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REVIEW

Targeting autophagy in cancer management – strategies and developments

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Abstract: Autophagy is a highly regulated catabolic process involving lysosomal degradation of intracellular components, damaged organelles, misfolded proteins, and toxic aggregates, reducing oxidative stress and protecting cells from damage. The process is also induced in response to various conditions, including nutrient deprivation, metabolic stress, hypoxia, anticancer therapeutics, and radiation therapy to adapt cellular conditions for survival. Autophagy can function as a tumor suppressor mechanism in normal cells and dysregulation of this process (ie, monoallelic *Beclin-1* deletion) may lead to malignant transformation and carcinogenesis. In tumors, autophagy is thought to promote tumor growth and progression by helping cells to adapt and survive in metabolically-challenged and harsh tumor microenvironments (ie, hypoxia and acidity). Recent in vitro and in vivo studies in preclinical models suggested that modulation of autophagy can be used as a therapeutic modality to enhance the efficacy of conventional therapies, including chemo and radiation therapy. Currently, more than 30 clinical trials are investigating the effects of autophagy inhibition in combination with cytotoxic chemotherapies and targeted agents in various cancers. In this review, we will discuss the role, molecular mechanism, and regulation of autophagy, while targeting this process as a novel therapeutic modality, in various cancers.

Keywords: autophagy inhibition, chemotherapy, tumor microenvironment

Background

Macroautophagy (conventionally called autophagy), which is complementary to the ubiquitin–proteasome system, is responsible for the vast majority of regulated protein recycling, in addition to the capture and degradation of mitochondria, Golgi complexes, polyribosomes, endoplasmic reticulum (ER), and other intracellular constituents, such as proteins, aggregates, and building blocks.[1–4] The process of autophagy occurs continuously at basal levels in healthy cells to eliminate long-lived, misfolded, and damaged constituents through autolysosomes (autophagolysosome).[2] Lysosomes are responsible for the degradation of extracellular macromolecules taken up by the cells through endocytosis or phagocytosis, as well as those from the cytoplasm through merging with autophagosomes.

Autophagy is characterized by the formation of double-layer membrane vesicles called autophagosomes that capture intracellular components and subsequently merge with lysosomes, leading to digestion of the cargo.[3] The process is used by normal and cancer cells as mechanisms of recycling building blocks and conserving energy, in addition to eliminating toxic materials for homeostasis and survival.[4]

Autophagy can be selective through the formation of autophagosomes around protein aggregates or damaged organelles by actions of autophagosomal membrane

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receptors (eg, NBR1, p62/SQSTM1, Nix).[4] There are two other forms of autophagy in eukaryotic cells called microautophagy and chaperone-mediated autophagy, which can also specifically target organelles, including mitochondria and intracellular molecules.[3] Microautophagy, the nonselective lysosomal degradation process, is involved in the maintenance of organelle size, membrane homeostasis, and cell survival.[5] Chaperone-mediated autophagy is mediated by a chaperone protein called heat shock cognate 70 (Hsc70), which binds to specific proteins containing certain amino acid sequences or motifs and targets them into the lysosome through the lysosome-associated membrane protein type 2A (LAMP2A) receptor.[6] Selective removal of mitochondria, ER, or peroxisomes by autophagy is referred to as mitophagy, ERphagy, and peroxyphagy, respectively.[7] Mitophagy is an important mitochondrial quality control mechanism that eliminates damaged mitochondria.[7] BNIP3 is also degraded upon formation of the autophagolysosome, and thus reduction of BNIP3 is considered an indication of mitophagic and ERphagic flux. PINK1 and Parkin selectively bind to damaged mitochondria, and target these organelles for autophagic degradation through the ubiquitination of mitochondrial protein.[7]

Autophagy-based degradation pathways may lead to autophagy-associated cell death, which is important for development, differentiation, aging, and cellular remodeling under certain environmental stress conditions.[8–11] All of these forms of autophagy can be induced by nutrient deprivation, hypoxia, and other cellular stresses including metabolic and therapeutic stress such as chemotherapy, radiation, natural polyphenolic compounds, and inhibitors of mammalian target of rapamycin (mTOR) kinase activity (ie, rapamycin).[9,10] Thus, dysregulation of autophagy disrupts physiological processes and has been implicated in the pathogenesis of various conditions, including cancer and neurological diseases, such as Alzheimer, Parkinson's, and Huntington's diseases.[11] When functioning properly, the autophagic process in neuronal cells prevents neurodegeneration by eliminating the accumulation of abnormal intracellular proteins. Thus, well-balanced regulation of autophagic machinery is critical for prevention against some neurological diseases and cancer.

