

Autophagy and cancer

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Abbreviations: 3-MA, 3-methyladenine; AMBRA1, activating molecule in Beclin-1-regulated autophagy protein 1; AMPK, 5' AMP-activated protein kinase or AMP-activated protein kinase; ATG, autophagy-related gene; BFA, bafilomycin A1; CQ, chloroquine; DAPkinase, death associated protein (DAP) kinase; EGCG, epigallocatechin-3-gallate; HCQ, hydroxychloroquine; HDAC inhibitor, histone deacetylases inhibitor; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog; RNAi, RNA interference; TSC, tuberous sclerosis complex

Abstract

Basal autophagy plays a critical role in maintaining cellular homeostasis and genomic integrity by degrading aged or malfunctioning organelles and damaged or misfolded proteins. However, autophagy also plays a complicated role in tumorigenesis and treatment responsiveness. It can be tumor-suppressing during the early stages of tumorigenesis (i.e., it is an anti-tumor mechanism), as reduced autophagy is found in tumor cells and may be associated with malignant transformation. In this case, induction of autophagy would seem to be beneficial for cancer prevention. In established tumors, however, autophagy can be tumor-promoting (i.e., it is a pro-tumor mechanism), and cancer cells can use enhanced autophagy to survive under metabolic and therapeutic stress. The pharmacological and/or genetic inhibition of autophagy was recently shown to sensitize cancer cells to the lethal effects of various cancer therapies, including chemotherapy, radiotherapy and targeted therapies, suggesting that suppression of the autophagic pathway may represent a valuable sensitizing strategy for can-

cer treatments. In contrast, excessive stimulation of autophagy may also provide a therapeutic strategy for treating resistant cancer cells having high apoptotic thresholds. In order for us to develop successful autophagy-modulating strategies against cancer, we need to better understand how the roles of autophagy differ depending on the tumor stage, cell type and/or genetic factors, and we need to determine how specific pathways of autophagy are activated or inhibited by the various anti-cancer therapies.

Keywords: autophagy; cell death; cell transformation, neoplastic; neoplasms; therapeutics

Introduction

Autophagy is a self-digestive process that ensures the lysosomal degradation of superfluous or damaged organelles and misfolded proteins (Levine and Klionsky, 2004). Basal autophagy helps maintain homeostasis by contributing to protein and organelle turnover, while additional autophagy is induced in stressed cells as a cell-survival mechanism. Autophagic defects have been implicated in various diseases and health states, including neurodegeneration, aging, infection, myopathy, Crohn's disease and cancer (Levine and Kroemer, 2008). However, the role of autophagy in cancer is quite complicated and still somewhat controversial; it appears to be tumor suppressive during cancer development, but contributes to tumor cell survival during cancer progression (Rouschop and Wouters, 2009). Furthermore, tumor cells can use autophagy to resist various anti-cancer therapies (Chen and Karantza-Wadsworth, 2009). Cancer cells experience higher metabolic demands and stresses than normal cells, due to their rapid proliferation and altered glycolytic metabolism (White and DiPaola, 2009), and thus may depend more heavily on autophagy for survival (Amaravadi *et al.*, 2011). In many experimental settings, the inhibition of autophagy has been shown to enhance the therapeutic benefits of various cancer therapies (Chen and Karantza-Wadsworth, 2009; Chen *et al.*, 2010). However, strategies to induce autophagic cell death have also shown promise as a means for killing certain types of cancer cells with high

