FOCUSED REVIEW

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Autophagy and cancer therapy

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Abbreviations: AMPK, AMP activated protein kinase; Atg, autophagy-related; DRAM, damage-regulated autophagy modulator; EM, electronic microscopy; ER, endoplasmic reticulum; LC-3, microtubule-associated protein light chain 3; mTOR, mammalian target of rapamycin; PAS, phagophore-assembly site; PE, phosphatidylethanol amine; PI, phosphatidylinositol; PI3K, phosphatidylinositol 3 kinase; 2-DG, 2-deoxyglucose; 3MA, 3 methyl adenine; 5-FU, 5-fluorouracil

Although autophagy has been shown to have a clear role as a tumor suppressor mechanism, its role in cancer treatment is still controversial. Because autophagy is a survival pathway activated during nutrient deprivation and other stresses, it is reasonable to think that autophagy can function as a tumor cell survival mechanism activated after cancer treatment. Such a mechanism could be widely important because most cancer treatments induce autophagy in tumor cells. Indeed, many papers have presented data suggesting that tumor cell autophagy induced by anticancer treatment inhibits tumor cell killing. However, it has also been proposed that autophagy is a cell death mechanism that could function as a backup when apoptosis is disabled. The fact that there are active clinical trials in patients both using autophagy inhibitors or inducers together with other cancer treatments underscores the importance of understanding and distinguishing between these opposing ideas. Here we discuss some of the recent work studying the role of autophagy with different cancer therapies.

Introduction

Autophagy is a catabolic mechanism of regulated intracellular turnover in which cells degrade their own cytoplasmic components within their lysosomes. Different types of autophagy have been described, with macroautophagy being the most studied. In this process, cells form de novo structures called phagophores, which elongate to engulf cytoplasmic proteins and even complete organelles in a double membrane structure named the autophagosome. Later, autophagosomes fuse with lysosomes to cause the degradation of their content. This review will focus on macroautophagy, referred hereon as autophagy.

Autophagy was described almost 50 years ago in mammalian cells.[1] However, the discovery of Atg proteins in yeast has led to a dramatic increase in research in this area.[2,3] Now we know that besides its role in homeostatic functions such as protein and organelle turnover occurring in virtually all eukaryotic cells, autophagy is rapidly upregulated in response to stresses like starvation, growth factor withdrawal or high energetic requirements.[4] The amino acids produced by autophagy can be used for de novo synthesis of proteins or to maintain cellular ATP production. Moreover, increasing evidence suggests that defects in the autophagic pathway have an important role in the pathogenesis of neurodegenerative diseases,[5-7] muscular diseases[8] and aging,[9] where autophagy functions as a mechanism to remove aggregated or damaged proteins and organelles. Autophagy has also shown to have an important function in the removal of intracellular bacteria and viruses[10-12] as well as in cancer.

The pro-survival and homeostatic functions of autophagy are evolutionarily conserved from yeast to mammals. During nutrient rich conditions, autophagy occurs at low levels (basal autophagy), and it provides tissues with a housekeeping mechanism of intracellular quality control through cytoplasmic turnover and removal of damaged or superfluous organelles.[13] Genetic studies with tissue specific deletions of Atg genes (*atg 5, atg 7* or *FIP200*) have found ubiquitinated protein aggregates in the cytoplasm of neurons, liver, heart, muscle and pancreas, which lead to tissue specific dysfunctions in these mice and thus imply that basal autophagy has an important function in intracellular quality control.[14] Basal autophagy has also been shown to be a tumor suppressor mechanism[13,15] and autophagy-related proteins decline with aging as does the efficacy of the autophagic process[16] further supporting the role of autophagy as an homeostatic mechanism.

When cells are subjected to nutrient deprivation, autophagy is rapidly induced to maintain the amino acid pool in the cytoplasm and survive starvation possibly through new protein synthesis, energy production and gluconeogenesis. In fact, amino acid levels in autophagy deficient mice (*atg 3, 5, 7, 9* and *atg16L1* knockout mice) are lower than in wild type mice after birth and although these mice appear normal, they die within 1 day since they cannot survive the neonatal starvation period due to termination of the transplacental nutrient supply.[14] Moreover, it has been shown that autophagy inhibition by knockdown of proteins involved in the autophagic pathway decreases viability of cells exposed to serum, amino acid[17-19] or growth factor deprivation.[20]

In contrast to the established pro-survival functions of autophagy, although it is widely accepted that cells can manifest

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increased autophagosome accumulation during death, it is not yet clear how important autophagy is as a cell death mechanism. Many studies suggest that if cellular damage is extensive, or if apoptosis is compromised, autophagy may be used to kill the cell.[8,21] However, it is not yet completely clear if autophagy per se can work as a true cell death mechanism in a physiological or pathological context. As with many aspects of autophagy research, the central problem in determining whether studies on autophagic cell death (or autophagy-dependent cell death) really show a requirement for the process of autophagy to cause death by itself has been the lack of reliable autophagic markers and the challenge of capturing a dynamic process with static measurements.[22] Pharmacological agents used to increase or inhibit autophagy have other effects too and functions independent of autophagy have been described for Atg proteins including caspase and apoptosis activation.[23-26] Thus, even if knockdown or genetic deletion of an Atg gene leads to reduced cell death in response to a death stimulus, it is often difficult to exclude the possibility that this was actually because the Atg protein in question was controlling a different death mechanism rather than affecting autophagy. So, although knockdown of Atg proteins decreases cell death in some models, it has sometimes been shown that autophagy might be regulating other forms of cell death such as apoptosis or necrosis.[27-29] Additionally, it could be that autophagy can promote cell death only when apoptosis is inhibited.[30-33] Together, these issues mean that the central question of whether autophagy sometimes functions as a mechanism of programmed cell death remains controversial.

Atg proteins and Regulators of Autophagy

To date, more than 30 *ATG* (autophagy-related) yeast genes and most of their orthologues in higher eukaryotes have been described, implying an evolutionary conserved process. Atg proteins have been classified into functional groups: (1) the Atg 1 kinase complex, (2) Atg 9, (3) class III phosphatidylinositol (PI) 3-kinase complex, (4) the Atg 12 conjugation system and (5) the Atg 8 conjugation system.[3,34,35]

During autophagy, many Atg proteins localize to an initial sequestering compartment. In yeast, this structure is known as the phagophore-assembly site (PAS) which leads to the formation of the isolation membrane or phagophore.[36] This structure eventually expands to envelope portions of the cytoplasm and to form an autophagosome. The Atg 1 kinase/ULK 1/2 complex includes Atg 13, FIP200 and Atg 101 in mammals. The function of this complex is to recruit other Atg proteins to the PAS and promote autophagosome formation through phosphorylation of specific substrates.[34] Atg 1/ULK1 also has an important function in Atg 9 regulation. Atg 9 is the only Atg transmembrane protein described and it is thought to be responsible for the delivery of membrane to the PAS from mitochondria in yeast or from trans-Golgi network and late endosomes in higher eukaryotes.[35,37]

One subgroup of Atg proteins (Atg 6/Beclin-1, Atg 14/Atg 14L) forms a complex with class III PI3K and UVRAG[38] to produce PtdIns3P. This enzymatic activity is essential for vesicle nucleation during autophagy.[3,39] Atg18 can bind to PtdIns3P and PtdIns(3,5)P2. Both Atg 2 and Atg 18 can interact with Atg 9 and are thought to regulate its retrieval from the PAS together with Atg 1.[3] Autophagosome extension is regulated by two ubiquitin-like systems. In the Atg 12 conjugation system, Atg 7 activates Atg 12, an ubiquitin-like protein, and binds it to Atg 10. Atg 12 is eventually conjugated to its target protein Atg 5. Atg 5 interacts with Atg16, forming the Atg 12-Atg 5-Atg 16 complex which later forms a multimer through the homo-oligomerization of Atg 16. In the Atg 8/LC3 conjugation system, Atg 8 is conjugated to phosphatidylethanolamine (PE). Atg 4 cleaves Atg 8, which later binds Atg 7. Activated Atg 8 is then transferred to Atg 3 and finally conjugated to PE. Atg 8-PE/LC3-II can be also recycled by Atg 4 to release free Atg 8 or it can be degraded after fusion of the autophagosome with the lysosome. The formation of the Atg 12-Atg 5 complex and Atg 8-PE are essential for autophagy progression and the amount of LC3-II is one of the most common assays used as an indicator of autophagy.[22,40] Mutation of any member of the conjugates, or of other components required for their formation, results in defects in autophagosome formation[40] and knockdown of these genes is commonly used to inhibit autophagy.