Molecular mechanisms regulating autophagy

The regulation of autophagy is an evolutionarily conserved and highly complex process, consisting of several basic phases, including 1) initiation; 2) nucleation; 3) maturation; and 4) merging with lysosomes, resulting in the degradation of sequestered material. The successive steps are regulated by about 30 autophagy-associated genes/proteins (Atg/Apg) in conjunction with various signaling pathways (Figure 1).[12] The initiation phase is regulated by a complex consisting of Atg1, ULK1, and Atg13.[13] This is the point where a cell's nutritional status and signaling through mTOR and adenosine monophosphate (AMP) kinase (AMPK) can regulate the rate of autophagic vessel formation.[14] Nucleation is controlled by a class 3 phosphatidylinositol-3-kinase (PI3K) called vacuolar protein sorting 34 (Vps34), which binds to a complex consisting of Atg6/Beclin-1, p150, and Atg14L.[15] Inhibition of Vps34 with 3-methyl adenine (3-MA) or small interfering (si)RNA to *Beclin-1* is commonly used as an experimental tool to inhibit autophagy. Maturation (elongation, curvature, and closure) is regulated via ubiquitin-like conjugation systems, which regulate LC3 (also known as Atg8/microtubule-associated protein 1 light chain 3 [LC3]-I/II). The first system generates LC3-II, which is the cleaved and lipidated (phosphatidylethonolamine [PE]) form of LC3 that is inserted into the autophagosomal membrane and often monitored by Western blot or immunocytochemistry as a marker for evaluating autophagy. The second system consists of Atg12 bound to Atg5 and Atg16L, which recruits LC3-II to the developing autophagosomal membrane. LC3 binding to the membranes is important for transport and maturation of the autophagosome, which later fuses its external membrane with lysosomes to degrade its cargo. LC3-II remains on mature autophagosomes until fusion with lysosomes is completed. LC3-II also binds to the adaptor protein p62/sequestosome-1 (SQSTM1), which is involved in trafficking proteins into the proteasome and serves to facilitate the autophagic degradation of ubiquitinated protein aggregates. P62/SQSTM1 is normally degraded during autophagy and accumulates when autophagy is impaired. 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 (LAMP2).

Autophagy appears to play a significant role in the tumor microenvironment. The observation that coculture of cancer cells with fibroblasts results in reduced numbers of mitochondria in the fibroblasts and increased numbers of mitochondria in cancer cells has led to the "Reverse Warburg Effect" theory.[13] This theory postulates that cancer cells induce a redox environment in the stroma, which induces mitophagy in the cancer-associated fibroblasts. The mitophagy releases glutamate from the fibroblast, which feeds the TCA cycle in cancer cells to efficiently produce