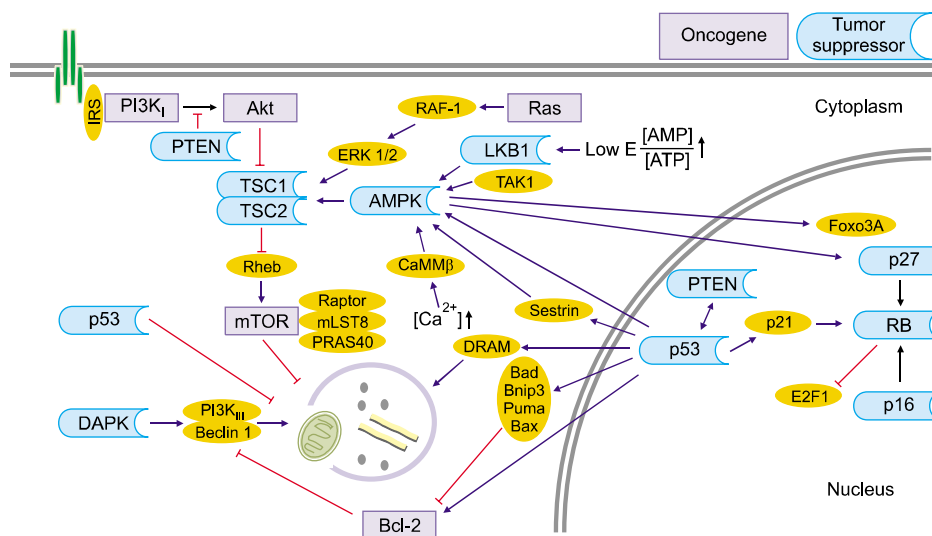


Figure 1. Oncogenes and tumor suppressors associated with the regulation of autophagy. Tumor suppressors (blue) except for cytoplasmic p53 are among the factors positively regulating autophagy, whereas oncogene products (pink) inhibit autophagy. Growth factor signaling activates the PI3K/Akt/mTOR axis resulting in autophagy inhibition. In contrast, class III PI3K activates autophagy. Low cellular energy levels with increased AMP/ATP ratio activate the LKB1-AMPK-mTOR pathway to also upregulate autophagy. p53 exhibits complex autophagy regulation, as nuclear p53 activated by genotoxic or oncogenic stress positively regulates autophagy by inhibiting mTOR in an activated AMPK- and TSC1/TSC2-dependent manner, whereas cytoplasmic p53 can suppress autophagy.

apoptotic thresholds (Gozuacik and Kimchi, 2007). Although we still have much to learn about the regulation of autophagy in cancer, it appears to provide a promising target for cancer treatment. This review examines the multiple roles of autophagy as a novel target for anticancer therapy and assesses the current efforts and remaining tasks in the development of autophagy-modulating strategies against cancer.

Roles of autophagy in cancer

Autophagy as a tumor-suppressing mechanism

Deficiencies in autophagy lead to the accumulation of damaged macromolecules and organelles (particularly mitochondria), subsequently inducing oxidative stress, DNA damage and chromatin instability (Levin and Klionsky, 2004; Chen and Karantz, 2011). Thus, autophagic defects are ultimately associated with the accumulation of oncogenic mutations and increased tumor susceptibility (Karanta-Wadsworth *et al.*, 2007; Mathew *et al.*, 2007). Some of the most important evidence for the role of autophagy in tumor suppression comes from studies on the Bcl-2-interacting protein, Beclin 1 (BECN1, also called ATG6). For example, mono-allelic deletion of Beclin 1 has been frequently observed in human breast (Futreal *et al.*, 1992), ovarian (Aita *et al.*, 1999) and prostate cancers (Gao *et al.*, 1995), and mice with a disrupted Beclin