Bcl-2 family members negatively regulate autophagy by binding to Beclin-1 during non starvation conditions.[41,42] JNK1 mediated phosphorylation of Bcl-2,[43] DAPK mediated phosphorylation of Beclin-1,[44] activation of BH3-only proteins or the use of BH3 mimetics[45] provoke the dissociation of this complex inducing autophagy. Other regulators of autophagy have been described, including myo-inositol-1,4,5 triphosphate (IP3), its receptor[46,47] and tumor suppressor genes, like p53 and ARF.[27,48]

Autophagy and Cancer Cell Metabolism

Autophagy is controlled by two energy-sensing pathways involved in the regulation of cell growth and metabolism, the class I PI3K/mTOR and the AMP activated protein kinase (AMPK) signaling pathways. Class I PI3K activates mTOR kinase in response to insulin and growth factors and negatively regulates autophagy. During nutrient availability (growth factors and amino acids), mTOR complex I (mTORCI) represses the Atg 1/ULK 1/2 complex.[49] Thus, mTOR inhibition by rapamycin or nutrient deprivation is a common method to induce autophagy. Amino acid starvation can also induce autophagy through the Raf kinase and eIF2a.[49] Conversely, AMPK, the primary energy-sensing kinase in the cell which is activated during energy depletion or hypoxia by increased ratios of AMP to ATP, can inactivate mTORC1 promoting autophagy.[50] It has been proposed that the ability of AMPK to promote cell survival during metabolic stress may be due to its autophagy stimulating effects,[51] which might be particularly important in cancer cells exposed to energetic and environmental stresses in the tumor microenvironment. Autophagy can also be induced by hypoxia and autophagic cells have been found to localize to hypoxic tumor regions where autophagy supports cell survival[52] through elimination of p62, an autophagic substrate,[53] damaged mitochondria and reactive oxygen species.[54]

Autophagy and Cancer

A direct link between autophagy and cancer was the discovery that Beclin-1 could function as a tumor suppressor.[15] *Beclin-1* is monoallelically deleted in a high percentage of human breast, ovarian and prostate cancers and decreased levels of the protein have been found in human breast, ovarian and brain tumors.[13,15,55,56] Furthermore, heterozygous disruption of *beclin-1* makes mice more prone to the development of spontaneous tumors including lymphomas, lung carcinomas, hepatocellular carcinomas and mammary precancerous lesions.[57,58] Moreover, Beclin-1 restoration in MCF-7 cells decreases their proliferation and tumor formation in nude mice.[15] UVRAG is also deleted in various human colon cancer cells and tissues, and is a tumor suppressor candidate since its overexpression in a human colon cancer cell line decreases proliferation and tumor size in nude mice.[38] Other autophagy related genes have also been implicated in tumorigenesis. *MAP1-LC3* is localized to 16q24.1, a locus frequently deleted in liver, breast, prostate and ovarian cancers.[59] *Atg 7* liver conditional knockout mice develop hepatomegaly, a condition that may lead to malignant transformation and their hepatocytes accumulate abnormal mitochondria as well as poli-ubiquitinated aggregates;[60] and although *atg 4C* knockout mice do not develop more spontaneous tumors than their wild-type littermates, they are more prone to develop chemically-induced fibrosarcomas.[61] Autophagy deficient tumors grow faster in orthotopic injections in nude mice, and tumor growth is further increased by defects in apoptosis.[52,53,62] In total, this evidence provides strong reason to think that autophagy is a tumor suppressor mechanism and that a reduction in autophagic levels might promote tumorigenesis. Moreover, autophagy is negatively regulated by PI3K/Akt/mTOR and oncogenic transformation is frequently initiated through mutations that increase signaling through this pathway.[4]

The mechanism by which autophagy suppresses tumorigenesis seems to involve its ability to reduce cellular damage by decreasing genomic instability, oxidative stress, eliminating damaged proteins and organelles,[52,53,63] and mediating oncogene-induced senescence.[64] At the same time, autophagy may also have tumor promotion abilities—e.g., to increase metastasis through its ability to protect cells from anoikis.[65]

Autophagy and Cancer Therapy

Autophagy maintains survival in tumor cells in response to metabolic stress restricting necrosis and inflammation, especially when apoptosis is disabled by, for example, elevated expression of anti-apoptotic Bcl-2 family proteins, as is the case of many cancer cells.[52,62] Autophagy induction also decreases mitochondrial mass by mitophagy (mitochondrial degradation by autophagy), and it has been reported that this reduces the susceptibility of cells to mitochondrial apoptosis inducing stimuli.[66] Thus, it seems reasonable to propose that tumor cells could induce autophagy to survive metabolic stress in the tumor microenvironment and also to avoid apoptosis induced by therapy.

On the other hand, it has also been proposed that autophagy could function as a cell death mechanism especially in cells with defective apoptosis[31,67] and that autophagy may even have differential effects depending on the cell type or the genetic background.[68] Since a limitation of current therapy is that many cancers develop resistance to apoptosis, therapies targeting alternative cell death pathways are attractive. Thus, it has been suggested that efforts to increase autophagy and thus induce autophagic cell death when tumor cells are capable of avoiding apoptosis should be pursued. This is, of course, the opposite of the strategy that has been suggested (i.e., that we should try to inhibit autophagy) in order to prevent autophagy-mediated resistance against therapy. So, whether autophagy is a protective mechanism that decreases tumor cell death upon treatment, or if it acts as an apoptosis promoting mechanism or as a cell death mechanism per se is an important issue that needs to be determined. This question is especially pressing just now because both approved and experimental anticancer therapies induce the accumulation of autophagosomes in tumor cells and manipulation of autophagy during cancer treatment is already being pursued. For example, both rapamycin, an autophagy inducer that inhibits mTOR and chloroquine, an autophagy inhibitor that blocks the autophagosome-lysosome fusion step, are being used in combination with other chemotherapy in clinical trials for different cancer types. Below we discuss some recent studies using autophagy manipulation together with cancer therapy, a summary of the methods used to test autophagy (**Table 1**) and of the role proposed for autophagy in these studies (**Table 2**) to try to make sense of these competing ideas.

