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Autophagy as a therapeutic target in cancer

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Abbreviations: 3D, three-dimensional; 3-MA, 3-methyladenine; 4-HPR, N-(4-hydroxyphenyl) retinamide; 5-FU, 5-fluorouracil; ACD, autophagic cell death; ADI-PEG20, arginine deiminase, pegylated; AML, acute myelogenous leukemia; AMP, adenosine monophosphate; ARHI, aplasia ras homolog member I; ATGs, autophagy related genes; ATP, adenosine triphosphate; BafA, bafilomycin A; BCG, Bacillus Calmette-Guerin; BH3, BCL-2 homology domain 3; BIF-1, Bax-Interacting factor-1; BRCA1, breast cancer susceptibility gene 1; CAMKKβ, calmodulin-dependent protein kinase kinase β; CINJ, Cancer Institute of New Jersey; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; CQ, chloroquine; CR, caloric restriction; DAPK, death-associated protein kinase; DCIS, ductal carcinoma in situ; DNA, deoxy-ribonucleic acid; DRAM, damage-regulated autophagy modulator; EGFR, epidermal frowth factor receptor; ER, endoplasmic reticulum; FOLFOX, 5-FU/leucovorin and oxaliplatin; HCQ, hydroxychloroquine; HDACi, histone deacetylase inhibitor; HIV, human immunodeficiency virus; iBMK, immortalized baby mouse kidney; IFN, interferon; IKK, IκB kinase; IL, interleukin; iMMECs, immortalized mouse mammary epithelial cells; IRE1, inositol-requiring kinase 1; JNK, c-jun N-terminal kinase; LC3, light chain 3 (also, ATG8); Maastricht Rad Onc, Maastricht Radiation Oncology; Mass Gen Hosp, Massachusetts General Hospital; mTOR, mammalian target of rapamycin; Nat U Hosp, Singapore, National University Hospital, Singapore; NCI, National Cancer Institute, USA; NK, natural killer; NINN, Mexico, National Institute of Neurology and Neurosurgery, Mexico; NSCLC, non-small cell lung cancer; NSLIJHS, North Shore Long Island Jewish Health System; PERK, protein kinase-like endoplasmic reticulum kinase; PI3K, phosphatidyl inositol-3-kinase; PRAS40, proline-rich AKT substrate of 40 kDa; PTEN, phosphatase and tensin-homologue deleted on chromosome 10; RNAi, RNA interference; ROS, reactive oxygen species; RT, radiation therapy; SAHA, suberoylanilide hydroxamic acid (also, vorinostat); SCLC, small cell lung cancer; TAK1, transforming growth factor-β-activating kinase 1; TFT, trifluorothymidine; TNF, tumor necrosis factor; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; TRAIL, TNF-related apoptosis inducing ligand; TSC, tuberous sclerosis complex; U, university; U Penn, University of Pennsylvania; U Texas HSC S Ant, University of Texas Health Science Center at San Antonio; UVRAG, ultraviolet radiation resistance-associated gene; VHL, Von Hippel-Lindau; XELOX, capecitabine (xeloda) and oxaliplatin

Autophagy is a self-catabolic process that maintains intracellular homeostasis and prolongs cell survival under stress via lysosomal degradation of cytoplasmic constituents and recycling of amino acids and energy. Autophagy is intricately involved in many aspects of human health and disease, including cancer. Autophagy is a double-edged sword in tumorigenesis, acting both as a tumor suppressor and a protector of cancer cell survival, and elucidation of its exact role at different stages of cancer progression and in treatment responsiveness is a complex and challenging task. Better understanding of autophagy regulation and its impact on treatment outcome will potentially allow us to identify novel therapeutic targets in cancer. In this review, we summarize current knowledge on the regulation and dual function of autophagy in tumorigenesis, as well as ongoing efforts in modulating autophagy for cancer treatment and prevention. This is a very exciting and highly promising area of cancer research, as pharmacologic modulation of autophagy appears to augment the efficacy of currently available anticancer regimens and opens the way to the development of new combinatorial therapeutic strategies that will hopefully contribute to cancer eradication.