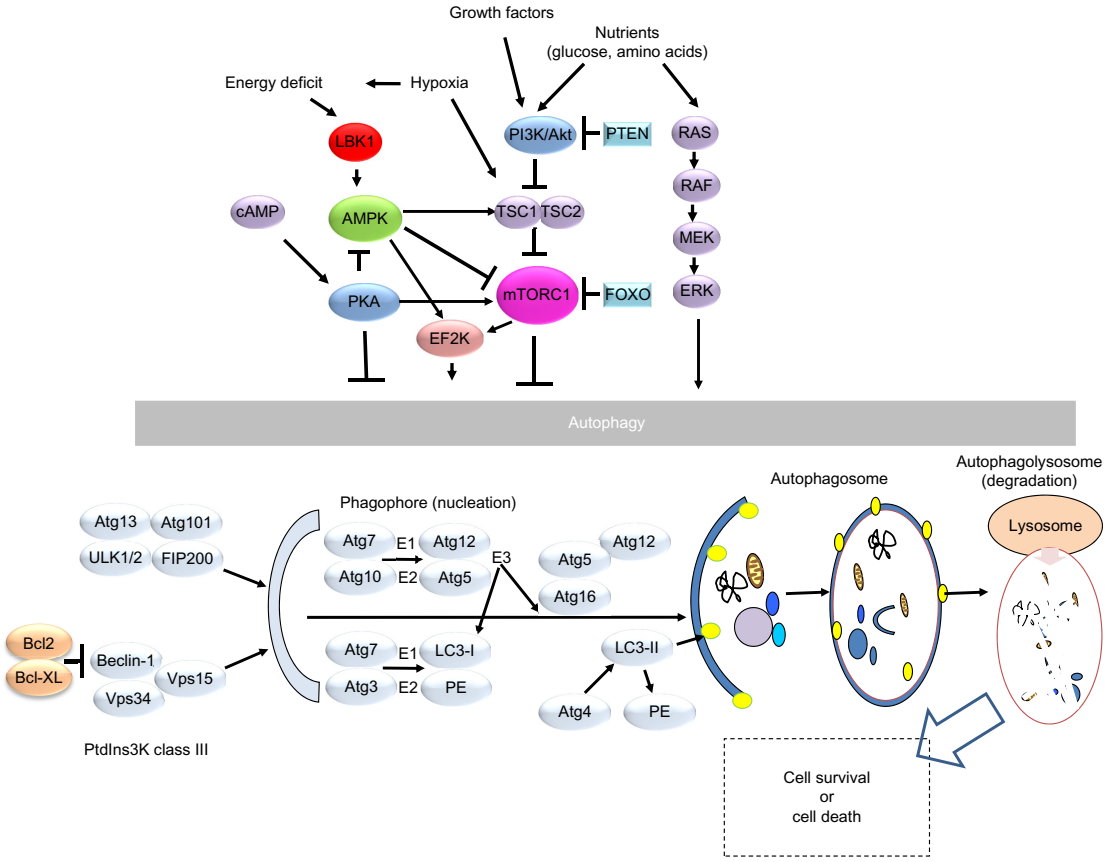

Figure 1 Regulation of autophagy.

Notes: mTOR is one of the most important regulators of autophagy. mTOR and other pathways including cAMP, LKB, AMPK, and PKA merge at mTORC1. AMPK inhibits mTORC1 by direct interaction or by indirect activation of the TSC2 protein. The mTORC1 substrate p70S6K is a positive regulator of autophagy. Another important upstream factor is AKT/PKB, which acts a negative regulator of the TSC1/2 complex. In addition to energy depletion and hypoxia, the RAS, RAF, MEK, and ERK pathway is also involved in regulation of autophagy. The autophagic processes require induction, phagophore assembly (nucleation), sequestration, autophagosome formation, and autophagolysosome formation. The initial phase involves the initiation of the ULK complex, including ULK1/2, Atg13, Atg101, and FIP200. The activation of the PtdIns3K complex (Beclin-1, Vps34, and Vps 15), Vps, is an essential step in phagophore assembly (membrane nucleation). The E1-like enzyme Atg7 activates Atg12 and LC3-I, and the E2-like enzymes Atg10 (for activation of Atg12) and Atg3 (for LC3-I). Atg5 is conjugated to the Atg12 protein and this complex acts as an E3 ubiquitin ligase to catalyse the conjugation of LC3-I to PE in the process of sequestration. The subsequent autophagosome formation is dependent on the Atg12–Atg5–Atg16 complex. Once autophagosome formation is completed, the Atg12–Atg5–Atg16 complex dissociates from autophagosomes to allow Atg4 access to LC3-II for deconjugation from the lipid PE. Later, the lysosome merges with the autophagosome to form an autolysosome, which degrades the cytosolic macromolecules, proteins, and organelles. Depending on the cellular status, stress signal, and duration, the process leads to either cell death or cell survival.

Abbreviations: AKT/PKB, protein kinase B; mTOR, mammalian target of rapamycin; TAK, thylakoid membrane protein kinase; LKB, liver kinase B; AMPK, adenosine monophosphate kinase; PKA, protein kinase A; TOR, target of rapamycin; LC3, microtubule-associated protein 1 light chain; PE, phosphatidylcholine; cAMP, cyclic adenosine monophosphate.

adenosine triphosphate (ATP). A by-product of the TCA cycle, ammonia, released from the cancer cells continues to stimulate stromal cell mitophagy.