1 gene had a higher incidence of lymphoma, lung cancer and liver cancer (Qu *et al.*, 2003; Yue *et al.*, 2003). Constitutive activation of the PI3K-Akt-mTOR axis, a common characteristic of cancer (LoPiccolo *et al.*, 2008), is known to suppress autophagy (Janku *et al.*, 2011), while promoting tumor cell growth, proliferation and survival (Martelli *et al.*, 2007) (Figure 1). A number of tumor suppressor proteins have been shown to promote autophagy, including Atg4c (Mariño *et al.*, 2007), Bax-interacting factor-1 (Bif-1) (Takahashi *et al.*, 2007), BH3-only proteins (Maiuri *et al.*, 2007), DAPkinase (Bialik and Kimchi, 2010), LKB1 (Liang *et al.*, 2007), ultraviolet radiation resistance-associated gene (UVRAG) (Liang *et al.*, 2006), PTEN (Arico *et al.*, 2001), TSC (Zhou *et al.*, 2009), nuclear p53 (Maiuri *et al.*, 2010) and AMPK (Luo *et al.*, 2010). Furthermore, autophagy has been shown to reduce intratumoral necrosis and local inflammation (Degenhardt *et al.*, 2006). Collectively, these results support the contention that autophagy plays a tumor-suppressing role in cancer, and suggest that reduced autophagic activity may constitute a hallmark of cancer (Hanahan and Weinberg, 2011).

Autophagy as a pro-survival and resistance mechanism

In established cancer cells, metabolic stress (which arises as a result of insufficient nutrient or oxygen supplies and/or the increased energetic demands

Table 1. Preclinical studies supporting autophagy inhibition for cancer treatment

Cancer type	Treatment	Accelerated cell death by autophagy inhibition			Reference
		Early step	Late step	siRNA	
Breast cancer	γ -radiation			Bcelin 1, ATG3, ATG4b, ATG4c, ATG5, ATG12	Apel <i>et al.</i> , 2008
	Tamoxifen	3-MA		ATG5, Beclin 1, ATG7	Schoenlein <i>et al.</i> , 2009
	Bortezomib			LC3, HDAC6, ATF4	Milani <i>et al.</i> , 2009
	Trastuzumab	3-MA	BFA	LC3	Vazquez-Martin <i>et al.</i> , 2009
	Sulforaphane		BFA		Kanematsu <i>et al.</i> , 2010
Colorectal cancer	Bortezomib			Beclin 1	Fels <i>et al.</i> , 2008
	γ -radiation			Beclin 1, ATG5, ATG7, UVRAG	Apel <i>et al.</i> , 2008
	5-FU	3-MA		ATG7	Li <i>et al.</i> , 2010
Glioma	Sulforaphane	3-MA			Nishikawa <i>et al.</i> , 2010
	Temozolomide		BFA		Kanzawa <i>et al.</i> , 2004
	4-HPR	3-MA	BFA		Tiwari <i>et al.</i> , 2008
	γ -radiation	3-MA	BFA	Beclin 1, ATG5	Lomonaco <i>et al.</i> , 2009
	Imatinib		BFA, RTA 203		Shingu <i>et al.</i> , 2009
	PI-103	3-MA	BFA, Monensin		Fan <i>et al.</i> , 2010
	Rapamycin		BFA		Fan <i>et al.</i> , 2010
Chronic myeloid leukemia (CML)	SAHA	3-MA	CQ		Carew <i>et al.</i> , 2007
	OSI-027 (mTOR)		CQ		Yogalingam and Pendergast, 2008
	Imatinib		CQ, BFA	ATG5, ATG7	Bellodi <i>et al.</i> , 2009
Multiple Myeloma (MM)	Imatinib	3-MA			Crowley <i>et al.</i> , 2011
	Bortezomib		BFA		Kawaguchi <i>et al.</i> , 2011
Prostate cancer	Androgen deprivation	3-MA		Beclin1	Li <i>et al.</i> , 2008
	ADI-PEG20 (arginine deiminase)		CQ	Beclin1	Kim <i>et al.</i> , 2009
	Saracatinib (Src kinase)	3-MA	CQ	ATG7	Wu <i>et al.</i> , 2010
Skin cancer	Cisplatin	3-MA		ATG5	Claerhout <i>et al.</i> , 2010
Lymphoma	Tamoxifen		CQ	shATG5	Amaravadi <i>et al.</i> , 2007
Gastrointestinal stromal tumors (GISTs)	Imatinib		CQ, Quinacrine	ATG7, ATG12	Gupta <i>et al.</i> , 2010
Gastric cancer	Quercetin		CQ	ATG5, Beclin1	Wang <i>et al.</i> , 2011