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Introduction

Macroautophagy, hereafter referred to as autophagy, is an evolutionarily conserved, genetically controlled cell survival pathway involving the degradation of cytoplasmic constituents, and the recycling of ATP and essential building blocks for the maintenance of cellular biosynthesis during nutrient deprivation or metabolic stress.[1] This cellular self-consumption process is characterized by sequestration of bulk cytoplasm, long-lived proteins and cellular organelles in double-membrane vesicles, called autophagosomes, which are ultimately delivered to and degraded in the lysosomes.[2] Autophagy is intricately implicated in both health and disease.[3] Basal autophagy plays a critical role in cellular homeostasis by eliminating excessive, damaged and/or long-lived proteins and organelles, thus preserving the quality of essential cellular components.[4,5] Under stress, autophagy is commonly induced as a temporary cell survival mechanism. Autophagy defects play a

role in the pathogenesis of diverse diseases, including myopathy,[6] neuronal degeneration,[7] microbial infection,[8] inflammatory bowel disease,[9,10] aging[11] and cancer.[12] Recent studies have shed light on the functional role of autophagy in different cellular processes and the potential of autophagy modulation as a novel therapeutic strategy for different pathologic conditions, including cancer.[13,14] Anticancer therapies, such as hormonal agents, chemotherapy and irradiation, frequently induce autophagy, in most cases as a prosurvival response potentially contributing to treatment resistance;[15-17] however, autophagy activation in particular genetic backgrounds and/or completion of the autophagic process beyond reversibility of cell viability can also lead to cell death, thus enhancing treatment efficacy.[18] The complex role of autophagy in tumorigenesis and treatment responsiveness makes it hard to decipher how to universally modulate autophagy for maximum therapeutic benefit, indicating that context- and cell type-specific approaches may be required.

In this review, we present the current knowledge on autophagy regulation, its role as a double-edged sword in tumorigenesis, and the implications of pharmacologic autophagy modulation for cancer treatment. Therapeutic regimens potentially altering the functional status of autophagy in tumors will also be reviewed, hopefully providing a reference for the future development of combinatorial treatments involving autophagy modulation as a cancer therapeutic and/or preventative strategy.

Regulation of Autophagy in Cancer

In normal cells, autophagy is regulated by a cellular network that involves upstream signaling pathways integrated by the mammalian target of rapamycin (mTOR) kinase, a master regulator.[12,14,19] Nutrient and/or growth factor availability activates the PI3K/AKT/mTOR axis, which inhibits autophagy thus stimulating cell growth and proliferation; whereas nutrient and/or growth factor limitation, hypoxia and other stressors deactivate this axis, leading to autophagy induction and suppression of cell growth and proliferation.[14,20] In tumor cells, autophagy regulation is in principal similar, but the regulatory network is even more complex due to frequently abnormal PI3K/AKT/mTOR and other signaling cascade activation and interactions between these pathways.[21,22]

PI3K-AKT-mTOR pathway. Abnormal, and often constitutive, activation of the PI3K-AKT-mTOR axis promoting tumor cell growth, proliferation and survival is a common occurrence in cancer.[23-25] This may be the result of one or multiple cellular events that are associated with cancer initiation and/or progression, such as phosphatase and tensin-homologue deleted on chromosome 10 (PTEN) mutation or loss, tuberous sclerosis complex (TSC) 1 and/or 2 mutation, tyrosine kinase growth factor receptor and/or type I PI3K mutation, AKT overexpression, mTOR inhibition and carcinogen exposure, leading to the abnormal activation of this signaling cascade and subsequent inhibition of autophagy (**Fig. 1**).[14] PI3K mutations, such as E542K, E545D and E545K, which result in constitutive kinase activation, are frequently detected in human cancers.[26-29] Activated PI3K results in sequential AKT and mTOR activation, ultimately suppressing autophagy. This is in contrast to the action of a different PI3K (type III) that binds to the Beclin 1 protein forming a complex involved in autophagy induction.[30]