Interpretation of autophagy markers

"Guidelines for the use and interpretation of assays for monitoring autophagy" has recently been published in *Autophagy* by a group of autophagy experts under the leadership of Dr Daniel Klionsky.[13] Although LC3-II expression, GFP-LC3 punctate formation, and transmission electron microscopy (TEM) are used commonly in in vitro studies, in clinical samples, autophagy is mostly evaluated by examining LC3-II and *Beclin-1* expression by immunohistochemistry and autophagy genes by quantitative reverse-transcription polymerase chain reaction.[13] Although TEM is the gold standard method to monitor autophagy, it is labor intensive; thus, evaluation of autophagy marker proteins in tissues using standard immunohistochemical techniques has become common practice. Increases in LC3-II levels are commonly used to detect the induction of autophagy in vitro and in vivo, but they can be misinterpreted if autophagy is blocked at the level of lysosomal fusion resulting in the buildup of autophagosomal vessels. To demonstrate autophagic flux, meaning autophagy that proceeds through to lysosomal degradation without blockage, inhibitors of lysosomal acidification and function,

such as bafilomycin A, chloroquine (CQ), and hydroxychloroquine (HCQ), are commonly utilized in in vitro settings.[13] Autophagic flux is confirmed if LC3-II is further increased in the presence of these inhibitors, but often, an additional molecule called p62 (also called SQSTM1) is evaluated to confirm this conclusion. The p62 protein binds to proteins that have been targeted for autophagosomal degradation by conjugation with single ubiquitin moieties or ubiquitin chains that are branched on Lys63, and that also, but with lower affinity, binds to proteins targeted for proteasomal degradation by conjugation with poly-ubiquitination chains that are branched on Lys48.[17,18] The ubiquitinated proteins are then brought into the developing autophagosome by p62, which binds to LC3-II on the inner autophagosomal membrane.[17,19] Upon fusion with the lysosome, p62 is degraded inside the autophagosome and, therefore, reduction of p62 is considered a biomarker of autophagy. Prevention of p62 reduction with lysosomal inhibitors is considered an indication of autophagic flux. While these manipulations provide interpretable endpoints in tissue culture studies, total levels of p62 in tissue are less meaningful due to simultaneous induction of p62 by stresses that induce autophagy and the reduction of p62 by autophagic flux.[20] Immunohistochemical analysis of either the A or B alleles of LC3, namely LC3A or LC3B, respectively, have been used to probe for the level of autophagy occurring in tissue.[21,22] Although early studies suggested that only LC3B was involved in autophagy, more recent studies have indicated a role for LC3A in this process.[23,24]

While autophagy appears to engulf areas of the cytoplasm, specificity for individual molecules and organelles can be conferred by p62 and other molecules, such as Bcl-2 19 kDa interacting protein (BNIP3). In the presence of organelle damage, BNIP3 located on the cytoplasmic side of the mitochondria and ER membranes forms homodimers that have high affinity for LC3-II on the inside of autophagosomal membranes.[25]

Autophagy as a tumor suppressor and cell survival mechanism

An increasing body of evidence indicates that autophagy may be deregulated, suppressed, or overactivated in tumors. Whether autophagy is a prosurvival (oncogenic) process or a prodeath (tumor-suppressive) pathway is still controversial.[26–32] However, studies indicate that this discrepancy depends on the cellular context (ie, mutations, genetic alterations), the status of activated or deactivated signaling pathways, and the extent of cellular stress. In breast cancer cells, Beclin-1 protein levels are often found to be low or undetectable, but high in all normal breast epithelial cells.[33] Beclin-1 is monoallelically deleted in 40%–70% of cases of human breast, prostate, and ovarian cancers.[33–36] Although biallelic mutations of *Beclin-1* have not yet been demonstrated in human cancer cells, the monoallelic deletion of the *Beclin-1* gene has also been observed in other cancer types, and autophagy is commonly inhibited in some of the aggressive tumors including ovarian and prostate cancer.[37,38] The first evidence linking dysfunctional autophagy and cancer was demonstrated by a study where the disruption of *Beclin-1* (*BECN1)* increased cellular proliferation and the frequency of spontaneous malignancies (ie, lung cancer, liver cancer, and lymphomas), as well as mammary hyperplasia, and accelerated the development of carcinogen-induced (ie, hepatitis B virus) premalignant lesions.[33] Furthermore, disruption of *Beclin-1* resulted in reduced autophagy in mice, indicating that *Beclin-1* is a haplo-insufficient tumor suppressor gene, providing evidence that autophagy is a novel mechanism of cell growth control and tumor suppression. These findings suggest that disruption of the function of *Beclin-1* and other autophagy genes may contribute to the pathogenesis of human cancers. Later studies have demonstrated that defective autophagy leads to a reduction in degraded cellular contents, the induction of reactive oxygen species (ROS), DNA damage, genetic instability, and inflammation, providing conditions for transformation and carcinogenesis.[35]