of rapidly dividing tumor cells) induces autophagy as the cells seek an alternative source of energy and metabolites (Onodera and Oshumi, 2005; Degenhardt *et al.*, 2006; Jones and Thompson, 2009; Rosenfeldt and Ryan, 2009; Rabinowitz and White, 2010). Furthermore, autophagy may also be induced as an adaptive cellular response to various cancer therapies, leading to chemoresistance and cancer cell survival (Chen and Karantza-Wadsworth, 2009; White and DiPaola, 2009). A number of studies in various cancers have examined the inhibition of autophagy through pharmacological means, such

as treatment with 3-methyladenine (3-MA, a PI3K III inhibitor), bafilomycin A (a specific inhibitor of vacuolar-type H⁺-ATPase), chloroquine (CQ) or hydroxychloroquine (HCQ) (lysosomotropic agents that impair fusion between autophagosomes and lysosomes), or by knockdown of autophagy-related genes, such as *ATG5*, *ATG6* and *ATG7*. Table 1 summarizes preclinical studies supporting autophagy inhibition as an anticancer strategy. Notably, these inhibitions sensitized cancer cells to a wide range of therapeutic modalities, including genotoxic chemo- and radio-therapy, hormonal

Table 2. Preclinical studies supporting autophagy induction for cancer treatment

Cancer type	Treatment	Delayed cell death by autophagy inhibition			Reference
		Early step	Late step	siRNA	
Prostate cancer	Phenethyl isothiocyanate	3-MA		ATG5	Bommareddy <i>et al.</i> , 2009
	Fisetin		CQ	Beclin 1	Suh <i>et al.</i> , 2010
Glioma	Sodium selenite	3-MA	CQ	ATG6, ATG7	Kim <i>et al.</i> , 2007; Kim and Choi, 2008
	Imatinib	3-MA		ATG5	Shingu <i>et al.</i> , 2009
	Cannabinoid	3-MA	HCQ, E64+Pepstatin A	ATG1, ATG5, AMBRA1	Salazar <i>et al.</i> , 2009
	β -Lapachone	3-MA	BFA	ATG5, ATG6	Park <i>et al.</i> , 2011
Lung cancer	Elisidepsin (PM02734)	3-MA		ATG5	Ling <i>et al.</i> , 2011
CML	Resveratrol		BFA	ATG5, LC3, p62	Puissant <i>et al.</i> , 2010

therapy and receptor tyrosine kinase inhibition (Chen *et al.*, 2010). In this respect, the inhibition of autophagy appears to represent a major therapeutic means for sensitizing cells to various anti-cancer therapies.

Autophagy as a pro-death mechanism: autophagic cell death

Under extreme stress, tumor cells with apoptosis-defective genetic backgrounds die *via* other mechanisms (Kondo and Kondo, 2006; Gozuacik and Kimchi, 2007). Sustained autophagic activation, which leads to turnover of proteins and organelles beyond a survival threshold, can kill some cancer cells with high apoptotic thresholds, thus enhancing treatment efficacy (Yang *et al.*, 2011). In this case, therefore, cell death *via* autophagy could provide an alternative therapeutic strategy (Gozuacik and Kimchi, 2007; Eisenberg-Lerner *et al.*, 2009).