The tumor suppressor PTEN is frequently mutated or lost in human tumors, also leading to AKT activation and autophagy inhibition.[31-33] The serine/threonine kinase mTOR, which is a downstream target of AKT, controls a series of autophagy-related genes and is commonly recognized as the master autophagy regulator.[14,34,35] AKT activates the mTOR complex via phosphorylation of TSC2 and PRAS40 (proline-rich AKT substrate of 40 kDa).[36] The deregulated PI3K/AKT/mTOR axis not only suppresses autophagy, but also induces protein translation, cell growth and proliferation, which are indisputable driving forces in tumorigenesis.[24,37] Pharmacologic inhibition of the PI3K/AKT/mTOR axis for cancer treatment results in reciprocal autophagy induction, raising the critical issue of the potential dual function of autophagy activation in treatment responsiveness. Type I PI3K inhibitors, such as lithium and carbamezapine; type III PI3K inhibitors, such as 3-MA, LY294002 and wortmannin; AKT inhibitors, such as perifostine and API-2; and mTOR inhibitors, such as rapamycin, RAD001 and CCI-779; have all been reported as promising anticancer agents.[14,38,39] However, the impact of drug-induced autophagy modulation on antitumor activity and how to best exploit this response for maximum therapeutic benefit are both questions that require further investigation.

LKB1-AMPK-mTOR pathway. The LKB1-AMPK-mTOR pathway acts as a central sensor regulating lipid and carbohydrate metabolism in metabolically active tissues, such as liver, muscle and fat. Interestingly, AMPK has also been implicated in cancer cell metabolism and tumorigenesis.[40,41] As the major upstream regulator, the serine/threonine kinase LKB1 (also known as STK11) activates AMPK by directly phosphorylating its α-subumit at Thr172.[42,43] Low energy or metabolic stress conditions, such as nutrient and oxygen deprivation, are associated with reduced cellular ATP levels and an elevated AMP/ATP ratio, which activates the tumor suppressor LBK1, resulting in AMPK activation and, ultimately, mTOR inhibition and autophagy induction (**Fig. 1**).[42] Furthermore, AMPK directly phosphorylates TSC2 and the regulatory associated protein of mTOR (Raptor), thus inhibiting mTOR in both TSC-independent and -dependent ways and activating autophagy as a cell survival mechanism in response to metabolic stress in an LKB1-dependent manner.[44,45] Calcium and calmodulin-dependent protein kinase kinase β (CAMKKβ) are also involved in AMPK activation in hypothalamic neurons,[46] T cells and endothelial cells,[47,48] indicating that calcium metabolism also plays a role in LKB1-AMPK-mTOR-mediated autophagy regulation. In parallel to upregulating autophagy, activation of the LKB1-AMPK-mTOR axis results in the phosphorylation and stabilization of $p27^{kip}$, a cyclin-dependent kinase inhibitor, to induce cell cycle arrest for energy conservation.[49] Finally, the transforming growth factor-β-activating kinase 1 (TAK1) was recently identified as another AMPK activator.[50] Further investigation of the LKB1-AMPK-mTOR signaling cascade will advance our understanding of the different mechanisms regulating autophagy and will hopefully contribute to the