DNA Damaging Agents

There are many reports in the literature that support the idea of autophagy as a survival mechanism protecting from cell death upon DNA damage, which is a mainstay of many cancer treatment protocols. In this regard, it has been suggested that a possible mechanism of protection could be an autophagy dependent increase in ATP production after treatment.[69] Thus, lysosomal inhibitors bafilomycin A1 and chloroquine as well as the PI3K inhibitor wortmannin increased cisplatin induced apoptotic cell death in human U251 glioma, rat C6 glioma and mouse L929 fibrosarcoma cell lines associated with an increase in Bax/Bcl-2 mRNA and protein ratio.[70] In another study, autophagy was induced in the MCF-7 breast cancer cell line treated with the DNA topoisomerase I inhibitor camptothecin and, when autophagy was inhibited, survival decreased due to an increase in apoptosis. In this work, autophagy inhibition by 3MA (3-methyl adenine, a PI3K inhibitor) treatment increased cell death only in estrogen receptor (ER)-positive non-invasive MCF-7 and T47D cell lines and not in metastatic ER-negative BT-549, MDA-MB-231, MDA-MB-435 cells.[71] In a different study, autophagy inhibition induced apoptosis in a 5-fluorouracil (5-FU) colon cancer resistant cell line. Autophagy inhibition by 3MA or Atg 7 knockdown was associated with apoptotic features in different colon cancer cells treated with 5-FU and with Bcl-X_L downregulation. In addition, 3MA treatment enhanced 5-FU induced decrease in tumor size in xenografts.[72]

A pivotal study suggesting a pro-survival role of autophagy in cancer treatment with DNA damaging agents was performed

Table 1. Methods used to assess autophagy

Methods used to assess or manipulate autophagy	Principle	Caveats
3 Methyl adenine (3 MA)	Inhibitor of class III PI3K.	Can also inhibit class I PI3K. At the concentrations used to inhibit autophagy, it can target other kinases and affect cellular metabolism, endocytosis and mitochondrial permeability transition.
Chloroquine, quinacrine, bafilomycin A1	Block autophagy at the autophagosome-lysosome fusion. Bafilomycin A1 inhibits vacuolar ATPase and inhibits lysosomal acidification.	Inhibition of lysosomal degradation can affect other cellular processes such as mitosis and endocytosis.
Acridine orange	Acidotropic dye.	Primarily stains lysosomes.[110]
Electron microscopy	Observation of double membrane structures with cytoplasmic content indicates autophagosome formation.	Considerable specialized expertise is required. Assessment and especially quantitation can be subjective. Difficult to distinguish between increased and blocked autophagy.
LC3-II accumulation or GFP-LC3 dots	When conjugated to phosphatidylethanol amine (PE), LC-3 becomes LC3-II and localizes to the autophagosomes.	LC3-II is also degraded in the lysosome or recycled by Atg 4. In order to assess autophagic flux, LC3-II analysis has to be used together with lysosomal inhibitors. Alternatively, p62 degradation (an autophagic substrate) can also be used to assess autophagic flux.
Atg protein knockdown	Autophagy reduction has been reported in Atg 3, 5, Beclin-1, Atg 7, Atg 9a, Atg 12, Atg 16L1, FIP200 and Ambra1 knockdown in different models.	Although this is the most specific approach to inhibit autophagy, functions independent of autophagy have been described for proteins like Atg 5.[24]

For a detailed review on these techniques, see reference 22.

using a mouse model of B cell lymphoma where Myc/p53 knock in tumors were generated and transferred to a syngeneic host. Upon p53 induction in this model, cells undergo autophagy and tumors regress due to apoptosis induction but eventually, animals experience tumor recurrence. In this model, chloroquine treatment or Atg 5/7 knockdown decreased viability of cells treated with the alkylating agent MNNG in vitro, and chloroquine delayed tumor recurrence after cyclophosphamide treatment in vivo.[73]

Other studies suggest that autophagy might be the mechanism of cell death upon tumor treatment or that autophagy might be involved in the induction of apoptosis. Thus, treatment of apoptosis deficient Bax/Bak$^{-/-}$ or Bcl-X_L/Bcl-2 overexpressing MEFs with etoposide induced non-apoptotic cell death with autophagic features. 3MA treatment or Atg 5/Beclin-1 knockdown decreased cell death suggesting that autophagy is involved in cell death in this model.[31] Finally, temozolomide, a DNA alkylating agent, induced autophagy and not apoptosis in malignant glioma cell lines. In this study, 3MA increased cell viability and bafilomycin treatment decreased it with associated loss of mitochondrial membrane potential, lysosomal permeabilization and consequently, apoptosis induction.[74] The authors suggest a differential effect of inhibiting autophagy at early and late stages. However, no genetic manipulation of autophagy was performed in this study and it is therefore possible that these effects were actually due to other effects of 3MA or bafilomycin. Although a precise mechanism for autophagic stimulation after treatment with DNA damaging agents has not been described, p53 and DRAM (damage-regulated autophagy modulator), a p53 target, could be involved in autophagy and cell death induced by these treatments.[27] Since p53 expression induces apoptosis that can be decreased by Atg 5 or DRAM knockdown, these studies suggest that p53-induced autophagy could promote apoptosis in cancer cells.

Radiation

Besides inducing apoptosis, mitotic catastrophe and senescence, radiation therapy also induces autophagy in cancer cells.[75,76] As with other treatments, the role of autophagy with radiation is not entirely clear; however, several reports suggest that autophagy promotion through mTOR inhibition might be implicated in cell demise with this treatment. Surprisingly, in some studies increased cell death is achieved when apoptosis is also inhibited.[77,78] Thus, rapamycin treatment enhanced radiation induced cell death in the MCF-7 breast cancer cell line, which is known to be apoptosis defective[79] and in PTEN$^{-/-}$ PC3 prostate cancer cells but not in PTEN$^{+/+}$ DU145 cells.[80] Caspase inhibition with z-VAD or Bax/Bak knockdown further enhanced cell death induced by rapamycin and radiation treatment in the PC3 cell line and Atg 5 knockdown decreased radiation induced cell death in both PC3 and DU145 cell lines. In a different study, Bax/Bak$^{-/-}$ MEFs were more sensitive to radiation than their wild-type counterpart. In this work, the authors found increased pro-autophagic Atg 5-Atg 12 complex and Beclin-1 proteins in apoptosis deficient MEFs and treatment with rapamycin enhanced both autophagy and radiation sensitization. Interestingly, Atg 5 and Beclin-1 overexpression increased sensitivity of both wild type and Bax/Bak$^{-/-}$ MEFs to radiation in long term clonogenic survival assays.[77] Moreover, radiosensitization can be induced in a lung cancer cell line through combined inhibition of caspase-3 and mTOR in clonogenic survival assays and in an in vivo xenograft model. In this

Table 2. Studies cited in this review, indicating the proposed role of autophagy in cell death and the methods used to determine it