However, the concept of “autophagic cell death”, also known as type II programmed cell death (Gozuacik and Kimchi, 2004), has been the subject of some controversy. It was initially regarded as a cell death mode that included the presence of autophagosomes (Schweichel and Merker, 1973). In many cases, however, autophagy is presumably activated by dying cells as part of an unsuccessful effort to cope with stress (i.e., as a pro-survival mechanism) (Boya *et al.*, 2005). Increases in autophagic markers (e.g., autophagosome accumulation and up-regulation of the LC3 II form) in dying cells following exposure to chemotherapy or molecular targeted therapeutics do not necessarily indicate increases in autophagic flux (Mizushima *et al.*, 2010). Instead, inefficient fusion between autophagosomes and lysosomes or reduced lysosomal degradation might lead to massive accumulation of autophagosomes. In this case, inhibition of autophagy would accelerate cell death rather than preventing

it (Boya *et al.*, 2005). Since the term “autophagic cell death” is highly prone to misinterpretation from a purely morphological perspective, the Nomenclature Committee on Cell Death very recently suggested that the term should only be used to indicate a cell death that is mediated by autophagy, as assessed based on biochemical and functional considerations (Galluzzi *et al.*, 2012). In other words, “autophagic cell death” should be a cell death mode that is suppressed by inhibition of autophagy by chemicals (e.g., 3-MA) and/or genetic means, such as gene knockout/mutation or RNAi targeting of essential autophagic modulators, such as *AMBRA1*, *ATG5*, *ATG12* or *Beclin 1* (Galluzzi *et al.*, 2012). Recently, Shen and Codogno (2011) also defined “autophagic cell death” as a form of programmed cell death in which autophagy per se serves as a cell death mechanism, in that it is cell death by autophagy, not cell death with autophagy. They proposed that autophagic cell death should meet the following criteria: 1) the cell death occurs without apoptosis; 2) there is an increase of the autophagic flux, not just the autophagic markers, in dying cells; and 3) suppression of autophagy *via* both pharmacological inhibitors and genetic approaches can rescue or prevent the cell death. Such autophagic cell death has been reported during the *in vivo* development of *D. melanogaster* (Berry and Baehrecke, 2007; Denton *et al.*, 2009) and in hippocampal neural stem cells of adult rat brain following insulin withdrawal (Yu *et al.*, 2008). However, the *in vivo* evidence of autophagic cell death in mammals has been relatively limited to date.

Targeting autophagy for cancer therapy

Induction of autophagy for cancer prevention

Various dietary phytochemicals, including β -carotene, lycopene, lutein, quercetin, resveratrol, curcumin and

epigallocatechin-3-gallate (EGCG) have demonstrated chemopreventive activities in many preclinical and clinical studies (Davis 2007; Pan and Ho, 2008). Their antioxidant, anti-inflammatory, and pro-apoptotic activities appear to be important for preventing, suppressing, or reversing the development of carcinogenesis (Tan *et al.*, 2011). Both *in vitro* and animal studies have demonstrated that a number of phytochemicals (curcumin, resveratrol, EGCG, sulforaphane and silibinin) show preferential cytotoxicity to malignant cancer cells over normal cells, suggesting that they might safely be used for both cancer chemoprevention and cancer therapy (Nair *et al.*, 2007; Mann *et al.*, 2009). Interestingly, a number of dietary phytochemicals, including quercetin, apigenin, genistein, hesperetin, and luteolin were recently shown to induce autophagy in both normal cells and several cancer cell types (Singletary and Milner, 2008; Hannigan and Gorski, 2009). Furthermore, pharmacological activation of autophagy by drugs, such as rapamycin analogs (mTOR inhibitors, (Harrison *et al.*, 2009)), class I PI3K inhibitors (Levine and Kroemer, 2008) and metformin (an AMPK activator, (Buzzai *et al.*, 2007)) were demonstrated to inhibit malignant transformation (Evans *et al.*, 2005). The possibility of preventing cancer by activating autophagy is very intriguing. However, it is not yet clear whether the enhancement of autophagy is a potentially critical mechanism for the chemopreventive and/or chemotherapeutic actions of these agents. Future work will be required to determine whether the up-regulation of autophagy is a safe and effective approach for cancer prevention.

