

Review

The Role of Autophagy in Cancer: Therapeutic Implications

Zhineng J. Yang, Cheng E. Chee, Shengbing Huang, and Frank A. Sinicrope

Abstract

Autophagy is a homeostatic, catabolic degradation process whereby cellular proteins and organelles are engulfed by autophagosomes, digested in lysosomes, and recycled to sustain cellular metabolism. Autophagy has dual roles in cancer, acting as both a tumor suppressor by preventing the accumulation of damaged proteins and organelles and as a mechanism of cell survival that can promote the growth of established tumors. Tumor cells activate autophagy in response to cellular stress and/or increased metabolic demands related to rapid cell proliferation. Autophagy-related stress tolerance can enable cell survival by maintaining energy production that can lead to tumor growth and therapeutic resistance. As shown in preclinical models, inhibition of autophagy restored chemosensitivity and enhanced tumor cell death. These results established autophagy as a therapeutic target and led to multiple early phase clinical trials in humans to evaluate autophagy inhibition using hydroxychloroquine in combination with chemotherapy or targeted agents. Targeting autophagy in cancer will provide new opportunities for drug development, because more potent and specific inhibitors of autophagy are needed. The role of autophagy and its regulation in cancer cells continues to emerge, and studies aim to define optimal strategies to modulate autophagy for therapeutic advantage. *Mol Cancer Ther*; 10(9); 1533–41. ©2011 AACR.

Introduction

Macroautophagy (hereafter referred to as autophagy) is a homeostatic and evolutionarily conserved process that degrades cellular organelles and proteins, and maintains cellular biosynthesis during nutrient deprivation or metabolic stress (1). Autophagy begins with the formation of double-membrane vesicles, known as autophagosomes, that engulf cytoplasmic constituents. The autophagosomes then fuse with lysosomes, where the sequestered contents undergo degradation and recycling (1). Autophagy is important in all cells for the removal of damaged or long-lived proteins and organelles. Autophagy defects are associated with susceptibility to metabolic stress, genomic damage, and tumorigenesis in mice, indicating a role for autophagy in tumor suppression (2). Monoallelic loss of the essential autophagy gene, *Beclin 1*, has been found in 40 to 75% of human breast, prostate, and ovarian cancers (2), suggesting that autophagy may play a role in preventing these tumors. Although autophagy is a mechanism of tumor suppression, it also confers stress tolerance that enables tumor cells to survive under adverse conditions (1). Stress in tumor cells is com-

pounded by the high metabolic demand associated with rapid cell proliferation. Autophagy is induced in tumor cells within hypoxic tumor regions (3). Stress-induced autophagy in tumor cells can lead to treatment resistance and tumor dormancy, with eventual tumor regrowth and progression (4). In preclinical models, inhibition of pro-survival autophagy by genetic or pharmacological means was shown to kill tumor cells and trigger apoptotic cell death (1, 5–9). Furthermore, autophagy inhibitors given in combination with chemotherapy suppressed tumor growth and triggered cell death to a greater extent than did chemotherapy alone, both *in vitro* and *in vivo* (Table 1). These data indicate that pro-survival autophagy may represent a major impediment to successful cancer therapy, and thus, it represents a novel therapeutic target. However, autophagy has been referred to as a double-edged sword because in certain cellular contexts, excessive or sustained tumor cell autophagy may be prodeath, particularly in apoptosis-defective cells (10). Understanding the role of autophagy in cancer treatment is critical, because many anticancer therapies have been shown to activate autophagy, although the consequences of autophagy activation in this context are unclear. The complex role of autophagy in cancer continues to emerge, and it is important to elucidate the mechanisms by which autophagy influences tumorigenesis as well as treatment response. Analysis of autophagic signaling may identify novel therapeutic targets for modulation and therapeutic advantage. In this review, we outline the multiple roles played by autophagy in tumor biology, including its emergence as a therapeutic target for both cancer prevention and therapy.