Effect of autophagy on cell death	Type of cancer	Treatment	Method used to evaluate autophagy (long/short term assays)	Tested in vivo?	Ref
Autophagic cell death	SV40 transformed Bax/Bak$^{-/-}$ MEFs or Bcl-X_L/Bcl-2 overexpressing MEFs	Etoposide, staurosporine	3MA, Atg 5 or Beclin-1 knockdown, EM, GFP-LC3 puncta. Short and long term assays.	No	31
	PC3 PTEN$^{-/-}$ and DU145 PTEN$^{+/+}$ prostate cancer cell lines	Radiation$^{+/-}$ caspase inhibitors and mTOR inhibition	GFP-LC3 puncta, Atg 5 and Beclin-1 knockdown. Long term assays.	No	80
	Bax/Bak$^{-/-}$ MEFs	Radiation	3 MA, Atg 5/Beclin-1 knockdown and overexpression, GFP-LC3 puncta, EM. Long term assays.	No	77
	H460 lung cancer cell line	Radiation$^{+/-}$ caspase inhibitors and mTOR inhibition.	LC3-II accumulation, EM, GFP-LC-3 puncta, RAD001 treatment (mTOR inhibitor), Atg5/ Beclin-1 knockdown. Long term assays.	Yes	78
	Breast cancer MCF-7 cell line	Bcl-2 knockdown	Acridine orange, LC3-II accumulation, EM, GFP-LC3 puncta. Short term assays.	No	67
	Androgen independent prostate cancer cell lines with high Bcl-2/BclX_L levels (PC-3, CL-1)	(-)-gossypol, a BH3-mimetic	LC3-II accumulation, EM, GFP-LC-3 puncta, 3 MA, Atg 5, Beclin-1 knockdown. Short term assays.	Yes	96
Autophagy promotes apoptosis	Non-transformed cells (MEFs and CCD-18Co non-transformed colon cancer cells)	ER stress inducers (A23186, tunicamycin, thapsigargin)	LC3-GFP puncta, Beclin-1, Atg7 knockdown, 3 MA, bafilomycin A1, wortmannin. Short term assays.	No	68
	p53 inducible cells or RKO colon cell line	p53 induction/ Actinomycin D treatment	LC3-II accumulation, LC3-GFP puncta, EM, protein degradation, Atg5 knockdown. Short and long term assays.	No	27
Autophagy promotes necrosis	Bax/Bak$^{-/-}$ or Bcl-X_L overexpressing MEFs	ER stress inducers (thapsigargin, tunicamycin, brefeldin A)	3 MA, Atg 5 knockdown, EM, LC3-GFP puncta. Short term assays.	No	97
Autophagy protects from apoptosis	Human U251 glioma, rat C6 glioma and mouse L929 fibrosarcoma cell lines	Cisplatin	Acridine orange staining, EM, lysosomal inhibitors bafilomycin and chloroquine, wortmannin. Short term assays.	No	70
	MCF-7 breast cancer cell line	Camptothecin	GFP-LC3 puncta, EM, bafilomycin, wortmannin. 3 MA. Beclin-1, Atg7 knockdown. Short term assays.	No	71
	Colon cancer cells (HCT116, DLD-1, DLD-1/5-FU resistant cell line)	5-fluorouracil	Atg 7 knockdown, 3 MA, LC-3 immunostaining, LC-3 processing and p62 western Blot. Short term assays.	Yes	72
	Myc/p53 knock-in model of lymphoma	MNNG, cyclophosphamide	Atg5, Atg7 knockdown, chloquine, EM, GFP-LC3 puncta, short term assays.	Yes	73
	Prostate cancer cell lines (LNCaP, PC3)	2-deoxyglucose	GFP-LC3, LC3-II accumulation, Beclin-1 knockdown. Short term assays.	No	85
	Pancreatic (1,420), melanoma (MDA-MB-435) and breast cancer (SKBR3) cell lines	2-deoxyglucose	LC-3 immunocytochemistry, LC3-II flux, Atg 7 knockdown, 3 MA, rapamycin. Short term assays.	No	86
	K562 CML cell line or primary CML cells	Imatinib	LC3-II accumulation, GFP-LC3 puncta, chloroquine, bafilomycin, Atg 5 or Atg 7 knockdown. Short and long term assays.	Yes	93
	Gastrointestinal stromal tumor cell lines	Imatinib	LC-3 accumulation, mCherry-GFP-LC3 puncta, chloroquine, quinacrine, Atg 7 or Atg 12 knockdown. Long term assays.	Yes	94
	Prostate cancer (DU145), colon cancer (HCT116)	ER stress inducers (A23186, tunicamycin, thapsigargin)	LC3-II accumulation, GFP-LC3 puncta, EM, Atg6/Atg8 kd. Short term assays.	No	68
	Wt MEFs	ER stress inducers (thapsigargin, tunicamycin, brefeldin A)	3 MA. Short term assays.	No	97

Table 2. Studies cited in this review, indicating the proposed role of autophagy in cell death and the methods used to determine it (continued)

Autophagy protects from cell death	Glioblastoma specimens and stem cells isolated from them	Radiation	LC-3 accumulation, acridine orange staining, 3 MA, bafilomycin A1, Beclin-1 or Atg 5 knockdown. Long term assays.	No	81
	U251 glioma cell line	Temozolomide /etoposide	LC-3 accumulation, 3 MA, bafilomycin A1, Beclin-1 knockdown. Short term assays.	No	69
Inhibition of autophagy protects from cell death when blocked at early stages but induces cell death when blocked at the lysosomal stage	Malignant glioma cells (U373-MG)	Temozolomide	Acridine orange, GFP-LC3 puncta, LC-3 mRNA levels, 3 MA, Bafilomycin A1. Short term assays.	No	74
	Malignant glioma cells (U87-MG, U373-MG, LNZ308, LN229)	Imatinib	LC3-II accumulation, LC3-GFP puncta, acridine orange, 3 MA, Atg 5 kd, bafilomycin, RTA203. Short and long term assays.	No	95
Autophagy promotes survival or death depending on the autophagy gene that is knocked down	MDA-MB-231 breast, HTB43 pharyngeal, A549 lung, HTB35 cervical cancer cell lines	Radiation	EM, GFP-LC3 puncta, Atg 3, 4b, 4c, 5, 12, Beclin-1 knockdown. Long term assays.	No	82
	Panc02 prostate cancer cell line	Paclitaxel or melphalan	GFP-LC3 puncta, LC3-II accumulation, Atg 5 or Beclin-1 knockdown, 3 MA. Short term assays.	No	89

work, sensitization to radiation could be inhibited in clonogenic survival assays by Atg 5/Beclin-1 knockdown.[78] This study suggests that autophagy might act as a cell death pathway, which is more efficient when apoptosis is inhibited.

In contrast, other work supports a protective role of autophagy against radiation therapy. Thus, glioblastoma specimens and stem cells isolated from the same samples undergo differential induction of autophagy after radiation treatment. In this work, treatment with 3-MA or bafilomycin A1 decreased viability more efficiently in isolated stem cells compared with bulk glioma cells,[81] suggesting that autophagy might be especially important in conferring resistance in glioma "stem" cells. Confusingly, knockdown of different autophagy related genes might have differential effects on survival depending on the gene and on the radiation dose used in different cancer cell lines.[82]

Treatments that Target Metabolic Pathways

Cancer cells have altered metabolism characterized by increased glycolysis even in the presence of oxygen.[83] Therefore, treatments like metformin, which reduces the levels of circulating glucose and inhibits mTOR through AMPK activation,[84] and 2-deoxyglucose (2-DG), a glucose analog that inhibits glycolytic hexokinase, are being used to target this characteristic of cancer cells. Since one of the functions of autophagy is to provide ATP and amino acids during starvation, its effects might be particularly important in these treatments.

As expected, 2-DG induces autophagy in prostate, melanoma, pancreatic and breast cancer cell lines and has important effects in hypoxic cells which cannot produce ATP aerobically.[85,86] In accordance with the protective and nutrient providing role of autophagy, Beclin-1 knockdown induces caspase-3 activation and increases cell death after 2-DG treatment of prostate cancer cell lines.[85] A study in pancreatic, melanoma and breast cancer cell lines suggested that 2-DG induces autophagy by inducing ER stress rather than by ATP decrease. 2-DG interferes with oligosaccharide synthesis leading to abnormal N-liked glycosylation and ER stress. Mannose, the main sugar in N-linked glycosylations, reversed ER stress and autophagy without affecting ATP levels. In these cells, 3MA treatment or Atg 7 knockdown increased cell death and caspase cleavage, while rapamycin decreased cell death,[86] supporting a protective role of autophagy.

