¹

Further studies will be required to clarify whether the reduced autophagic capacities of malignant cells are instrumental in their active proliferation or, rather, a consequence. This would pave the way for a better understanding of the tumor suppression functions of autophagy.

AUTOPHAGY AND GROWTH FACTORS

It has recently been reported that growth factors are involved in the suppression of autophagy. These results are consistent with previous observations that: (1) growth factors enhance cell growth and proliferation; this is closely linked to the concomitant stimulation of anabolism and, conversely, the suppression of catabolism,⁷² (2) growth factors regulate the uptake of nutrients, such as glucose and amino acids, which are downregulators of autophagy;⁷³ and (3) growth factors can regulate the activity of the class I PI3K/Akt/mTOR pathway that coordinates the regulation of both autophagy and growth factor signaling.⁷⁴

The role of autophagy in cell survival in response to growth factor withdrawal has recently been investigated in cells without the essential bax/bak apoptotic machinery (*bax*^{-/-}*bak*^{-/-} cells).⁷⁵ Because IL-3 deprivation rapidly triggers the activation of apoptosis in

wild-type hematopoietic cell lines, the *bax*^{-/-}*bak*^{-/-} cells model has made it possible to investigate the effect of IL-3 withdrawal on autophagy without interference from apoptosis. IL-3 deprivation induces autophagy, which in turn promotes prolonged cell survival independently of growth factors. Indeed, inhibition of the autophagy genes *atg5* and *atg7* and the pharmacological blockade of autophagy both accelerate cell death, even in the presence of an abundant supply of extracellular nutrients. Cells can be rescued from death by the bioenergetic substrate, methylpyruvate, suggesting that in the absence of growth factors cells use autophagy-induced cell catabolism to maintain a sufficient level of ATP production and ensure cell survival. Conversely, growth factor stimulation promotes cell survival by maintaining the ability of cells to take up the extracellular nutrients necessary for ATP production. However, this autophagy-mediated survival mechanism is self-limiting, and persistent growth factor deprivation leads to cell death within a few weeks. This is probably due to severe degradation of essential organelles and macromolecules induced by prolonged stimulation of autophagy.

An increase in autophagy has been also reported in sympathetic neurons deprived of glial cell line-derived neurotrophic factor (GDNF), which is associated with nonmitochondrial cell death.⁷⁶ It has also been shown that inhibiting the Platelet Derived Growth factor (PDGF)-signaling pathway, using anti-PDGF neutralizing antibody, promotes autophagy in malignant glioma cells.⁷⁷ However, in these systems, the role of autophagy in protecting against or promoting cell death has not been fully clarified.

While numerous studies have focused on the effects of growth factors on both cell growth and proliferation in cancer cells, less is known about the functional role of growth factors in the inhibition of autophagy in tumor cells. As one function of autophagy is to remove damaged macromolecules and organelles, we can surmise that inhibiting autophagy in cancer cells by activating the growth factor signaling pathway would lead to an accumulation of such damage, and thereby contribute to the development of cancer. In support of this hypothesis, it has been shown that mitochondria with deleterious mitochondrial DNA mutations are targets of autophagy in a context of serum deprivation, whereas supplementation with IGF-1 growth factor counteracts this effect.⁷⁸

AUTOPHAGY IN CELL SURVIVAL AND CELL DEATH

This topic has been extensively discussed in several recent reviews.^{75,79-86} The aim of this section is to summarize what is known about autophagy signaling in the survival and death of cancer cells.

Autophagy in cancer cell survival. Cancer cells may encounter situations where the supply of nutrients and oxygen is limited. Autophagy could be a quick way for cancer cells to cope with these harsh conditions before other adaptive mechanisms, including gene expression and metabolic adaptation, kick in to protect the cells.

Although the autophagy response to starvation is generally less pronounced in cancer cells than in normal cells, many cancer cell types still are able to increase autophagy when deprived of growth factors or nutrients.⁹ Starvation-induced autophagy is a cell survival mechanism that can protect cancer cells from cell death by repressing the induction of apoptosis,⁸⁷ and by maintaining metabolic activity through lysosomal recycling of intracellular nutrients.⁷⁴ Interestingly mTOR, which integrates amino acid and growth factor signaling (see previous sections), also senses energy stress, through the activity of AMPK,⁸⁸ and hypoxia, via the hypoxia-inducible gene *REDD1*.⁸⁹ Both AMPK and *REDD1* have an inhibitory effect on mTOR.