Authors' Affiliation: Department of Medicine and Division of Oncology, Mayo Clinic, and Mayo Cancer Center, Rochester, Minnesota

Corresponding Author: Frank A. Sinicrope, Department of Medicine and Division of Oncology, Mayo Clinic and Mayo Cancer Center, 200 First St. SW, Rochester, MN 55905. Phone: 507-266-0132; Fax: 507-255-6318; E-mail: sinicrope.frank@mayo.edu

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Table 1. Preclinical and ongoing clinical studies using the autophagy inhibitors chloroquine and hydroxychloroquine in cancer treatment

Tumor type	Development status	Therapeutic combination
Colorectal cancer	<i>In vitro, in vivo</i>	CQ + bortezomib (63) CQ + vorinostat (56)
Gastrointestinal stromal tumor	Phase II	HCQ + XELOX + bevacizumab
Prostate cancer	<i>In vitro, in vivo</i>	CQ + imatinib (75)
Vulvar cancer	<i>In vitro</i>	CQ + Src kinase inhibitors (57)
Chronic myelogenous leukemia	<i>In vitro</i>	CQ + cetuximab (34)
Lymphoma	Phase II	CQ + vorinostat (73)
Pancreatic cancer	<i>In vivo</i>	HCQ + imatinib
Prostate cancer	Phase II	CQ + cyclophosphamide (27)
Lung cancer	Phase I/II	HCQ only
Glioblastoma multiforme	Phase II	HCQ + gemcitabine
Multiple myeloma	Phase I/II	HCQ + docetaxel
Renal cell carcinoma	Phase I	HCQ + erlotinib
Breast cancer	Phase II	HCQ + temozolomide + radiation
Chronic lymphocytic leukemia	Phase II	HCQ + bortezomib
Advanced solid tumor	Phase I	HCQ only
	Phase I	HCQ only
	Phase I	HCQ + sirolimus or vorinostat
	Phase I	HCQ + temsirolimus
	Phase I	HCQ + sunitinib
	Phase I	HCQ + temozolomide

Abbreviations: CQ, chloroquine; HCQ, hydroxychloroquine.

Regulation of Autophagy

Autophagy involves the formation of autophagosomes that assemble around and encapsulate damaged organelles or cellular debris, and then fuse with lysosomes to degrade their contents (11). The initiation of autophagy is controlled by the ULK1 (human homolog of ATG1) kinase complex, which consists of ULK1, Atg13, and Atg17, and integrates stress signals from mTOR complex 1 (mTORC1; refs. 12 and 13). When mTORC1 kinase activity is inhibited, autophagosome formation can occur. This involves vacuolar sorting protein 34 (Vps34), a class III phosphoinositide 3-kinase (PI3K) that forms a complex with Beclin 1 (11). The production of PtdIns3P by the Beclin 1/Vps34 complex is essential for the recruitment other autophagy-related gene (Atg) products that are critical for autophagosome formation (11). During the initiation phase, formation of the Atg5-Atg12-Atg16 complex promotes the recruitment and conversion of cytosolic-associated protein light chain 3 (LC3-I) to the membrane-bound, lipidated form, LC3-II (14). LC3 is conjugated to phosphatidylethanolamine and incorporated into the membrane by an Atg7- and Atg3-dependent activation and transfer cascade that follows cleavage of LC3 by the cysteine protease Atg4 (15). Upon completion of autophagosome formation, and with the exception of a proportion of LC3-II that remains bound to the luminal

membrane, the Atg proteins are then recycled in the cytosol (11). LC3-II remains on mature autophagosomes until fusion with lysosomes is completed, and it is commonly used to monitor autophagy. LC3-II also binds to the adaptor protein p62/sequestosome1 (SQSTM 1), which is involved in trafficking proteins to the proteasome and serves to facilitate the autophagic degradation of ubiquitinated protein aggregates (16). p62/SQSTM 1 is normally degraded during autophagy and accumulates when autophagy is impaired, as has been shown in autophagy-deficient mice (17). Late events in autophagy involve the final maturation and fusion of autophagosomes with lysosomes to form an autolysosome, a step that requires small Rab GTPases and lysosome-associated membrane protein 2 (18, 19).