The process of autophagy is thought to play a role in cancer initiation and progression, invasion and metastasis, and cancer stem cells and tumor dormancy. The induction of autophagy could lead to the survival of cancer cells in the highly metabolically challenged tumor microenvironment. In this environment, various stresses, including hypoxia, acidity (low pH), increased metabolic products, reduced availability of nutrients due to high mitotic activity, and limited angiogenesis, activate autophagic scavenging of long-lived proteins and organelles to provide recycled components for reuse and repair. Extensive degradation by autophagy could lead to the degradation and loss of critical cellular components, which would cause a form of cell death (autophagic cell death), leading to the elimination of defective cells.[4]

Signaling pathways involved in the regulation of autophagy

A major regulator of autophagy is the mTOR pathway, downstream of protein kinase B (PI3K/AKT), which is often found to be overactivated in various human cancers. Excessive amino acids induce mTOR activity, which inhibits autophagy. High levels of AMP-active AMPK, which inhibits mTOR,

induces autophagy.[14] It is becoming clear that many signaling pathways and critical cellular proteins, including class 3 PI3K (Vps34), AMPK, Ras, Raf, MEK, ERK, JNK, p53, PTEN, p70S6K, eEF-2 kinase (EF2K), nuclear factor-kappa B (NF-κB), and others, are involved in the regulation of autophagy.[29] As previously stated, class 3 PI3K forms a complex with *Beclin-1* and regulates nucleation.[39–41] Both Bcl-2 and Bcl-XL antiapoptotic proteins can regulate autophagy by binding and inhibiting *Beclin-1*, and may calibrate autophagy to levels that are compatible with survival.[42] Class 1 PI3K, the PI3K/AKT/mTOR pathway, inhibits autophagy, whereas inhibitors of this pathway such as PTEN, TORCH1, AMPK, and rapamycin (a pharmacological mTOR inhibitor) induce autophagy.[39,43] TOR has been shown to inhibit Atg1/ULK1. Rapamycin induces autophagy by restoring Atg1's kinase activity.[44] The RAS/RAF/MEK/ERK pathway can also promote autophagy.[45] Recent findings suggest that NF-κB inhibition can lead to an autophagic cell death.[46,47] Tumor necrosis factor-alpha (TNFα), insulin-like growth factor-1, and 7-ketocholesterol can regulate the expression of *ATG* genes, including *LC3* and *Beclin-1*. In addition, p53 tumor suppressor protein is a transcription factor that responds to cellular stress and inhibits tumor formation. Recent studies also suggest that cytoplasmic p53 may inhibit autophagy, and that damage-regulated autophagy modulator (DRAM), a p53 target, can modulate autophagy.[48] Constitutive Ras activation induces autophagy to promote cell survival and tumor growth by altering mitochondrial function, underlying the role of Ras in tumors, which have mutated K-ras (ie, 90%–100% of pancreatic cancers).[49] These observations represent critical findings regarding how signaling pathways control autophagy, and modulation of autophagy by targeting these pathways may be used as a therapeutic intervention depending on the cellular context.

Targeting autophagy for cancer therapy and management

Emerging data derived from in vitro and in vivo studies suggest that autophagy plays a critical role in maintaining cellular homeostasis and survival under stress conditions by eliminating damaged or malfunctioning organelles and toxic proteins, as well as by recycling building blocks and generating resources for ATP and energy. Autophagy is induced by various conditions in the harsh tumor microenvironment, including hypoxia (HIF-1α-dependent and -independent), metabolic stress, anticancer therapeutics, and radiation therapy.[50–59] Thus, autophagy also plays a role in tumor cell survival and progression, as well as in response to anticancer therapies, which led to the hypothesis that the modulation of autophagy can be used as a therapeutic modality or can enhance the efficacy of cancer therapeutics. Currently, various clinical trials are investigating autophagy inhibitors in combination with cytotoxic chemotherapies and targeted agents in solid and hematological cancers (Table 1).