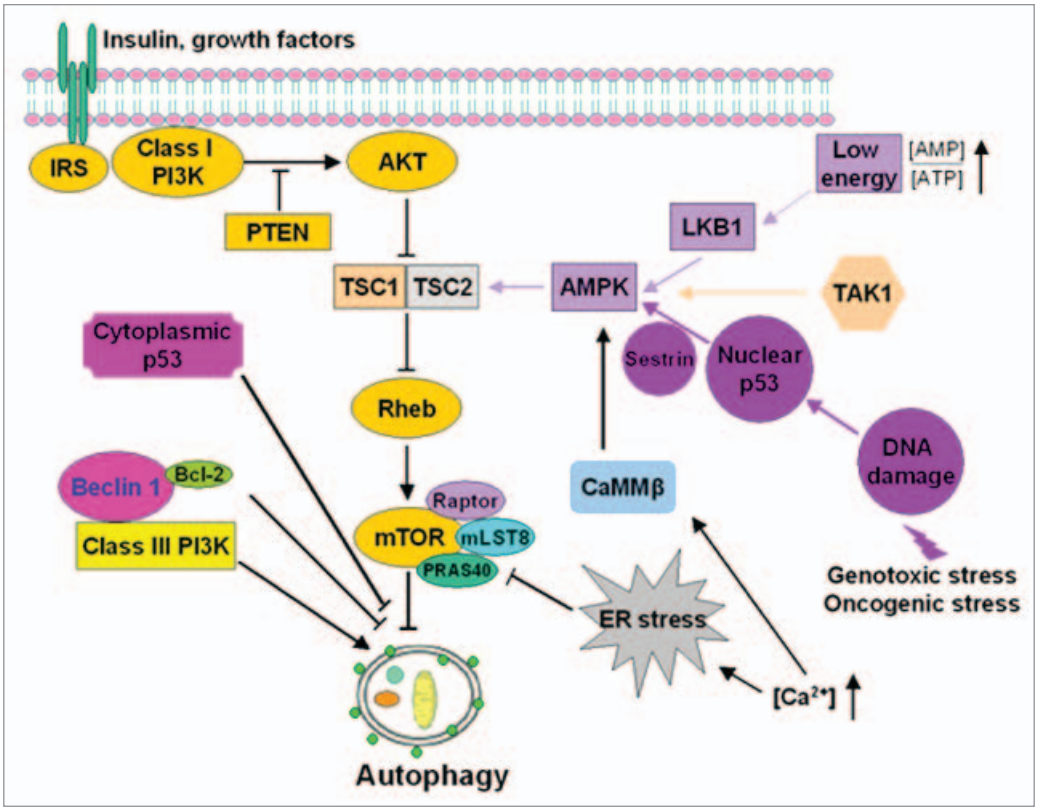

Figure 1. Autophagy regulation. Growth factor signaling activates the PI3K/AKT/mTOR axis resulting in autophagy inhibition. In contrast, class III PI3K activates autophagy. Low cellular energy levels with increased AMP/ATP ratio activate the LKB1-AMPK-mTOR pathway to also upregulate autophagy. Furthermore, increased calcium ion levels induce autophagy by two mechanisms, i.e., the Ca^{2+}-CaMKKβ-AMPK pathway and direct Ca^{2+}-induced ER stress. 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.

development of novel treatment regimens that include cancer cell metabolism as a therapeutic target.

p53. p53 deletion and/or mutation is observed in nearly 50% of human cancers.[51] The tumor suppressor p53 is normally activated under genotoxic or oncogenic stress and leads to cell cycle arrest, senescence or apoptosis.[52,53] p53 has opposing effects on autophagy regulation based on its cellular localization, and thus the p53-autophagy interaction is highly context-dependent. Transcriptional activation, and thus nuclear localization, of p53 is a positive autophagy regulator by inhibiting mTOR in an activated AMPK- and TSC1/TSC2-dependent manner (**Fig. 1**).[54] p53 also activates autophagy by triggering other downstream targets, such as sestrin and the damage-regulated autophagy modulator (DRAM).[55,56] Loss of cytoplasmic p53 function by genetic or pharmacologic manipulation paradoxically activates autophagy as well, suggesting that the non-nuclear p53 pool is a potential autophagy repressor.[57] Autophagy induction by p53 loss promotes p53-deficient cell survival by sustaining high ATP levels under conditions of hypoxia and nutrient depletion.[58] Interestingly, although cytoplasmic p53 inhibits autophagy, nuclear p53 mutants fail to block autophagy. This dual interplay between p53 and autophagy is still under investigation. A possible explanation is that the role of p53 in autophagy regulation depends on the particular stress and microenvironment that a cell is exposed to, thus likely changing upon cancer progression. In the early stages of tumorigenesis, p53-mediated autophagy induction may act as a gatekeeper in response to genotoxic or oncogenic stress by eliminating malfunctioning cells. In advanced tumors, where p53 is commonly deleted or mutated, p53 deregulation stimulates autophagy as an amino acid and energy recycling mechanism to support tumor cell viability.[59]

BCL-2. Beclin 1 (BECN1, also called ATG6) was originally identified as a novel BCL-2-interacting protein in a yeast two-hybrid system.[60] The autophagic function of BECN1 1 is evolutionarily conserved from yeast to human,[61-66] and involves the binding of BCL-2 to other autophagy regulators, such as VPS34, p150, UVRAG, BIF1, ATG14L and Rubicon to form huge protein complexes and initiate double-membrane autophagosome formation.[67-71] Allelic *BECN1* deletion is frequently observed in human breast, ovarian and prostate cancers and leads to tumor development in mice, whereas ectopic BECN1 expression, which restores autophagy, inhibits tumorigenesis; indicating that BECN1 is a tumor suppressor and establishing a connection between defective autophagy and cancer.[62,72-77]