Nascent tumor cells encounter stressful conditions, including energy stress and hypoxia that induce *REDD1*.^{90,91} The regulation of autophagy in cancer cells warrants further investigation. Paradoxically, the pharmacological activation of AMPK by AICAR has an inhibitory effect on autophagy in mammalian cells (see refs. 27 and 29 for discussion).⁹² This suggests that the complexity of the signaling circuits that control autophagy cannot always be extrapolated from charts depicting signaling pathways.

Autophagy in cell death. Although autophagic cell death (type II cell death) was recognized many years ago as a cell death mechanism that can proceed independently of apoptosis (type I cell death),⁹³ the role of autophagy during mammalian cell death has only recently been supported by knocking down *atg* genes.^{6,7} Moreover type I and type II cell death are probably nonexclusive mechanisms in mammals, because they are sometimes observed in the same dying cell.⁹⁴ This situation is reminiscent of that observed in insect tissues during the cell death that occurs in the *Drosophila* salivary gland during development.⁹⁵

Several signaling pathways have been shown to modulate the autophagic response during cancer cell death. Both Akt and mTOR regulate autophagy and cell death in glioma cells,⁹⁶ and in tamoxifen-treated MCF-7 cells via ceramide production.⁹⁷ However, ceramide can trigger autophagy and cell death by other mechanisms, including the upregulation of the expression of the BH3-only protein BNIP3 in glioma cells.⁹⁸ The role of ceramide in autophagy and cell death is in line with its tumor suppressor function.⁹⁹

Activation of the MAP-Erk1/2 signaling pathway is instrumental in colon cancer cells to stimulate autophagy and type II cell death in response to soybean B-group triterpenoid saponins.^{58,100} TNF- α stimulates autophagy and apoptosis in T-lymphoblastic leukemia cells.¹⁰¹ Inhibition of autophagy by 3-methyladenine protects these cells against death. In addition, TNF- α stimulates autophagic cell death independently of caspase activation.¹⁰² Recently, the interaction of Atg5 with the death domain of Fas-associated death domain protein (FADD) has been shown to play a crucial role in INF γ -induced cell death independently of detectable activation of caspase 8.¹⁰³ Elucidating signaling events downstream of FADD in Atg5-induced cell death would contribute to a better understanding of how autophagic cell death is controlled. Interestingly, the death domain of FADD can activate a cell death pathway involving both apoptosis and autophagy that is selectively inactivated at the earliest stages of epithelial cancer development.⁶⁰

Autophagic cell death has been observed in mouse fibroblastic cells and human monocytoïd cells in response to the inhibition of caspase 8.⁶ The accumulation of autophagic vacuoles is dependent on the presence of receptor-interacting-protein (RIP), a protein associated with the cytoplasmic domain of the death receptor, and on the activation of JNK. In addition, RIP is a substrate for caspase 8, which cleaves and inactivates it. This study suggests that caspase 8 may also have a role in processes other than apoptosis. Another interesting observation is the implication of c-Jun, a downstream effector of JNK, in controlling autophagic cell death, which suggests that transcriptional activity is required to induce cell death.

DAPI controls autophagy and cell death downstream of interferon- γ (INF γ) in human cervix carcinoma cells.⁵⁹ Interestingly, DRP-1 has been also shown to control tamoxifen-induced autophagy and

cell death in the human breast MCF-7 cancer cells.⁵⁹

POTENTIAL IMPLICATIONS OF AUTOPHAGY SIGNALING IN CANCER BIOLOGY

Tumor antigen presentation and angiogenesis are two important events modulated during tumor progression. Very few studies have directly investigated the role of autophagy in angiogenesis and tumor antigen presentation. Nevertheless on the basis of recent publications, it can be speculated that the role of autophagy in angiogenesis and tumor antigen presentation is still underestimated.

Autophagy and tumor immunity. Several decades ago, it was proposed that the immune system is capable of recognizing and eliminating primary tumors, a concept known as tumor immunosurveillance.¹⁰⁴ So far, there have been no direct observations indicating that the downregulation of autophagic activity in malignant cells might play a significant role in tumor-immune system interactions, perhaps because autophagy has not been investigated in this context. However, recent reports have shown that autophagy is an immunologically-regulated process that can be modulated by certain immune mediators. For instance IFN γ stimulates autophagy,^{59,105} whereas IL-13 is a strong antagonist of autophagy via the class I PI3-kinase pathway.⁵² Alternatively, autophagy has recently been shown to constitute an alternative pathway to antigen presentation,¹⁰⁶ which allows MHC class II restricted presentation of endogenous peptides,¹⁰⁷⁻¹¹¹ and cross-presentation has also been shown to involve chaperone-mediated autophagy.^{112,113}