A major regulator of autophagy is the mammalian target of rapamycin (mTOR) pathway, which consists of 2 distinct signaling complexes known as mTORC1 and mTORC2 (11). mTOR is activated downstream of PI3K-AKT, a pathway that is commonly dysregulated in human cancer (Fig. 1; ref. 20). Activation of mTOR can also occur due to loss of tumor suppressors (LKB1, PML, PTEN, and TSC1/2) or through gain-of-function mutations in receptor tyrosine kinases (21). Cellular stress leads to downregulation of mTOR1 activity that triggers autophagy (11), and in this regard, mTOR inhibitors, including rapamycin, have been

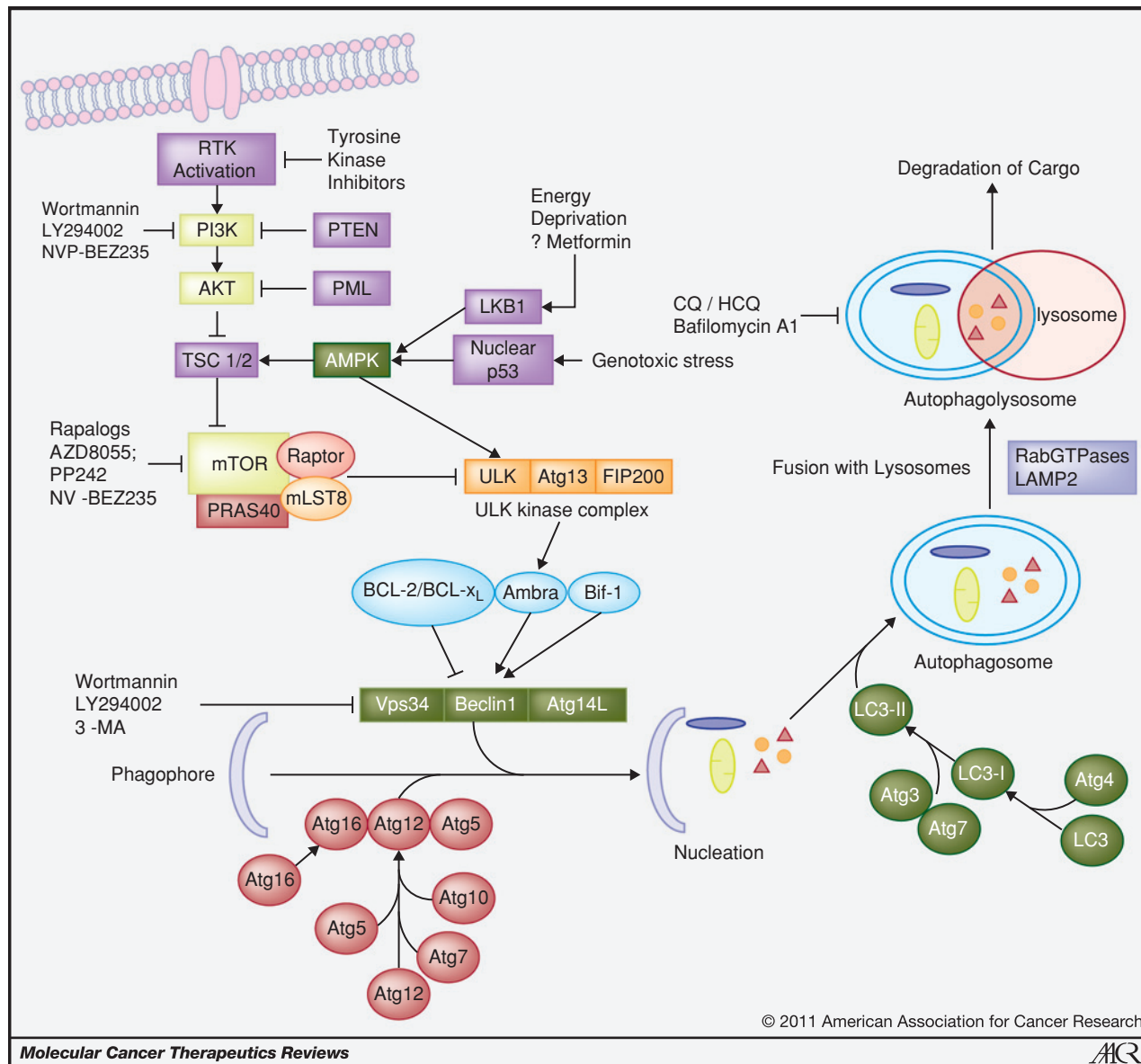


Figure 1. Overview of the autophagy pathway. The initiation of autophagy is controlled by the ULK1 kinase complex, which integrates stress signals from mTORC1. When mTORC1 kinase activity is inhibited, autophagosome formation can occur from the phagophore and involves Vps34, a class III PI3K that forms a complex with Beclin 1. Beclin 1 interacts with factors (Ambra, Bif1, and Bcl-2) that modulate its binding to Vps34, whose lipid kinase activity is essential for autophagy. In addition to these 2 complexes, autophagosome formation requires the participation of 2 ubiquitin-like protein conjugation systems (Atg12 and LC3) that are essential for the formation of the phagophore. In addition, the LC3 system is required for autophagosome transport and maturation. Mature autophagosomes fuse their external membranes with those from lysosomes to degrade their cargo and recycle essential biomolecules. Autophagy can be inhibited by drugs that target early or late stages in the pathway. CQ and HCQ inhibit autophagy at a late stage by blocking lysosomal acidification, thus preventing the lysosome from digesting its engulfed cargo.

shown to induce autophagy in tumor cells (20). mTOR negatively regulates autophagy by causing phosphorylation of Atg13, which reduces its interaction with ULK1 and inhibits formation of a trimeric complex required for autophagosome formation (12). A decrease in intracellular energy results in activation of adenosine monophosphate kinase (AMPK), a central metabolic sensor that has important functions in regulating lipid and glucose metabolism. Activation of AMPK serves to

repress mTOR and initiate autophagy (12). A recent study found that AMPK can directly phosphorylate ULK1, which is required for mitochondrial homeostasis and cell survival during starvation (22). Autophagy can be induced by hypoxia, a stimulus for AMPK, that is mediated by hypoxia-inducible factor (HIF) and its target gene *BNIP3* (21).

Autophagy can be potentially induced by the unfolded protein response, a component of the endoplasmic