BCL-2 is an anti-apoptotic protein commonly overexpressed in breast cancers, which contributes to treatment resistance by inhibiting chemotherapy and hormonal therapy-induced apoptosis. BCL-2 is also involved in autophagy inhibition by binding

to the BH3 domain of BECN1 and negatively regulating the autophagy-promoting BECN1-VPS34 complex.[70] Dissociation of BECN1 from BCL-2, and thus autophagy activation, in response to nutrient limitation depends on BCL-2 phosphorylation by the starvation-activated c-JUN N-terminal kinase (JNK).[78]

BECN1 and BCL-2 complex formation is primarily dependent on the BH3 domain of BECN1 and the BH3-receptor domain of BCL-2.[79] Pharmacologic interference with this interaction by BCL-2 knockdown, BH3-mimetics or JNK activation results in autophagy induction and provides a novel therapeutic strategy targeting cancer cells with high treatment resistance due to BCL-2 overexpression.

ER stress and Ca^{2+} signaling. The endoplasmic reticulum (ER) is a cellular organelle responsible for secreted and membrane protein folding. Cellular stressors, such as hypoxia, low glucose and deregulation of calcium homeostasis, result in misfolded protein accumulation in the ER, triggering ER stress and cell death, typically via apoptosis.[80,81] ER stress activates autophagy as a compensatory mechanism, whereas autophagy inhibition enhances ER stress-induced cell death.[82,83]

ER stress is accompanied by calcium release into the cytosol, thus activating Ca^{2+}-regulated signaling pathways. Calcium- and CAMKKβ-dependent AMPK activation is one mechanism connecting calcium release from the ER to autophagy, as discussed earlier in this review.[84, 85] Recent studies also reported autophagy induction by protein kinase-like endoplasmic reticulum kinase (PERK) and inositol-requiring kinase 1 (IRE1)-dependent mechanisms.[86,87] Given the interest in ER stress inducers as potential anticancer agents, it is worthwhile to exploit how to best manipulate the resulting autophagy upregulation for maximizing anticancer benefits.

Other autophagy regulators. The death-associated protein kinase (DAPK) is a cytokeleton-associated, calmodulin-regulated serine/threonine protein kinase.[59] DAPK or DAPK-related protein kinase 1-associated autophagy is observed in many human malignancies, including B and T cell lymphomas and breast, lung, head and neck, gastric, cervical and prostate cancers. Conversely, DAPK inhibition results in autophagy suppression.[88,89]

IκB kinase (IKK) stimulates autophagy by multiple NF-κB-independent mechanisms, including starvation-induced JNK1 and AMPK phosphorylation, and likely plays a pivotal role in autophagy induction by physiological and pharmacological stimuli.[90]

Role of Autophagy in Cancer: A Double-edged Sword

The dual function of autophagy in cancer, as both a tumor suppressor and a protector of cancer cell survival, has been widely recognized and remains a rigorously investigated topic.[14,91] Elucidation of the specific role that autophagy plays at different stages in cancer progression and determination of its cell type and genetic context-dependency will lead to the development of novel, and hopefully more effective, cancer therapeutic and preventative strategies.

Autophagy as a tumor suppressor mechanism. Morphological evidence of autophagosome accumulation in dying cells linked autophagy to cell death and led to the definition of an autophagosome-associated, non-apoptotic form of programmed cell death (PCD) as autophagic cell death (ACD) or type II PCD, potentially functioning as a tumor suppressor mechanism similar to apoptosis.[92] The tumor suppressive role of autophagy was validated by the discovery and characterization of the autophagy-related genes (*Atg*),[93] among which B*eclin 1* (*Atg6*) was the first to be reported as a plausible tumor suppressor, as allelic *BECN1* deletion is frequently observed in human breast, ovarian and prostate cancers,[62,73-77] and aging *Becn1*$^{+/-}$ mutant mice develop tumors (lymphomas, lung and liver cancers), as well as mammary hyperplasia and hepatitis B virus-induced premalignant lesions.[65,66]

Genetic alteration of other autophagy-related genes has also been causally associated with tumorigenesis. *Atg4C* knockout mice exhibit increased susceptibility to chemical carcinogen -induced fibrosarcomas,[94] whereas deletion of the essential autophagy regulator *Atg5* results in natural killer (NK) cell malignancies.[95] Furthermore, loss of Bax-interacting factor-1 (BIF-1), which is a positive apoptosis and autophagy regulator, suppresses programmed cell death and promotes colon adenocarcinomas,[96] and nonsense mutations in the BECN1-binding protein UVRAG (ultraviolet radiation resistance-associated gene), which also positively regulates autophagy, are found in colon and gastric cancers.[97,98]