Metformin has been shown to decrease cancer cell viability and tumor growth in xenograft models.[84] Interestingly, metformin decreases ATP levels and inhibits mitochondrial complex 1 activity in a prostate cancer cell line but not in immortalized human primary prostate epithelial cells. Furthermore, the combination of 2-DG and metformin increased cell death compared to the drugs alone. In this model, 2-DG but not metformin induced autophagy, despite the fact that metformin caused AMPK phosphorylation. Moreover, metformin inhibited 2-DG induced autophagy and increased apoptosis in the LNCaP prostate cancer cell line.[87] In a different study, metformin or mTOR knockdown decreased cisplatin induced cell death in glioma and fibrosarcoma cell lines while bafilomycin A1 treatment or AMPK knockdown increased cell death with this treatment, suggesting a protective role for AMPK mediated autophagy with this treatment.[70] Therefore, a potential caveat of the use of metformin is that when it is used in combination with other therapies it could increase autophagy through AMPK activation and induce a mechanism of protection against certain treatments. Accordingly, metformin or AICAR (a different AMPK activator) treatment selectively decreased tumor xenograft growth of the $p53^{-/-}$ HCT116 colon cancer cell line and not in the same cell line expressing wild type p53.[88] The authors found autophagy induction in $p53^{+/+}$ but not in $p53^{-/-}$ cells in response to metformin or AICAR treatment, indicating that p53 induced autophagy might be responsible for resistance to metformin treatment in mice xenografts

and suggesting that metformin might be useful in the treatment of p53-defficient tumors. Interestingly, in the same study, both $p53^{+/+}$ and $^{-/-}$ cells induced autophagosome formation in response to rapamycin, suggesting that p53 is required for AMPK but not for rapamycin mediated autophagy.

Taxanes

The microtubule depolymerizing agent paclitaxel also induces autophagy in cancer cells. For example, when human pancreatic cancer cells are treated with paclitaxel, they undergo autophagy that can be decreased by Atg 5 or Beclin-1 knockdown or 3MA treatment. Interestingly, both Beclin-1 knockdown and 3MA treatment increased the amount of apoptotic cells after treatment with these drugs while Atg 5 knockdown decreased it.[89] It is unclear why two knockdowns (Beclin-1 and Atg 5), which should, in principle, have the same inhibitory effects on autophagy have different effects in this case. This study therefore provides a good example of the potential for confusion when considering the role of autophagy in cancer therapy—a drug is demonstrated to induce autophagy as measured by LC-3 puncta, which was blocked by knockdown of Atg proteins that are generally considered to be essential for autophagy. However, when apoptosis was measured by staining with the Annexin V marker, one knockdown appears to increase apoptosis while the other one inhibits it. The simplest explanation for such results (assuming that the assay of autophagy was in fact measuring what the authors thought it was—i.e., in this case, that the reduced level of GFP-LC3 puncta is a measure of inhibited autophagy and the drug that was proposed to increase autophagy was in fact increasing the flux) is that the effects of the knockdown are not really indicative of a protective effect of autophagy but rather that one or both knockdowns affected other pathways and that the increased or decreased apoptosis was due to these effects instead. In a different study, based on the fact that cervical cancer tissues have lower levels of Beclin-1 than normal tissues, the authors overexpressed Beclin-1 in a cervical cancer cell line. Beclin-1 overexpression decreased viability in cells treated with paclitaxel, cisplatin, 5-FU or epirubicin and Beclin-1 knockdown had no effect on cell viability with the same drugs.[90] No assessment of autophagic features was performed in this work.

Targeted Therapies

The PI3K/Akt/mTOR pathway is important for driving cell growth and proliferation in multiple tumor types.[91] Several receptor tyrosine kinases including the epidermal growth factor receptor can activate PI3K activity. Therefore, rapamycin, an mTOR inhibitor, various analogs and other drugs that target specific protein kinases in this pathway have been developed in order to inhibit this proliferation signals. Since the PI3K network regulates autophagy, drugs that target this pathway induce autophagy in cancer cells and rapamycin alone or in combination with the PI3K inhibitor LY294002 kills glioma cells non-apoptotically and with increased vacuole formation[92]—i.e., a potential example of autophagic death. Rapamycin can also sensitize cancer cells to chemotherapeutic agents such as adriamycin, cisplatin and hormonal therapies or enhance the efficacy of radiation therapy.[91] However, it is not clear whether this is an induction of autophagic cell death per se or if these effects are due to other mTOR effects that are separate from autophagy.

On the other hand, rapamycin pre-treatment can also protect from apoptotic stimuli.[66] Rapamycin induces a decrease in mitochondrial proteins and in cytochrome *c* release, which can be inhibited by 3MA treatment and is probably due to mitophagy. To test if rapamycin-mediated protection was due to mitophagy, the authors compared the protective effect seen with intrinsic apoptosis pathway inducers with that seen when using TNFα or Fas to induce apoptosis through the extrinsic pathway. This led to the conclusion that rapamycin pre-treatment and autophagy could protect cells in a manner that is dependent on the mitochondrial apoptosis pathway. Thus, even for an agent that is one of the most widely used ways to manipulate autophagy, the question of whether rapamycin-induced autophagy can help kill tumor cells during treatment or if it protects against apoptotic stimuli is unclear and might be different in different tumors. And, as with the other drugs discussed above, this is a question that really needs to be answered since rapamycin and its analogs are widely used in clinical trials both alone and in combination with other therapies, including hydroxychloroquine, which was chosen for a combination therapy study because it can inhibit autophagy.

Imatinib is a bcr-abl, c-abl, c-kit, PDGFRα and β inhibitor, and is the standard therapy for patients with chronic myeloid leukemia (CML), which harbor the bcr-abl fusion gene encoding a constitutively active tyrosine kinase. Imatinib treatment induces autophagy in CML cell lines. Furthermore, chloroquine or bafilomycin treatment as well as Atg 5 or 7 knockdown enhanced cell death induced by imatinib and chloroquine treatment decreased the amount of cells that can be recovered from the bone marrow cells of leukemic mice.[93] Imatinib treatment also induces autophagy in gastrointestinal stromal tumor cell lines, which commonly harbor activating KIT or PDGFRα mutations, and display an arrest in cell growth dependent on the presence of the drug. When autophagy genes are knocked down in these cells, or they are treated with anti-malarial agents chloroquine or quinacrine that both inhibit autophagy at the lysosomal fusion step, replating efficiency decreased in vitro as well as xenograft tumor growth in vivo.[94] The simplest explanation for these data is that the autophagy induced by the drug was protective and that overcoming it leads to tumor cell death. In a different study, glioma cells treated with imatinib also induced autophagy. Interestingly, when autophagy was inhibited at the autophagosome formation stage (3MA, Atg 5 knockdown), cell death decreased upon imatinib treatment and when autophagy was inhibited at the lysosome fusion stage (with bafilomycin A1 or RTA203), cell death increased with an associated loss in mitochondrial membrane potential. This study suggests that autophagy inhibition at different stages might have different or even opposing effects.[95] However, the effect of 3MA was no longer seen in colony formation assays, underscoring the importance of performing both short and long term assays to assess cell death

and raising the possibility that rather than altering whether or not the cells died, what was being changed here was the kinetics with which they died.

Bcl-2 is an anti-apoptotic protein that is overexpressed in half of human malignancies and its overexpression is associated with resistance to chemotherapy and radiation.[67] As noted above Bcl-2 also directly regulates autophagy through its ability to bind Beclin-1 so one might expect that therapeutics designed to target Bcl-2 would also affect autophagy. Bcl-2 knockdown with siRNAs induced non-apoptotic cell death in the MCF-7 breast cancer cell line that can be decreased by Atg 5 knockdown. Moreover, (-) gossypol, a natural BH_3-mimetic Bcl-2 inhibitor induced non-apoptotic death with autophagic features in androgen independent prostate cancer cell lines with increased Bcl-2/Bcl-X_L and induced apoptosis in androgen dependent cells with lower Bcl-2/Bcl-X_L levels. In this model, Atg 5/Beclin-1 knockdown or 3MA treatment decreased (-)-gossypol induced cell death in androgen independent cells with high Bcl-2 protein levels but increased cell death in androgen dependent cells with lower Bcl-2/Bcl-X_L levels.[96]