In vitro and in vivo studies demonstrated that the pharmacological inhibition (by HCQ, CQ, or 3-methyladenine [3-MA]) and genetic knockdown of autophagy genes augment the efficacy of various cancer therapeutics and targeted therapies. These studies led to the hypothesis that suppression of the autophagic pathway can be used as a sensitizing strategy for anticancer therapeutics.[4,12,29] These include a number of antineoplastic therapies, DNA-damaging agents (eg, doxorubicin, temozolomide, etoposide), radiation therapy, histone deaceltylase (HDAC) inhibitors, suberoylanilide hydroxamic acid inhibitors, arsenic trioxide, TNFα, interferon-γ, imatinib, rapamycin, and antiestrogen hormonal therapy (eg, tamoxifen), which have been shown to induce autophagy in some human cancer cell lines.[50–59] In addition, recent data showed that human cancer cell lines with *H-ras*- or *K-ras*-activating mutations have high basal levels of autophagy and increased ROS levels, and that suppression of autophagy inhibits cell growth, indicating that autophagy is required for tumor cell survival, and that inhibition of this

Table 1 Clinical trials targeting autophagy in cancer patients

Tumor type	Clinical status	Therapeutic combination
Advanced solid tumor	Phase I	HCQ + temozolomide
	Phase I	HCQ + sirolimus or vorinostat
	Phase I	HCQ + sunitinib
Metastatic melanoma	Phase I	HCQ + temsirolimus
Colorectal cancer	Phase II	HCQ + bevacizumab + XELOX
Glioblastoma multiforme	Phase I/II	HCQ + temozolomide + radiation
Lung cancer	Phase I/II	CQ + cisplatin, etoposide
	Phase I/II	HCQ + carboplatin, paclitaxel
	Phase I/II	HCQ + bevacizumab
	Phase II	HCQ + erlotinib
Prostate cancer	Phase II	HCQ + docetaxel
Breast cancer	Phase II	HCQ only
	Phase I/II	CQ only
	Phase II	CQ + paclitaxel, docetaxel, ixabepilone
Renal cancer	Phase I	HCQ only
Pancreatic cancer	Phase I	HCQ only
	Phase I/II	HCQ + gemcitabine
Multiple myeloma	Phase I/II	HCQ + bortezomib
Leukemia (CLL)	Phase II	HCQ only
Leukemia (CML)	Phase II	CQ + imatinib

Abbreviations: HCQ, hydroxychloroquine; XELOX, capecitabine plus oxaliplatin; CQ, chloroquine; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia.

process in *Ras*-driven cancers may be an effective treatment approach.[61] In cancer cells that survive after chemotherapy and/or radiation therapy, autophagy induction leads to tumor dormancy in residual cancer cells that may contribute to tumor recurrence.[60] Thus, targeting autophagy may prevent the dormant state and provide an effective strategy to induce better antitumor efficacy.

Clinical trials of autophagy inhibitors are using United States Food and Drug Administration (FDA)-approved antimalarial drugs such as HCQ and CQ, lysosmal inhibitors, which are known to inhibit autophagy (Table 1). The early results of some of these clinical studies indicate that autophagy inhibition in combination with anticancer therapies seem to be safe and can augment the efficacy of various anticancer therapies.[62–66]

One of the clinical trials evaluated whether blocking autophagy with HCQ enhanced cell death induced by alkylating chemotherapy in patients with advanced solid malignancies and melanoma (49 patients [73%] had metastatic melanoma).[62] These patients were given oral HCQ (200–1,200 mg daily) in combination with temozolimide (oral 150 mg/m^2 daily) for 1–2 weeks. This study evaluated the maximum tolerated dose (MTD), safety, pharmacokinetics, and pharmacodynamics of HCQ in combination with temozolomide. Patients tolerated the HCQ and temozolimide combination well with no dose-limiting toxicities.[62] For the Phase II study, patients were given 600 mg HCQ in combination with temozolomide twice daily, and some toxicities including grade 2 fatigue (55% of patients), nausea (48%), anorexia (28%), constipation (20%), and diarrhea (20%) were observed. Overall, 3/22 (14%) and 6/22 (27%) patients with metastatic melanoma had partial responses and stable disease.[62] Two out of six patients with refractory *BRAF* wild-type melanoma experienced a near complete response and prolonged stable disease. The study also evaluated and demonstrated the induction of autophagy in patient-derived peripheral blood mononuclear cells in response to combined therapy. Overall, the study indicated that the inhibition of autophagy by temozolomide may provide beneficial outcomes in melanoma patients.