reticulum (ER) stress pathway. The binding of misfolded proteins to the ER chaperone Bip/GRP78 leads to the release of 3 ER membrane-associated proteins: PKR-like eIF2 α kinase (PERK), activating transcription factor-6 (ATF6), and inositol-requiring enzyme 1 (IRE1; ref. 23). Whereas PERK and ATF6 are autophagy inducers, IRE1 negatively regulates autophagy. Other factors that link cellular stress with autophagy include the transcription factor NF- κ B and its upstream regulators IKK complex and TAK1, which integrate diverse stress signals, such as starvation and ER stress, with the autophagy pathway (24).

The tumor suppressor p53 protein can modulate autophagy depending on its cellular localization. Nuclear p53 acts as a transcription factor that transactivates several autophagy inducers, including DRAM1 and Sestrin2, to activate autophagy (25), whereas cytoplasmic p53 inhibits autophagy by an unknown mechanism. Inducers of autophagy can stimulate proteasome-mediated degradation of p53 (26). Recently, some novel regulators of autophagy have been found. Ataxia-telangiectasia mutated (*ATM*) is a cellular damage sensor that coordinates the cell cycle with DNA damage-response checkpoints and DNA repair, and engages the TSC2/mTORC1 signaling axis to regulate autophagy (27). Additionally, high mobility group box 1 (HMGB1) is an immune modulator and regulator of stress-induced autophagy that directly interacts with Beclin 1 (28).

Role of Autophagy in Cancer

Autophagy in tumor suppression

Autophagy is a homeostatic mechanism that when disrupted can promote and accelerate tumorigenesis. Autophagy functions as a tumor suppression mechanism by removing damaged organelles/proteins and limiting cell growth and genomic instability (17). Beclin 1 is a protein required for autophagy induction, and *Beclin 1*^{+/-} mice were shown to be tumor prone, indicating that *Beclin 1* is a haploinsufficient tumor suppressor gene (2). In contrast, excessive stimulation of autophagy due to Beclin 1 overexpression can inhibit tumor development (29). A potential molecular link between defective autophagy and tumorigenesis involves the accumulation of p62/SQSTM 1 protein aggregates, damaged mitochondria, and misfolded proteins that lead to the production of reactive oxygen species (ROS). This causes DNA damage that can lead to genomic instability (17). Knockdown of p62/SQSTM 1 in autophagy-defective cells prevented ROS and the DNA damage response (17). The relationship between defective autophagy and p62/SQSTM 1 accumulation with tumorigenesis was also shown in a study in which p62/SQSTM 1^{-/-} mice were protected from *Ras*-induced lung carcinomas compared with wild-type animals (30). Autophagy may also protect against tumorigenesis by limiting necrosis and chronic inflammation, which are associated with the release of proinflammatory HMGB1 (28). Together, these findings establish a role for autophagy as a mechanism of tumor suppression.