Other Therapies

Endoplasmic reticulum (ER) stress inducers A23186, tunicamycin and thapsigargin induced apoptosis and autophagy in colon and prostate cancer cell lines. Interestingly, autophagy functioned as a protective mechanism from apoptosis in transformed cells and as an apoptosis promoting mechanism in non-transformed cells.[68] On the other hand, Bax/Bak$^{-/-}$ MEFs survived short term treatment with thapsigargin or brefeldin A, but died after prolonged treatment with necrotic features. 3MA treatment or Atg 5 knockdown in these cells decreased cell death suggesting a possible role of autophagy in promoting necrosis. In this same study, 3MA increased cell death in apoptosis-competent wild type cells treated with ER stress inducers which suggests that autophagy may have opposing or different effects in determining the fate of a cell in apoptosis-competent and deficient cells.[97]

Autophagy in the Progression of Resistance to Treatment

Autophagy has also been implicated in the development of cancer cell resistance to certain treatments. For instance, in one study, the MCF-7 breast cancer cell line was exposed to increasing concentrations of tamoxifen until it could survive and proliferate in the presence of the drug.[98] These cells showed increased levels of basal autophagy and increased autophagy in response to tamoxifen treatment when compared to the parental MCF-7 cell line. Moreover, treatment with 3MA or Beclin-1 knockdown induced apoptosis in these cells after tamoxifen treatment. Similar results were found in a different breast cancer cell line that was made resistant to trastuzumab.[99] In this study, resistant cells showed increased autophagy compared to parental cells and autophagy inhibition by LC-3 knockdown restored sensitivity to trastuzumab treatment. These results suggest that autophagy could be involved in the progression of breast cancer to antiestrogen or trastuzumab resistance.

Autophagy has also been involved in the resistance to death receptor induced apoptosis and when inhibited, it can restore sensitivity to the treatment. Thus, wild type MEFs have been shown to be resistant to TNFα, while autophagy deficient Atg 5 knockout MEFs are sensitive to this treatment.[100] In a different study, Bax knockout HCT116 cells were shown to be resistant to TRAIL mediated apoptosis and autophagy inhibition by Beclin-1 or Vps34 knockdown restored sensitivity to this treatment.[101] This work also suggests that the mechanism by which autophagy protects from apoptosis is the autophagic degradation of the active fragment of caspase-8. In general, these results suggest that autophagy inhibition together with cancer treatment could decrease survival of tumor cells that develop resistance to treatment or could help in killing tumor cells that otherwise would be resistant to the treatment.

Conclusions

The confusion in the literature about even such fundamental questions such as whether or not drug-induced autophagy kills or protects tumor cells may arise because in some studies, pharmacological inhibitors are used without confirmation by genetic manipulation of autophagy, and might be observing effects independent of autophagy. For instance, 3MA can inhibit loss of mitochondrial membrane permeabilization,[102] and can even induce autophagy in cells grown in full medium[103] or suppress proteolysis in Atg 5 deficient cells.[104] It is also important to mention that different methods and appropriate controls should be used in order to distinguish between basal autophagy, induction of autophagy and suppression of downstream steps of autophagy.[22] Moreover, in order to assess viability, long-term assays should be used together with short-term assays measuring metabolic activity, membrane integrity, etc. This allows an investigator to determine if autophagy just delays the kinetics of cell death or the actual amount of cell death.[105] In vivo experiments should also be incorporated to further assess the physiological significance of the results. Because the in vivo environment will itself alter the autophagic response in tumors, it is quite possible that different effects of autophagy manipulation will be seen when comparing in vitro and in vivo studies with the same tumors and the same drugs. Finally, immune competent models should be used when possible (or better still comparisons between immune-competent and immune-deficient animals undertaken) since autophagy could have an important function in the modulation of the immune response to tumor cells.[4,89,106]

Because the same stimulus has been shown to induce different effects of autophagy on cell survival in different cell types,[68,96,97] it is possible that the genetic background of a cell could determine its response to autophagy during chemotherapy. In this regard, the mechanism of transformation in a tumor could be particularly important. For example, it has been suggested that in cells without increased anti apoptotic capacity e.g., with low Bcl-2/Bcl-X_L levels, Bax overexpression can decrease autophagy through caspase-3 dependent cleavage of Beclin-1. However,

in cells with high levels of antiapoptotic Bcl-2 family proteins, Bax can disrupt the Bcl-2/Bcl-X_L interaction with Beclin-1 and increase autophagy.[107] It has also been reported that when Bcl-2 is overexpressed, autophagy can switch from a mechanism that protects against growth factor deprivation to one that induces apoptosis.[29] On the other hand, autophagy may have differential effects on cell survival depending on the treatment in the same cells, possibly due to different signaling pathways activated by distinct treatments or even different "types" of autophagy. In this regard, it has been suggested that autophagy could be selective or non-selective. Thus, non-selective autophagy would be the one induced by metabolic stress or low nutrient levels resulting in the generation of ATP and metabolites for cell survival.[108] On the other hand, accumulating evidence supports the idea that autophagy selectively degrades specific targets, including proteins, mitochondria, peroxisomes, endoplasmic reticulum or bacteria and selective autophagy could have differential roles in each biological context.[108,109]

Another possibility is that tumor cells with activating mutations in cell survival pathways like the PI3K/Akt/mTOR pathway could have constitutive low levels of autophagy, which could result in a different outcome. For instance, Akt activation impairs autophagy in cells with defects in apoptosis, eliminating a survival mechanism that results in necrosis.[62] In a different study, expression of a constitutively active Akt inhibits the survival supportive function of autophagy in IL-3 dependent Bax/Bak$^{-/-}$ cells deprived of growth factor.[29] A different possibility that has been proposed[74,95] is that blocking autophagy at different steps i.e., at the autophagosome formation step or at the autophagosome-lysosome fusion step could have different outcomes on cell survival—a rationale for such differences may be that it is not the process of autophagy that determines death but rather the presence of high levels of autophagosomes or low levels of ATP due to decreased lysosomal degradation of the autophagosome content. So, a manipulation that blocks formation of autophagosomes might lead to quite different results than a manipulation that blocks their fusion with lysosomes and subsequent degradation. Additionally, differential effects from autophagy inhibition might arise from compensation of other types of autophagy; since chaperone mediated autophagy (CMA) can be upregulated as a compensatory mechanism in Atg 5 knockout cells.[100]

Thus, besides knowing that virtually any cancer treatment can induce autophagy, that changes in the environment to which tumors are exposed to, such as altered hypoxia also affect autophagy; and thus, that autophagy is undoubtedly happening and varying in tumors, we still don't have a clear understanding of its role in cancer cell survival. It is important to determine if autophagy has differential effects dependent on the genetic background of the cell, on the type of cancer or on the type of treatment. Moreover, it is also important to determine if the effects on cell survival or death, if any, are important enough to allow us to improve treatment by manipulating them or is only likely to have a slight effect e.g., on the kinetics of cell death. These issues are of significant importance since, as we mentioned previously, autophagy manipulation is already being used in clinical trials along with different chemotherapeutic drugs. These considerations make it particularly important we should avoid drawing conclusions based on limited or inadequate studies. It is therefore beholden upon those of us who are working in this area to develop better, more specific, methods to manipulate autophagy, measure the effects of these manipulations and to avoid potential artifacts (e.g., as caused by reliance on short term assays leading to an investigator missing an effect on tumor cell survival/death that was simply delayed). And, while adopting more rigorous approaches it is critical that future studies should examine multiple agents in the same tumor cells and to examine different types of tumor cells to determine if there really are underlying differences in chemosensitivity due to autophagy manipulation. Examination of potential differences in vivo and especially, the development of methods to assess autophagy in the context of clinical trials and the identification of biomarkers that will help discriminate between the conflicting roles for autophagy will be essential to applying these ideas effectively in the clinic.

Until we address these issues, interpretations of clinical studies may be compromised or, even worse, potentially useful treatments may be discarded or not pursued because of a mistaken impression about the value of manipulating autophagy in the therapeutic context.