Thus, investigating the connection between autophagy and immunity should provide new insights into the mechanism involved in tumor progression. This emerging field may also make it possible to develop new approaches to cancer vaccines, because some anticancer drugs may act by triggering autophagy,⁸ and in this manner could change the way cancer cells present cellular antigens.¹⁰⁸

Autophagy and angiogenesis. Several lines of evidence indicate that the stimulation of autophagy signaling inhibits tumor angiogenesis.^{114,115} Indeed, rapamycin and Rad001, two inhibitors of mTOR, radiosensitize the GL261 mice glioma model by inhibiting angiogenesis.¹¹⁴ Accordingly, the role of PTEN in angiogenesis has been demonstrated by experiments showing that the loss of *PTEN* is specifically responsible for enhancing tumor angiogenesis in mouse endothelial cells.¹¹⁵ Although these results suggest that autophagy may contribute to anti-angiogenic responses, the relationship between angiogenesis and autophagy remains to be fully elucidated.

CONCLUSION

The mTOR signaling network and MAP-Erk1/2 are two major signaling pathways that transduce growth factor signals and regulate autophagy,²⁷ and they are often impaired in cancer cells.^{36,116,117} Although dysregulation of these signaling pathways also has effects other than those on autophagy,²⁶ it is worthwhile considering the interplay of their role in modulating autophagy, cell survival and cell death during tumor progression. The regulation of autophagy is not limited to protein kinases and phosphatases, but also extends to other signaling molecules that regulate autophagy, such as sphingolipid signaling molecules.^{97,98} calcium¹¹⁸ and myo-inositol-1,4,5-triphosphate (IP3) and related metabolites.¹¹⁹

The autophagic capacities of cancer cells are blunted but not totally abolished.^{4,5} Along these lines, in *beclin 1* mutant mice, autophagosome formation is significantly reduced but still detectable.

In contrast, the hepatomegaly detected in *atg7*-conditional knock-out mice was found to correspond to an increase in hepatocyte swelling, even though neither enhanced cell proliferation nor tumorigenesis was observed in these animals.¹²⁰ In line with these findings, the total elimination of *beclin 1* blocks the proliferation of breast cancer cells.¹²¹ Beclin 1 could, of course, have an autophagy-independent role, but we cannot rule out the possibility that a low level of autophagy is required in cancer cells. This would be consistent with the observation that autophagic vacuoles have been observed in various human tumor biopsies,¹⁰ and with the ability of cancer cells to stimulate autophagy in response to drugs and, at least in some cases, to stimulate autophagy during starvation.^{9,85} Moreover it has been shown that, in a rat model of pancreatic cancer, autophagic capacities are increased at the adenoma stage before subsequently decreasing at the carcinoma stage.¹²² Analyzing autophagy in hamartoma syndromes with an established molecular link to dysregulation of mTOR³³ could be helpful for investigating the function of autophagy in specific tumor syndromes with an increased risk of malignancies.

Although this has not yet been validated experimentally in vivo, it seems possible that even a low level of autophagy could be a survival backup mechanism for cancer cells, giving them a selective advantage during tumor progression and metastasis in response to adverse conditions. Signaling involving integrins, Src, and focal adhesion kinase (FAK) has been shown to regulate autophagy in normal rat hepatocytes.^{123,124} Thus, the changes in the repertoire of cell adhesion molecule receptors (integrins, selectins, CAM and cadherins) that are often observed in cancer cells¹²⁵ may contribute to modifying their autophagic response in response to the cellular and extracellular matrix environments.

The need of cancer cells to keep the level of autophagy low is probably an Achilles' heel that can be exploited to induce cell death in response to cancer therapy.⁸ It has recently been shown in glioma cells⁹⁶ that interventions at various points in the autophagy signaling cascade can provide ways of producing unchecked autophagy that could contribute to eradicating cancer cells. Although the hardwiring of the signaling circuitry is now becoming clearer, its dynamic cross-talk, feedback controls and rewiring in cancer cells remain to be discovered.¹²⁶ A better understanding of the mechanism by which cancer treatments induce autophagy via signaling is required before any safe attempt can be made to drive cancer cells to death. Another way that leads to unchecked autophagy and autophagy gene-dependent cell death has been recently demonstrated by disrupting the interaction between Beclin 1 and Bcl-2.¹²⁷ This study provides evidence of a new function of Bcl-2 in keeping autophagy within the physiological range. Knowing more about the key mechanisms that regulate and initiate autophagy could provide some clues about the consequences of manipulating autophagy in cancer cells.

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