Autophagy in tumor cell survival

Evidence indicates that the predominant role of autophagy in cancer cells is to confer stress tolerance, which serves to maintain tumor cell survival (1). Knockdown of essential autophagy genes in tumor cells has been shown to confer or potentiate the induction of cell death (7). Cancer cells have high metabolic demands due to increased cellular proliferation, and in *in vivo* models, exposure to metabolic stress was shown to impair survival in autophagy-deficient cells compared with autophagy-proficient cells (17). Cytotoxic and metabolic stresses, including hypoxia and nutrient deprivation, can activate autophagy for recycling of ATP and to maintain cellular biosynthesis and survival. Autophagy is induced in hypoxic tumor cells from regions that are distal to blood vessels, and HIF-1 α -dependent and -independent activation have been described (21). HIF-1 α can also increase the expression of angiogenic factors, such as vascular endothelial growth factor, platelet-derived growth factor, and nitric oxide synthase (21). Increased basal levels of autophagy were detected in human pancreatic cancer cell lines and tumor specimens, and they were shown to enable tumor cell growth by maintaining cellular energy production. Inhibition of autophagy in these cells led to tumor regression and extended survival in pancreatic cancer xenografts and genetic mouse models (31). In cancer cells that survive chemotherapy and/or radiation, activation of autophagy may enable a state of dormancy in residual cancer cells that may contribute to tumor recurrence and progression (4). Inhibition of autophagy in tumor cells has been shown to enhance the efficacy of anticancer drugs (Table 1), supporting its role in cytoprotection.

Recent data indicate that human cancer cell lines bearing activating mutations in *H-ras* or *K-ras* have high basal levels of autophagy even in the presence of abundant nutrients (32). In these cells, suppression of essential autophagy proteins was shown to inhibit cell growth, indicating that autophagy maintains tumor cell survival and suggesting that blocking autophagy in tumors that are addicted to autophagy, such as *Ras*-driven cancers, may be an effective treatment approach.

Autophagy as a mechanism of cell death

In addition to the cytoprotective function of autophagy, which is supported by abundant evidence, induction of autophagic cell death has been proposed as a mechanism of cell death, given that features of autophagy have been observed in dying cells. In cancer cells, autophagy accompanied by nonapoptotic cell death has been described (33, 34). Prolonged stress and sustained autophagy may eventually lead to cell death when protein and organelle turnover overwhelm the capacity of the cell. Induction of autophagic cell death by anticancer drugs may occur depending on the cell type and genetic background. In *VHL*-deficient renal cell carcinoma cells, a novel small molecule (STF-62247) was shown to promote cell death through induction of autophagy (35). However, *in vivo*

evidence is limited, and whether induction of autophagic death in tumor cells can be achieved for cancer therapy remains unknown.

Autophagy and cell senescence

Autophagy and senescence represent 2 distinct cellular responses to stress that can serve as tumor suppressors. Recently, autophagy was shown to mediate *Ras* oncogene-induced senescence (36). Cellular senescence represents a state of cell cycle arrest maintained by the expression of cell cycle inhibitors (p16^{Ink4a}, p21^{Cip1}, and p27^{Kip1}) in metabolically viable cells (37). The senescence phenotype can be induced by oncogenes, DNA-damaging drugs, or oxidative stress, and their ability to induce senescence is enhanced by functional *p53* and *Rb* tumor suppressor genes (37). Senescence has been suggested as a mechanism for autophagy-mediated tumor dormancy (4, 38). Conversely, the inhibition of autophagy in tumor cells was shown to delay the senescence phenotype (36). A subset of Atgs (ULK1 and ULK3) is upregulated during senescence, and overexpression of ULK3 was shown to induce autophagy and senescence (36).