Both cell autonomous and non-cell autonomous mechanisms have been implicated in autophagy-mediated tumor suppression,[12] namely preservation of genome stability and cellular homeostasis and limitation of inflammation, respectively.[99-102]

Autophagy as a protector of cancer cell survival. Counterintuitive to its tumor suppressive role, autophagy has also been extensively documented to support cancer cell survival under stress.[91,103] For example, autophagy induction in immortalized, apoptosis-defective, IL-3-dependent bone marrow cells in response to growth factor withdrawal prolongs cell survival, as supported by the observed cell death acceleration upon autophagy inhibition.[104,105] Defective autophagy due to allelic *Becn1* deletion or constitutive AKT activation enhances the susceptibility of apoptosis-incompetent immortalized baby mouse kidney (iBMK) cells to metabolic stress.[99,102] Similarly, partially autophagy-defective *Becn1*$^{+/-}$ immortalized mouse mammary epithelial cells (iMMECs) are more sensitive to metabolic stress and show accelerated acinar lumen formation in 3D-culture.[100,106]

Autophagy-mediated support of tumor cell survival may play a critical role in cancer progression at later stages, such as dissemination and metastasis, which account for most cancer-associated deaths. In favor of this hypothesis, starvation-induced autophagy is accompanied by suppression of protein synthesis, cell division and motility in an energy conservation effort that sustains cells in a dormant state with the capacity to resume cell growth and proliferation upon regular growth condition restoration.[12,91,99,107] For example, autophagy induction in mammary epithelial cells upon their detachment from extraceullular matrix (ECM) sustains cell viability in an anoikis-resistant manner, whereas autophagy upregulation in ovarian cancer cells by the tumor suppressor ARHI (aplasia Ras homolog member I) promotes dormant cell

survival in vivo.[108,109] Finally, many studies have clearly documented that in cancer cells, autophagy is upregulated in response to metabolic and genotoxic stress induced by hormonal deprivation, chemotherapy and radiation as a cell survival mechanism, likely contributing to treatment resistance, but also providing a novel therapeutic target in cancer.[12,14,91,110-112]

Autophagy-mediated cancer cell survival is not unexpected given the functions of autophagy in normal cells: basal autophagy maintains cellular homeostasis by removing protein aggregates and damaged organelles, whereas starvation-induced autophagy prolongs cell survival by recycling amino acid and energy, both important functions for cellular fitness and viability preservation.[4,5] Cancer cells are often under higher metabolic stress than normal cells, which in turn increases tumor dependence on autophagy for survival (autophagy addiction), implying a therapeutic window for preferential cancer cell targeting by pharmacologic autophagy modulation. Metabolic stressors uniquely encountered by tumor cells include: (1) oncogene-induced accelerated cell growth and/or proliferation, which increase metabolic demands even in the presence of ample external nutrients,[113] (2) recurrent nutrient and oxygen deprivation during rapid tumor growth or anticancer treatment,[14,107] and (3) inefficient glucose utilization for energy production due to anaerobic glycolysis (Warburg effect).[12,114] Whether cancer cells are addicted to autophagy in an oncogene- and/or tumor type-dependent manner is currently under investigation.

Autophagy Modulation for Cancer Treatment

The goal of anticancer therapy is to effectively compromise tumor cell growth and survival, so as to cause cancer regression and prevent (or at least delay) cancer recurrence, thus improving patient quality of life and survival. Apoptosis is commonly inactivated in cancer, often in association with disease progression, and renders tumors resistant to chemotherapy- and radiation-induced cell death, undisputedly contributing to treatment resistance and earlier patient demise. During the last decade, our understanding of the proteins and molecular mechanisms responsible for apoptosis regulation, initiation and execution has expanded tremendously,[14,115] leading to rationally designed approaches for apoptosis reactivation, and thus restoration of cell death potential, in cancer cells.[116,117]