Inhibiting autophagy to enhance the efficacy of anticancer therapies

Despite recent advances in cancer treatment, many tumors still exhibit unsatisfactory responses to chemotherapy and/or radiation, either recurring or continuing to grow after treatment (Chen *et al.*, 2010). Autophagy is commonly up-regulated in both tumor and normal cells exposed to cancer therapies, but the greater reliance of tumor cells (versus normal cells) on the cytoprotective effects of autophagy provides a novel therapeutic opportunity (White and DiPaola, 2009). Indeed, autophagy is induced as a pro-survival strategy in human cancer cells treated with HDAC inhibitors (Carew *et al.*, 2007), arsenic trioxide (Smith *et al.*, 2010), TNF- α (Moussay *et al.*, 2011), IFN- γ (Ní Cheallaigh *et al.*, 2011), rapamycin (Fan *et al.*, 2010), and anti-estrogen hormonal therapy (Qadir *et al.*, 2008), suggesting that the inhibition of autophagy could sensitize cancer cells to these therapies. Consistent

with this notion, siRNA-mediated suppression of ATG5, ATG6, or ATG7 as well as co-treatment with 3-MA or bafilomycin A1 sensitized various cancer cells to various anti-cancer drugs or therapies (Chen *et al.*, 2010).

CQ derivatives act as lysosomotropic agents by increasing pH and inhibiting autophagosomal maturation (White and DiPaola, 2009). These oral drugs cross the blood-brain barrier (Amaravadi *et al.*, 2011) and have been prescribed for malaria (O'Neill *et al.*, 1998), rheumatoid arthritis (Kremer, 2001), and HIV (Romanelli *et al.*, 2004). Recently, CQ and HCQ were shown to enhance the cytotoxic effects of chemotherapy or standard cancer therapy *in vitro* (Amaravadi *et al.*, 2011). In addition, combined treatments with CQ or HCQ plus metabolic stressors (e.g., an angiogenesis inhibitor or 2-deoxyglucose) (Ramakrishnan *et al.*, 2007; DiPaola *et al.*, 2008) or targeted therapeutic drugs (e.g., imatinib, an inhibitor of Bcr-Abl) have been found to potentiate cell death (Shingu *et al.*, 2009; Calabretta and Salomoni, 2011). HCQ is less toxic than CQ (Gunja *et al.*, 2009) but has similar ability to induce autophagy (Amaravadi *et al.*, 2011). Thus, multiple clinical trials are currently assessing the effects of combined treatments with various anti-cancer drugs plus HCQ for patients with various refractory malignancies (<http://clinicaltrials.gov>) (White and DiPaola, 2009; Levy and Thorburn, 2011). Since the pharmacological modulation of autophagy appears to enhance the efficacy of current anticancer regimens, these new combinatorial strategies may hopefully contribute to cancer eradication in the future.

Induction of autophagic cell death as a therapeutic strategy

Since defects in apoptosis are often observed in many solid tumor cells and can increase the resistance of tumor cells to various conventional cancer therapies, the targeting of alternative cell death pathways is an attractive strategy for improving anti-tumor therapy (Schleicher *et al.*, 2010). Thus, the induction of cell death by autophagy may serve as a novel therapeutic strategy for eliminating cancer cells, especially those with high thresholds to apoptosis (Gozuacik and Kimchi, 2007). Although several agents, including arsenic trioxide (Kanzawa *et al.*, 2003) and vitamin D analog EB1089 (Høyer-Hansen *et al.*, 2005), were previously reported to induce autophagic cell death in cancer cells *in vitro*, in these cases, unfortunately, autophagic cell death was often determined based on morphological characteristics, meaning that some may not represent true autophagic cell death