Autophagy Modulation for Cancer Therapy

Autophagy inducers

Conventional cytotoxic drugs and irradiation have been shown to induce autophagy (5, 39–43). Other anticancer drugs that can induce autophagy include the BCR-ABL tyrosine kinase inhibitor imatinib (44), the anti-epidermal growth factor receptor (EGFR) cetuximab (45), proteasome inhibitors (46), TNF-related apoptosis-inducing ligand (TRAIL; ref. 47), and the HDAC inhibitors vorinostat (suberoylanilide hydroxamic acid) and OSU-HDAC42 (48). Arsenic trioxide was shown to induce autophagy in leukemia and glioma cells via regulation of the mitochondrial stress sensor BNIP3 malignant glioma (33, 49). Furthermore, agents with diverse mechanisms of action, including tamoxifen (50), cyclooxygenase inhibitors (51), and the protease inhibitor nelfinavir (52), have also been shown to induce autophagy in tumor cells. The consequences of promoting autophagy in tumor cells are incompletely understood and may depend on multiple factors, including the extent of induction, duration, and cellular context. Excessive or sustained autophagy has the potential to induce tumor cell death (35); however, the relevance of this finding with regard to the *in vivo* situation is unknown. mTOR is a central coordinator of cell growth that is involved in both protein translation and autophagy. Rapamycin is a naturally occurring allosteric mTOR inhibitor, and its analogs temsirolimus (CCI-779), everolimus (RAD-001), and deforolimus (AP-23573) selectively target mTORC1 to stimulate autophagy. With the exception of renal cell and neuroendocrine carcinomas and lymphoma, rapamycin and its analogs (rapalogs) have had limited success in the clinical setting (53). Rapamycin and rapalogs do not

inhibit mTORC2 and are unable to abrogate the S6K-IRS1-mediated negative feedback loop that can result in rebound AKT activation (54). These limitations led to the development of ATP-competitive inhibitors of both mTORC1 and mTORC2 (e.g., Torin1, PP242, AZD8055, and WYE132) and the dual PI3K-mTOR inhibitor NVP-BEZ235. In preclinical studies, dual inhibitors of mTORC1 and mTORC2 showed antitumor activity (55–57) and were shown to be more potent inducers of autophagy compared to mTORC1 inhibitors (58, 59). Of note, the dual PI3K-mTOR inhibitor PI-103 was shown to induce autophagy in glioma cells, and inhibitors of autophagy cooperated with PI-103 to induce apoptosis. Furthermore, the PI3K-mTOR inhibitor NVP-BEZ235, which is in clinical trials, synergized with chloroquine (CQ) to induce apoptosis in glioma xenografts (60). The effect of induction of prosurvival autophagy by these dual inhibitors on their antitumor effects is unknown.

The antidiabetic, biguanide drug metformin has been shown to inhibit mTOR signaling through its upstream mediator, AMPK, and to have a cytostatic effect in certain cancer cell types (61). Although metformin was shown to induce autophagy in colon cancer cells (62), it inhibited 2-deoxyglucose-induced autophagy, decreased Beclin 1 expression, and triggered a switch from cell survival to cell death in prostate cancer cells (61). This finding is inconsistent with the ability of metformin to activate AMPK, which is expected to induce autophagy. Other autophagy stimulators include the selective serotonin reuptake inhibitor fluoxetine, the norepinephrine reuptake inhibitor maprotiline (63), and the antiepileptic drug valproic (64). In studies using cell-based screening assays, the antihypertensive drugs verapamil, minoxidil, and clonidine were found to induce autophagy through an mTOR-independent pathway involving calpain (65).

Autophagy inhibitors

Multiple studies have shown that genetic knockdown of Atgs or pharmacological inhibition of autophagy can effectively enhance tumor cell death induced by diverse anticancer drugs in preclinical models (Table 1; refs. 7–9). Inhibition of autophagy in preclinical models improved response to alkylating agents in tumor cells (5). In apoptosis-defective leukemic and colon cancer cell lines, inhibition of autophagy was shown to sensitize resistant cells to TRAIL-mediated apoptosis (47). Furthermore, inhibition of autophagy enhanced apoptosis induction by cetuximab, an antibody against EGFR (45).