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References

1. Deter RL, Baudhuin P, De Duve C. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. J Cell Biol 1967; 35:11-6.
2. Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 2007; 8:931-7.
3. Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 2009; 10:458-67.
4. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008; 132:27-42.
5. Rubinsztein DC, DiFiglia M, Heintz N, Nixon RA, Qin ZH, Ravikumar B, et al. Autophagy and its possible roles in nervous system diseases, damage and repair. Autophagy 2005; 1:11-22.
6. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 2006; 441:885-9.
7. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 2006; 441:880-4.
8. Nishino I. Autophagic vacuolar myopathy. Semin Pediatr Neurol 2006; 13:90-5.
9. Martinez-Vicente M, Cuervo AM. Autophagy and neurodegeneration: when the cleaning crew goes on strike. Lancet Neurol 2007; 6:352-61.
10. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. Cell 2004; 119:753-66.
11. Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, et al. Autophagy defends cells against invading group A Streptococcus. Science 2004; 306:1037-40.
12. Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, et al. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. Science 2005; 307:593-6.
13. Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. Nat Rev Cancer 2007; 7:961-7.
14. Kuma A, Mizushima N. Physiological role of autophagy as an intracellular recycling system: with an emphasis on nutrient metabolism. Semin Cell Dev Biol 2010; 21:683-90.
15. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999; 402:672-6.
16. Madeo F, Tavernarakis N, Kroemer G. Can autophagy promote longevity? Nat Cell Biol 12:842-6.
17. Cheng Y, Li H, Ren X, Niu T, Hait WN, Yang J. Cytoprotective effect of the elongation factor-2 kinase-mediated autophagy in breast cancer cells subjected to growth factor inhibition. PLoS One 5:9715.

18. Boya P, Gonzalez-Polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, et al. Inhibition of macroautophagy triggers apoptosis. Mol Cell Biol 2005; 25:1025-40.
19. Gonzalez-Polo RA, Boya P, Pauleau AL, Jalil A, Larochette N, Souquere S, et al. The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci 2005; 118:3091-102.
20. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, et al. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005; 120:237-48.
21. Gozuacik D, Kimchi A. Autophagy and cell death. Curr Top Dev Biol 2007; 78:217-45.
22. Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. Cell 140:313-26.
23. Cao Y, Klionsky DJ. Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. Cell Res 2007; 17:839-49.
24. Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, et al. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat Cell Biol 2006; 8:1124-32.
25. Thorburn A. Apoptosis and autophagy: regulatory connections between two supposedly different processes. Apoptosis 2008; 13:1-9.
26. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol 2007; 8:741-52.
27. Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 2006; 126:121-34.
28. Scott RC, Juhasz G, Neufeld TP. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Curr Biol 2007; 17:1-11.
29. Altman BJ, Wofford JA, Zhao Y, Coloff JL, Ferguson EC, Wieman HL, et al. Autophagy provides nutrients but can lead to Chop-dependent induction of Bim to sensitize growth factor-deprived cells to apoptosis. Mol Biol Cell 2009; 20:1180-91.
30. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, et al. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science 2004; 304:1500-2.
31. Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004; 6:1221-8.
32. Shimizu S, Konishi A, Nishida Y, Mizuta T, Nishina H, Yamamoto A, et al. Involvement of JNK in the regulation of autophagic cell death. Oncogene 29:2070-82.
33. Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, et al. Autophagic programmed cell death by selective catalase degradation. Proc Natl Acad Sci USA 2006; 103:4952-7.
34. Mizushima N. The role of the Atg1/ULK1 complex in autophagy regulation. Curr Opin Cell Biol 22:132-9.
35. Xie Z, Klionsky DJ. Autophagosome formation: core machinery and adaptations. Nat Cell Biol 2007; 9:1102-9.
36. Hamasaki M, Yoshimori T. Where do they come from? Insights into autophagosome formation. FEBS Lett 584:1296-301.
37. Webber JL, Young AR, Tooze SA. Atg9 trafficking in Mammalian cells. Autophagy 2007; 3:54-6.
38. Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, et al. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. Nat Cell Biol 2006; 8:688-99.
39. Funderburk SF, Wang QJ, Yue Z. The Beclin 1-VPS34 complex—at the crossroads of autophagy and beyond. Trends Cell Biol 2010; 20:355-62.
40. Geng J, Klionsky DJ. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. 'Protein modifications: beyond the usual suspects' review series. EMBO Rep 2008; 9:859-64.
41. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005; 122:927-39.
42. Levine B, Sinha S, Kroemer G. Bcl-2 family members: dual regulators of apoptosis and autophagy. Autophagy 2008; 4:600-6.
43. Wei Y, Sinha S, Levine B. Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation. Autophagy 2008; 4:949-51.
44. Zalckvar E, Berissi H, Mizrachy L, Idelchuk Y, Koren I, Eisenstein M, et al. DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-X_L and induction of autophagy. EMBO Rep 2009; 10:285-92.
45. Maiuri MC, Criollo A, Tasdemir E, Vicencio JM, Tajeddine N, Hickman JA, et al. BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X(L). Autophagy 2007; 3:374-6.
46. Sarkar S, Rubinsztein DC. Inositol and IP3 levels regulate autophagy: biology and therapeutic speculations. Autophagy 2006; 2:132-4.
47. Criollo A, Vicencio JM, Tasdemir E, Maiuri MC, Lavandero S, Kroemer G. The inositol trisphosphate receptor in the control of autophagy. Autophagy 2007; 3:350-3.
48. Balaburski GM, Hontz RD, Murphy ME. p53 and ARF: unexpected players in autophagy. Trends Cell Biol 20:363-9.
49. Wang RC, Levine B. Autophagy in cellular growth control. FEBS Lett 584:1417-26.
50. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006; 124:471-84.
51. Shaw RJ. LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. Acta Physiol (Oxf) 2009; 196:65-80.
52. Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev 2007; 21:1621-35.
53. Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al. Autophagy suppresses tumorigenesis through elimination of p62. Cell 2009; 137:1062-75.
54. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 2008; 283:10892-903.
55. Miracco C, Cosci E, Oliveri G, Luzi P, Pacenti L, Monciatti I, et al. Protein and mRNA expression of autophagy gene Beclin 1 in human brain tumours. Int J Oncol 2007; 30:429-36.
56. Karantza-Wadsworth V, White E. Role of autophagy in breast cancer. Autophagy 2007; 3:610-3.
57. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003; 112:1809-20.
58. Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc Natl Acad Sci USA 2003; 100:15077-82.
59. Jin S. Autophagy, mitochondrial quality control and oncogenesis. Autophagy 2006; 2:80-4.
60. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, et al. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J Cell Biol 2005; 169:425-34.
61. Marino G, Salvador-Montoliu N, Fueyo A, Knecht E, Mizushima N, Lopez-Otin C. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. J Biol Chem 2007; 282:18573-83.
62. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation and tumorigenesis. Cancer Cell 2006; 10:51-64.
63. Mathew R, Kongara S, Beaudoin B, Karp CM, Bray K, Degenhardt K, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. Genes Dev 2007; 21:1367-81.
64. Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, et al. Autophagy mediates the mitotic senescence transition. Genes Dev 2009; 23:798-803.
65. Fung C, Lock R, Gao S, Salas E, Debnath J. Induction of autophagy during extracellular matrix detachment promotes cell survival. Mol Biol Cell 2008; 19:797-806.
66. Ravikumar B, Berger Z, Vacher C, O'Kane CJ, Rubinsztein DC. Rapamycin pre-treatment protects against apoptosis. Hum Mol Genet 2006; 15:1209-16.
67. Akar U, Chaves-Reyez A, Barria M, Tari A, Sanguino A, Kondo Y, et al. Silencing of Bcl-2 expression by small interfering RNA induces autophagic cell death in MCF-7 breast cancer cells. Autophagy 2008; 4:669-79.
68. Ding WX, Ni HM, Gao W, Hou YF, Melan MA, Chen X, et al. Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. J Biol Chem 2007; 282:4702-10.
69. Katayama M, Kawaguchi T, Berger MS, Pieper RO. DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. Cell Death Differ 2007; 14:548-58.
70. Harhaji-Trajkovic L, Vilimanovich U, Kravic-Stevovic T, Bumbasirevic V, Trajkovic V. AMPK-mediated autophagy inhibits apoptosis in cisplatin-treated tumour cells. J Cell Mol Med 2009; 13:3644-54.
71. Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. Cell Death Differ 2007; 14:500-10.
72. Li J, Hou N, Faried A, Tsutsumi S, Kuwano H. Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. Eur J Cancer 46:1900-9.
73. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, et al. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 2007; 117:326-36.
74. Kanzawa T, Germano IM, Komata T, Ito H, Kondo Y, Kondo S. Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. Cell Death Differ 2004; 11:448-57.
75. Gewirtz DA, Hilliker ML, Wilson EN. Promotion of autophagy as a mechanism for radiation sensitization of breast tumor cells. Radiother Oncol 2009; 92:323-8.
76. Zois CE, Koukourakis MI. Radiation-induced autophagy in normal and cancer cells: towards novel cytoprotection and radio-sensitization policies? Autophagy 2009; 5:442-50.
77. Kim KW, Mutter RW, Cao C, Albert JM, Freeman M, Hallahan DE, et al. Autophagy for cancer therapy through inhibition of pro-apoptotic proteins and mammalian target of rapamycin signaling. J Biol Chem 2006; 281:36883-90.
78. Moretti L, Attia A, Kim KW, Lu B. Crosstalk between Bak/Bax and mTOR signaling regulates radiation-induced autophagy. Autophagy 2007; 3:142-4.
79. Paglin S, Lee NY, Nakar C, Fitzgerald M, Plotkin J, Deuel B, et al. Rapamycin-sensitive pathway regulates mitochondrial membrane potential, autophagy and survival in irradiated MCF-7 cells. Cancer Res 2005; 65:11061-70.
80. Cao C, Subhawong T, Albert JM, Kim KW, Geng L, Sekhar KR, et al. Inhibition of mammalian target of rapamycin or apoptotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells. Cancer Res 2006; 66:10040-7.
81. Lomonaco SL, Finniss S, Xiang C, Decarvalho A, Umansky F, Kalkanis SN, et al. The induction of autophagy by gamma-radiation contributes to the radioresistance of glioma stem cells. Int J Cancer 2009; 125:717-22.
82. Apel A, Herr I, Schwarz H, Rodemann HP, Mayer A. Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. Cancer Res 2008; 68:1485-94.