(Kroemer and Levine, 2008). However, several other reports, have demonstrated concrete examples of autophagic cell death in response to certain agents, further showing that the inhibition or knockdown of essential regulators of autophagy could prolong cell survival. Some cancer cells (especially those lacking essential apoptotic modulators, such as BAX, BAK or caspases) were found to show autophagic cell death *in vitro* when treated with certain chemotherapeutic agents, such as etoposide, fenretinide and dexamethasone (Shimizu *et al.*, 2004; Fazi *et al.*, 2008; Grander *et al.*, 2009; Laane *et al.*, 2009). Certain phytochemicals, including fisetin and resveratrol, have also been shown to induce autophagic cell death in certain types of cancer cells (Puissant *et al.*, 2010; Suh *et al.*, 2010). Furthermore, we recently reported that sodium selenite induces mitophagic cell death (cell death by excessive mitophagy) selectively in malignant glioma cells, but not in normal astrocytes (Kim *et al.*, 2007; Kim and Choi, 2008), suggesting that selenite may prove useful for cancer therapy *via* a mitochondria-selective type of autophagic cell death. Table 2 lists other examples of autophagic cell death in cancer cells. Autophagic cell death seems to be induced in a cell type-, genetic background-, and stimulus-specific manner. Future studies will be needed to clarify whether the induction of autophagic cancer cell death will prove useful in the clinic.

Remaining tasks

Requirement for a novel and reliable method for measuring dynamic autophagic processes

Our current understanding of autophagy is undoubtedly incomplete. While we have a fairly good understanding of the regulatory mechanisms governing the early steps of autophagy, including autophagosome formation (initiation, nucleation and elongation) (Mizushima and Yoshimori, 2007), those governing the later steps of autophagy, such as autophagosomal maturation and lysosomal degradation, are less well understood. The widely used LC3-based autophagic assay primarily measures the early phases of autophagic activity, which control autophagosome formation (Kabeya *et al.*, 2000). Recently, several different assays have been used to monitor autophagic flux, including the LC3 turnover assay, measurements of the total levels of autophagic substrates (e.g., LC3, p62 and GFP-LC3) (Mizushima and Yoshimori, 2007), analysis of the mRFP-GFP-LC3 color change (Kimura *et al.*, 2007), and measurement of free GFP generated from GFP-LC3 (Hosokawa *et al.*,

2006). However, the utilities and limitations of these assays may vary somewhat in different cell types and experimental contexts (Mizushima *et al.*, 2010). For us to clearly understand the entire dynamic process of autophagy and identify therapeutic targets for its modulation, we need to develop new assays that can be used to examine autophagic activity all the way to the final lysosomal step. In addition, it would be useful to quantitatively measure activity changes among key autophagic effectors, and we should seek to generate standard autophagic assays suitable for routine clinical use. Currently, there are no available data on autophagic markers in biopsies obtained before and after treatment, which further indicates that we need new techniques for assessing autophagy in clinical samples, such as tumor biopsies and peripheral-blood mononuclear cells.

Rational therapeutic design based on the functional status of autophagy in cancer cells

Although only some of the links between autophagy and cancer have been elucidated in detail, pharmacological modulation of autophagy based on its functional status in tumors may provide novel opportunities for cancer management. However, the complex roles of autophagy in tumorigenesis and treatment responses make it difficult to decipher how we should modulate autophagy for maximum therapeutic benefit, given that context- and cell type-specific approaches may be required. Chronically autophagy-deficient tumors may be particularly sensitive to genotoxic and/or metabolic stress-inducing anticancer agents, such as DNA-damaging and anti-angiogenic drugs, due to the absence of autophagy-mediated survival mechanism (Chen and Karantza, 2011). In autophagy-competent tumors, concurrent autophagic inhibition would be expected to increase the efficacy of many anti-cancer modalities (Amaravadi *et al.*, 2011). However, in autophagy-competent tumor cells with particular genetic backgrounds (for example, those having high thresholds of apoptosis), sustained or overstimulation of autophagy can lead to cell death, and enhanced treatment efficacy (Buytaert *et al.*, 2006; Yang *et al.*, 2011). Therefore, gaining detailed information on the cellular functional status of autophagy (together with apoptosis) in certain cancer types may facilitate the rational design of therapeutic regimens *via* the modulation of autophagy (Table 3). Context-specific pharmacological modulation of autophagy thus holds great promise as a novel therapeutic approach against cancer.