The inhibition of autophagy has been shown to significantly augment the anticancer efficacy of the HDAC inhibitor, vorinostat.[63] The combination of the autophagy inhibitor HCQ (taken orally on a daily basis from days 2–21 of a 21-day cycle) and vorinostat (400 mg, days 1–21) in patients with advanced solid tumors (27 patients) resulted in some side effects, including grade 1 to 2 nausea, diarrhea, fatigue, weight loss, anemia, and elevated creatinine. Fatigue and gastrointestinal side effects were the dose-limiting toxicities, and 600 mg HCQ and 400 mg vorinostat was established as the MTD and recommended Phase II regimen. In the treatment group, one patient with renal cell carcinoma had a durable partial response and two patients with colorectal cancer had prolonged stable disease. Currently, additional clinical studies are being conducted to further investigate the clinical significance of autophagy inhibition as a new strategy to enhance the efficacy of HDAC inhibitors.

Another preclinical study in multiple myeloma demonstrated that autophagy inhibition with HCQ significantly potentiates the efficacy of the proteasome inhibitor, bortezomib.[75] In a Phase I study,[64] a HCQ and bortezomib combination was evaluated in relapsed or refractory myeloma patients (25 patients; eleven (44%) refractory to prior bortezomib treatment). Patients received HCQ (600 mg twice daily) with standard doses of bortezomib,[51] and of 22 patients evaluable for response, three (14%) had partial responses, three (14%) had minor responses, and ten (45%) had prolonged stable disease. The combined therapy showed therapy-associated increases in autophagy, and indicated the treatment's feasibility and potential benefits as a useful strategy for improving outcomes in myeloma patients.

Another Phase I study[65] evaluated the safety and preliminary clinical activity of the HCQ and temsirolimus (an mTOR inhibitor) combination in patients with advance solid tumors, following preclinical demonstration that HCQ enhanced cell death in combination with temsirolimus (CCI-779). This dose escalation study evaluated the effects in an initial set of 27 patients, followed by a cohort of 12 patients with metastatic melanoma. Patients receiving the combination of HCQ and temsirolimus experienced grade 3 or 4 toxicity of anorexia (7%), fatigue (7%), and nausea (7%). Although the MTD was not reached for HCQ, a Phase II study was conducted with a dose of 600 mg HCQ (twice daily) in combination with 25 mg weekly of temsirolimus. Patients experienced grade 1 or 2 toxicities, such as rash, stomatitis, and weight loss. Overall, 14/21 (67%) of all patients and 14/19 (74%) of patients with melanoma achieved stable disease. Thirteen melanoma patients treated with HCQ 1,200 mg/day in combination with TEM had a median progression-free survival of about 3.5 months. Evaluation of peripheral blood monocytes and tumor biopsies demonstrated autophagy inhibition only in patients treated with the highest dose of HCQ (1,200 mg daily). This study suggested that the combination of HCQ with temsirolimus was safe and well tolerated and, more importantly, it demonstrated significant antitumor activity and stable disease in 75% of cases of metastatic melanoma,

indicating that autophagy inhibition is a feasible and promising strategy in these patients.

Another Phase I clinical study[66] conducted among newly diagnosed glioblastoma (GB) patients evaluated the MTD and efficacy of HCQ in combination with radiation therapy and temsirolimus. Patients received HCQ (oral daily doses from 200–800 mg), radiation therapy, and temsiroliumus. The MTD for HCQ was 600 mg/day in the three-regimen combination. The subsequent Phase II cohort (number [n] =76) was also conducted, and in this study, patients had a median survival of 15.6 months. However, overall survival was not significantly improved.