Pharmacological inhibitors of autophagy can be broadly classified as early- or late-stage inhibitors of the pathway. Early-stage inhibitors include 3-methyladenine, wortmannin, and LY294002, which target the class III PI3K (Vps34) and interfere with its recruitment to the membranes. Late-stage inhibitors include the antimalarial drugs CQ, hydroxychloroquine (HCQ), bafilomycin A1, and monensin. Bafilomycin A1 is a specific inhibitor of vacuolar-ATPase (66), and monensin and CQ/HCQ are lysosomotropic drugs that prevent the acidification of

lysosomes, whose digestive hydrolases depend on low pH. Autophagosomes and lysosomes move along microtubules, and microtubule-disrupting agents (taxanes, nocodazole, colchicine, and vinca alkaloids) inhibit fusion of autophagosomes to lysosomes. Other inhibitors of autophagy that block autophagosome degradation include the tricyclic antidepressant drug clomipramine and the anti-schistosome agent lucanthone (67, 68).

The ability of autophagy inhibition to enhance chemosensitivity and tumor regression has been confirmed in animal models. In a *Myc*-induced murine lymphoma model, inhibition of autophagy by CQ enhanced cyclophosphamide-induced tumor cell death to an extent similar to that shown by shRNA knockdown of *Atg5*, and it delayed the time-to-tumor recurrence (5). In a colon cancer xenograft model, the addition of CQ to vorinostat was shown to significantly reduce tumor burden and to increase apoptosis (69). Similarly, CQ enhanced the therapeutic efficacy of the Src inhibitor saracatinib in a prostate cancer xenograft mouse model (70). Saracatinib decreased tumor growth by 26% compared with control-treated mice, and CQ plus saracatinib further inhibited tumor growth by 64% (70). This combination also led to at least a 2-fold increase in the number of apoptotic tumor cells in the group treated with saracatinib plus CQ, suggesting that suppression of autophagy drives cells into apoptosis (70). Autophagy inhibition by 3-methyladenine increased apoptosis induction by 5-fluorouracil in association with tumor regression in colon cancer xenografts (42). These data indicate that autophagy inhibition can enhance the antitumor efficacy of chemotherapeutic agents that use diverse cellular mechanisms. Of the known autophagy inhibitors, only CQ and HCQ have been evaluated in humans, because they are commonly used as antimalarial drugs and in autoimmune disorders. These drugs cross the blood-brain barrier, and HCQ is preferred to CQ in humans because of its more favorable side-effects profile (71). On the basis of preclinical data, several phase I/II trials are being conducted to evaluate the combination of HCQ with cytotoxic drugs in a variety of tumor types (Table 1). Challenges include the long half-life of HCQ and the need for micromolar concentrations to inhibit autophagy, which may limit its efficacy in human studies. A recently reported phase I trial of HCQ in combination with adjuvant temozolomide and radiation in patients with glioblastoma found that the maximum tolerated dose of HCQ was 600 mg per day, and this dose achieved concentrations of HCQ required for autophagy inhibition in preclinical studies (72). In this trial, investigators observed a dose-dependent inhibition of autophagy, as indicated by increases in autophagic vesicles (revealed by electron microscopy), and detected elevations in LC3-II in peripheral blood mononuclear cells (72). In a phase I trial of 2-deoxyglucose, an agent that blocks glucose metabolism, autophagy occurred in association with a reduction in p62/SQSTM1 in peripheral blood mononuclear cells (73). These biomarker data suggest the potential for evaluating autophagy

modulation during therapy and correlating such modulation with treatment outcome.

Intracellular proteins are degraded within lysosomes during autophagy and by the ubiquitin-proteasome pathway (74). Given the primary role of these pathways in protein degradation, it has been postulated that their combined blockade may lead to ER stress-induced cytotoxicity through the accumulation of unfolded protein aggregates that can activate autophagy through JNK (75) or PERK/eIF2 α (76). The combination of the proteasome inhibitor bortezomib and CQ was shown to suppress tumor growth to a greater extent than did either drug alone in colon cancer xenografts (6). Phase I/II clinical trials evaluating this combination are ongoing in patients with relapsed/refractory multiple myeloma.