Table 3. Proposed anti-cancer therapeutic strategy modulating autophagy

Cell types	Functional status		Therapeutic strategy modulating autophagy
	Autophagy	Apoptosis	
Normal	+	+	Activation of autophagy
Cancer	I	+	Induction of apoptosis <i>via</i> inhibition of autophagy
	II	+	Induction of autophagic cell death <i>via</i> over-stimulation of autophagy
	III	-	Induction of apoptosis using genotoxic and/or metabolic stress-inducing anti-cancer agents
	IV	-	Induction of non-apoptotic cell death using genotoxic and/or metabolic stress-inducing anti-cancer agents

+: functional, -: defective

Establishment of effective combinatorial therapeutic strategies using inhibitors of autophagy

Several agents that were previously identified as inducers of autophagy (mainly by LC3-based autophagic assays), including thapsigargin (Høyer-Hansen *et al.*, 2007) and imatinib (Ertmer *et al.*, 2007), were recently reported to inhibit the lysosomal steps (autophagosomal-lysosomal fusion or lysosomal degradation) in different experimental settings (Yogalingam and Pendergast, 2008; Ganley *et al.*, 2011). Inhibitors of autophagy (e.g., HCQ) are already being used in clinical trials along with various chemotherapeutic agents. Thus, when seeking to modulate autophagy in order to increase treatment efficacy, we should first evaluate whether the potentially co-treated anti-cancer drug increases or inhibits the autophagic flux in the target cancers.

The abrogation of autophagy often tends to sensitize therapeutic agent-resistant cancer cells to various anti-cancer treatments (Chen *et al.*, 2010). Notably, however, blocking autophagy at different steps (i.e., inhibiting the autophagosome-formation step with 3-MA versus inhibiting the autophagosome-lysosome fusion step with CQ or HCQ) can have different or even opposing outcomes. Temozolomide- or imatinib-induced cytotoxicity was attenuated by the inhibition of autophagy at an early stage, but augmented by inhibition of autophagy at a late stage (Kanzawa *et al.*, 2004; Shingu *et al.*, 2009). At present, we do not clearly understand how the inhibition of autophagy stage-specifically affects cytotoxicity. Comparative studies on the effects of various combined therapies with agents that inhibit different autophagic stages may contribute to the rational design of combined regimens against cancer.

Although autophagic manipulation by inhibitors such as CQ and HCQ is already being used in clinical trials, these agents are known to have additional effects, such as immunomodulation and the possible induction of DNA damage at higher

doses (Chen and Karantza, 2011). Thus, the side-effects of these autophagic inhibitors (e.g., potential induction of genomic instability, and adverse effect on the central nervous and cardiovascular systems) may make them suitable for only certain clinical situations. Furthermore, when we target the autophagic system for anti-cancer efforts, we must carefully consider the multifaceted nature of autophagy and its diverse crosstalk with other biological processes, including metabolism and the cell death pathways.

Conclusion

The molecular mechanisms underlying the regulation of autophagy and the role of autophagy in cancer cells are not completely understood. However, the pharmacological modulation of autophagy appears to have significant clinical potential as a novel therapeutic strategy for cancer eradication. The induction of autophagy may be useful for cancer chemoprevention in normal cells or triggering an alternative cell death mechanism in certain cancer cells, especially those with compromised apoptotic functions. In addition, suppression of the autophagic pathway may be combined with conventional antitumor regimens to achieve increased efficacy, representing a valuable therapeutic strategy for radio- and chemosensitization. In the future, we should seek to identify novel biomarkers for assessing dynamic autophagic processes and to establish new methods to assess autophagy in clinical samples. These efforts will help us understand the complicated role of autophagy in cancer and facilitate the rational design of combinatorial strategies aimed at modulating autophagy. Furthermore, future efforts toward universally modulating autophagy for maximum therapeutic benefit should focus on elucidating the genetic and physiological conditions that determine the pro-survival or pro-

death functions of autophagy.

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