83. Hsu PP, Sabatini DM. Cancer cell metabolism: Warburg and beyond. Cell 2008; 134:703-7.
84. Gonzalez-Angulo AM, Meric-Bernstam F. Metformin: a therapeutic opportunity in breast cancer. Clin Cancer Res 16:1695-700.
85. DiPaola RS, Dvorzhinski D, Thalasila A, Garikapaty V, Doram D, May M, et al. Therapeutic starvation and autophagy in prostate cancer: a new paradigm for targeting metabolism in cancer therapy. Prostate 2008; 68:1743-52.
86. Xi H, Kurtoglu M, Liu H, Wangpaichitr M, You M, Liu X, et al. 2-Deoxy-D-glucose activates autophagy via endoplasmic reticulum stress rather than ATP depletion. Cancer Chemother Pharmacol 2010; In press.
87. Ben Sahra I, Laurent K, Giuliano S, Larbret F, Ponzio G, Gounon P, et al. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. Cancer Res 70:2465-75.
88. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res 2007; 67:6745-52.
89. Tang D, Kang R, Cheh CW, Livesey KM, Liang X, Schapiro NE, et al. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. Oncogene 2010; 29:5299-310.
90. Sun Y, Liu JH, Jin L, Lin SM, Yang Y, Sui YX, et al. Overexpression of the Beclin1 gene upregulates chemosensitivity to anticancer drugs by enhancing therapy-induced apoptosis in cervix squamous carcinoma CaSki cells. Cancer Lett 294:204-10.
91. Rao RD, Buckner JC, Sarkaria JN. Mammalian target of rapamycin (mTOR) inhibitors as anticancer agents. Curr Cancer Drug Targets 2004; 4:621-35.
92. Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB, et al. Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. Cancer Res 2005; 65:3336-46.
93. Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, et al. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. J Clin Invest 2009; 119:1109-23.
94. Gupta A, Roy S, Lazar AJ, Wang WL, McAuliffe JC, Reynoso D, et al. Autophagy inhibition and antimalarials promote cell death in gastrointestinal stromal tumor (GIST). Proc Natl Acad Sci USA 2010; 107:14333-8.
95. Shingu T, Fujiwara K, Bogler O, Akiyama Y, Moritake K, Shinojima N, et al. Inhibition of autophagy at a late stage enhances imatinib-induced cytotoxicity in human malignant glioma cells. Int J Cancer 2009; 124:1060-71.
96. Lian J, Wu X, He F, Karnak D, Tang W, Meng Y, et al. A natural BH3 mimetic induces autophagy in apoptosis-resistant prostate cancer via modulating Bcl-2-Beclin1 interaction at endoplasmic reticulum. Cell Death Differ 2010; In press.
97. Ullman E, Fan Y, Stawowczyk M, Chen HM, Yue Z, Zong WX. Autophagy promotes necrosis in apoptosis-deficient cells in response to ER stress. Cell Death Differ 2008; 15:422-5.
98. Samaddar JS, Gaddy VT, Duplantier J, Thandavan SP, Shah M, Smith MJ, et al. A role for macroautophagy in protection against 4-hydroxytamoxifen-induced cell death and the development of antiestrogen resistance. Mol Cancer Ther 2008; 7:2977-87.
99. Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA. Autophagy facilitates the development of breast cancer resistance to the anti-HER2 monoclonal antibody trastuzumab. PLoS One 2009; 4:6251.
100. Wang Y, Singh R, Massey AC, Kane SS, Kaushik S, Grant T, et al. Loss of macroautophagy promotes or prevents fibroblast apoptosis depending on the death stimulus. J Biol Chem 2008; 283:4766-77.
101. Hou W, Han J, Lu C, Goldstein LA, Rabinowich H. Autophagic degradation of active caspase-8: A crosstalk mechanism between autophagy and apoptosis. Autophagy 6:891-900.
102. Xue L, Borutaite V, Tolkovsky AM. Inhibition of mitochondrial permeability transition and release of cytochrome *c* by anti-apoptotic nucleoside analogues. Biochem Pharmacol 2002; 64:441-9.
103. Wu YT, Tan HL, Shui G, Bauvy C, Huang Q, Wenk MR, et al. Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. J Biol Chem 285:10850-61.
104. Mizushima N, Yamamoto A, Hatano M, Kobayashi Y, Kabeya Y, Suzuki K, et al. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. J Cell Biol 2001; 152:657-68.
105. Thorburn A. Studying autophagy's relationship to cell death. Autophagy 2008; 4:391-4.
106. Thorburn J, Horita H, Redzic J, Hansen K, Frankel AE, Thorburn A. Autophagy regulates selective HMGB1 release in tumor cells that are destined to die. Cell Death Differ 2009; 16:175-83.
107. Luo S, Rubinsztein DC. Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-x_L. Cell Death Differ 17:268-77.
108. Wilkinson S, Ryan KM. Autophagy: an adaptable modifier of tumourigenesis. Curr Opin Genet Dev 20:57-64.
109. Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. Mol Cell 2009; 34:259-69.
110. Klionsky DJ, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy 2008; 4:151-75.