Another randomized, double-blind, placebo-controlled study[67] for GB multiforme patients (n=30) evaluated the benefits of adding CQ (150 mg/day) beginning on postoperative day 5 for 12 months to the treatments (chemotherapy or radiotherapy). CQ cotherapy seemed to improve midterm survival when given in addition to conventional therapy for GB multiforme. CQ cotherapy and radiation improved the response of brain metastasis with no increase in toxicity; however, CQ alone did not improve the response rate or overall survival.[68] These results suggest that larger studies of CQ are needed to make firm conclusions, and that the efficacy can be achieved with consistent inhibition of autophagy with CQ, rather than with HCQ. In a clinical trial in dogs (n=30),[69] HCQ and doxorubicin chemotherapy appeared to provide clinical benefit in non-Hodgkin's lymphoma with a ~30% complete remission rate.

Overall, in vivo preclinical studies with animals and clinical trials with patients indicate that autophagy inhibitors may enhance the efficacy of conventional therapies in various cancers, including hematological cancers. However, larger clinical trials and a comprehensive analysis of the data will eventually provide more definitive answers regarding the effects of manipulation of authophagy in patients.

Alternate approaches to inhibiting autophagy as a therapeutic strategy

Because mTOR is a major negative regulatory axis for autophagy, several drugs that directly inhibit mTOR (rapamycin, temsirolimus, everolimus) and its pathways have been used to induce autophagy. The inhibition of mTOR mimics cellular starvation by blocking signals required for cell growth and proliferation.[70] Some studies demonstrate that the PI3K/AKT/mTOR pathway promotes melanoma tumor growth and survival.[70,71] Knockdown of the expression of the essential autophagy gene (*ATG7*) results in cell death, indicating that the survival of melanoma cells is autophagy-dependent. Conversely, inhibition of mTOR with temsirolimus induces autophagy, which can promote tumor survival, and thus these agents may potentially limit their own efficacy.[71] In support of this, inhibition of autophagy with HCQ synergizes with temsirolimus and leads to melanoma cell death via apoptosis.[71] Combination treatments with temsirolimus and HCQ suppressed melanoma growth and induced cell death in both spheroid cultures and in tumor xenografts.[71] These data suggest that inhibition of the mTOR and autophagy pathways promotes apoptosis and could be a new therapeutic paradigm for the treatment of melanoma. In addition, other strategies have been used to induce autophagy. For instance, knockdown of *Bcl-2* expression by siRNA induces autophagic cell death in breast cancer cell lines and in vivo in orthotopic xenograft models of ER(–) and ER(+) breast cancer.[72,73] Also, targeting protein kinase C (PKC) delta and tissue transglutaminase, which inhibits autophagy in pancreatic cancer cells, can increase apoptosis.[74] Inhibition of NF-kB, MAPK, JNK, P38, or the induction of ERK (another regulator of autophagy) has been used to modulate autophagy in different cancer models to alter responses to the various therapies, including chemo and radiation therapy. Overall, data suggest that the multiple and different strategies for the modulation of autophagy could improve therapy in some tumors, and that they can be utilized as cotherapy.

Conclusion

Recent findings suggested that autophagy is a promising target in the context of cancer therapy. Autophagy is thought to act as a prosurvival pathway that protects cancer cells from various stresses and anticancer therapy-induced stress, thus the inhibition of autophagy is considered in combination with some of the anticancer therapeutic strategies. The results of the first clinical trials conducted with autophagy inhibition along with conventional therapies indicate some clinical benefits in some cancers. Overall, these studies suggest that the efficacy of standard therapies may be enhanced when combined with autophagy inhibitors, which warrant further evaluation. Further understanding of the pathways regulating autophagy will likely offer new targets for the treatment of certain aggressive forms of tumors. Once the results of current clinical trials are obtained and analyzed, the role of autophagy will be better understood in tumors, and it would indicate whether modulation of the autophagic process or its related pathways that protect cancer cells from undergoing autophagy may be added to conventional therapies for better efficacy. In addition to currently available autophagy inhibitors, such as US FDA-approved malaria

drugs (ie, HCQ, CQ), identification of novel, highly specific, and effective compounds to negatively regulate autophagy in cancer cells are needed.

Disclosure

The authors report no conflicts of interest in this work.

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