Interplay of autophagy and apoptosis

When autophagy is inhibited, apoptosis is promoted in cancer cells with intact apoptotic signaling (10). Disabled apoptosis is a frequent occurrence in human cancers, and tumors under stress generally die by other cell-death mechanisms. Autophagy may be an alternative mode of cell death in apoptosis-resistant cells (10, 50); however, the conditions under which autophagy can function as a primary cell-death mechanism remain to be defined. Cross-talk between autophagy and apoptosis exists at many levels because both pathways share mediators, ranging from the core machinery to upstream regulators (10). Recent findings suggest a link between autophagy and the extrinsic apoptotic pathway that is mediated by p62/SQSTM1 (77). p62/SQSTM1 was shown to bind caspase-8 to enable its aggregation and activation and to enhance TRAIL-mediated apoptosis (77). TRAIL is a cytokine with activity against multiple tumor types in which it induces apoptosis. TRAIL and death receptor agonists are currently being evaluated in phase I/II studies.

Antiapoptotic Bcl-2 family proteins are overexpressed in multiple tumor types where they contribute to both intrinsic and acquired treatment resistance. Although Bcl-2 family proteins function to inhibit apoptosis, data indicate that they can also inhibit autophagy (78). The mechanism of this effect reflects the fact that Bcl-2/Bcl-x_L proteins can bind to and disrupt the autophagic function of Beclin 1, which contains a BH3 domain (Fig. 1; ref. 78). Small-molecule antagonists of Bcl-2/Bcl-x_L, known as BH3 mimetics (ABT-737/263, obatoclax), can competitively disrupt the Beclin 1–Bcl-2/Bcl-x_L interaction to trigger autophagy (78). These data suggest that inhibition of cytoprotective autophagy by BH3 mimetics may further enhance apoptosis. In this regard, the selective cyclooxygenase-2 inhibitor celecoxib was shown to induce apoptosis that was enhanced by the BH3 mimetic ABT-737 and was further augmented by inhibition of autophagy in colon cancer cells (51).

Targeting autophagy for cancer prevention

Because autophagy plays a role in tumor suppression, the induction of autophagy may be an important

strategy for cancer prevention. PI3K-AKT-mTOR signaling is frequently dysregulated in human tumors, and the inhibition of mTOR signaling can induce autophagy. In accordance with this observation, treatment with the mTOR inhibitor rapamycin was associated with a 90% reduction in carcinogen-induced lung tumors in a murine model (79). In another study, inhibition of mTOR signaling by metformin attenuated tumorigenesis in the same tumor model (80). Furthermore, continuous, low-dose rapamycin treatment in APC^{Min/+} mice with enhanced AKT-mTOR signaling was shown to markedly inhibit intestinal neoplasia (81). Defective autophagy has been linked to colonic tumor formation through a mechanism involving the aberrant activation of *Wnt* signaling from impaired degradation of Dishevelled by autophagy (82). Other pharmacological activators of autophagy may also be beneficial for cancer chemoprevention, and further studies are awaited.

Conclusions

Autophagy serves a dual role as a mechanism of tumor suppression and as an adaptive stress response in tumor cells that helps them maintain their survival in a setting of increased metabolic demands, a hypoxic microenvironment, or cancer therapy. Maintenance of cell survival by autophagy can promote the growth of established tumors. Abundant preclinical evidence indicates that stress-induced autophagy in tumor cells is predominantly cytoprotective and that inhibition of autophagy can enhance tumor cell death by diverse anticancer therapies. These data establish autophagy as a novel therapeutic target whose modulation presents new opportunities for cancer treatment. Although several drugs can inhibit autophagy,

most of these drugs lack specificity and antitumor activity. CQ is the most widely tested in preclinical models, and multiple ongoing phase I and II clinical trials are evaluating HCQ alone or in combination with cytotoxic chemotherapy or targeted agents, mostly in patients with solid tumors. Targeting autophagy in cancer will provide new opportunities for drug development because more potent and specific inhibitors of autophagy are clearly needed. High-throughput screening of chemical libraries to identify small-molecule inhibitors of autophagy is ongoing. Biomarkers to measure autophagy modulation during treatment should be an important component of drug development efforts. Although important strides have been made, several key issues remain unresolved, including how autophagy is regulated in tumor cells, the interplay between autophagy and apoptosis, and the specific mechanism by which autophagy confers treatment resistance. An increased understanding of autophagy in cancer is important for its optimal exploitation for therapeutic advantage. Given the role of autophagy in tumor suppression, activation of autophagy may be an important strategy for cancer chemoprevention.

Disclosure of Potential Conflicts of Interest

Frank A. Sinicrope is a consultant to and member of the advisory board of Merck Serono.

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