Despite recent advances in cancer treatment, many tumors still exhibit unsatisfactory responsiveness to biological agents, chemotherapy and/or radiation, either recurring or continuing to grow during or after treatment.[85,118,119] Autophagy upregulation is a common occurrence in response to cancer therapies, expected to occur in both tumor and normal cells, but likely playing a more critical role in the survival of the already metabolically stressed cancer cells, as explained above, and thus contributing to treatment resistance. At the same time, however, this prosurvival function of autophagy provides a novel therapeutic opportunity, as concurrent autophagy inhibition may preferentially sensitize tumor cells to anticancer agents by depriving them of an essential survival mechanism that may be dispensable for normal cell viability under similar conditions.[91,120] Drug-induced autophagy may also be therapeutically beneficial by itself, limiting tumor cell proliferation and resulting in autophagic cell death in a cell type- and genetic background-specific manner.[14,62,85] Thus, context-specific pharmacologic autophagy modulation holds great promise as a novel therapeutic approach adding another weapon to the currently available armamentarium against cancer.[14,121]

Autophagy inhibition as a therapeutic strategy in cancer. In cancer cells, autophagy is generally, and often preferentially as compared to normal cells, induced as a prosurvival function in response to treatment-associated genotoxic and metabolic stress.[110,120,122] Thus, concurrent autophagy inhibition is expected to mostly have a synergistic effect with chemotherapy and/or radiation on cancer cell elimination. The impact of autophagy inhibition on anticancer therapy has been evaluated in multiple tumor models, including glioma,[123,124] myeloma,[125] breast,[126,127] colon[128,129] and prostate cancers.[130] For example, and as described in more detail in **Table 1**, which summarizes preclinical studies supporting autophagy inhibition as an anticancer strategy, treatment-induced autophagy mediated resistance to the HER2 monoclonal antibody trastuzumab, whereas LC3 knockdown via shRNA resulted in resistant cells being re-sensitized to treatment;[127] autophagy inhibition by either pharmacological agents or RNAi targeting essential autophagy regulators potentiated imatinib mesylate-induced cell death in chronic myelogenous leukemia (CML) cells;[131] autophagy suppression also enhanced the therapeutic efficacy of cisplatin and 5-FU in esophageal and colon cancers, respectively.[132,133]

In the absence of drugs specifically targeting autophagy regulators, indirect autophagy inhibition by the lysosomotropic antimalaria drugs chloroquine (CQ) and hydroxychloroquine (HCQ), which interfere with lysosomal acidification and thus, block the autophagic process at its final step, are currently under clinical investigation in combination with standard treatment in multiple tumor types. **Table 2** summarizes such ongoing clinical trials involving CQ- and HCQ-mediated autophagy modulation for cancer therapy. Treatment with CQ or HCQ is likely not the same as direct autophagy inhibition, given that CQ and HCQ are known to exert additional functions, such as immunomodulation and possibly DNA damage as alkylating agents at higher doses. Nevertheless, the results of the above mentioned clinical trials are eagerly awaited as an initial proof-of-principle that autophagy inhibition has a role in cancer treatment. In the event that these studies fail to show the trends anticipated, before declaring autophagy inhibition as an unsuccessful therapeutic strategy in cancer, it will be necessary to assess whether tumors with particular oncogenic changes are better candidates than others for such treatment, in which case trials should be redesigned to specifically target these malignancies.

Autophagy induction as an alternate anticancer strategy. Although autophagy inhibitors combined with standard treatment are emerging as promising anticancer agents, certain cancer cell lines and xenograft tumors were sensitized to therapeutic regimens involving autophagy induction rather than inhibition. This was commonly observed in an apoptosis-defective background, where cancer cells were committed to non-apoptotic cell death modes, including necrosis, necroptosis and possibly

Table 1. Preclinical studies supporting autophagy inhibition for cancer treatment

Cancer type	Primary treatment (target)	Autophagy inhibition method (target)	References
Breast cancer	Trastuzumab (HER2 receptor)	3-MA (PI3K III), BafA (lysosome), RNAi (LC3)	127
	Camptothecin (DNA topoisomerase II)	3-MA (PI3K III), BafA (lysosome), RNAi (BECN1, ATG7)	126
	Tamoxifen (estrogen receptor)	3-MA (PI3K III), RNAi (BECN1, ATG5, ATG7)	163
	Bortezomib (26S proteasome)	RNAi (LC3, ATF4, HDAC6)	164
	Faslodex (estrogen receptor)	RNAi (BECN1)	165
	RT (DNA)	RNAi (BECN1, ATG3, ATG4B, ATG4C, ATG5, ATG12)	128
	Sulforaphane (histone deacetylation)	BafA (lysosome)	166
Cervical cancer	Bortezomib (26S proteasome)	RNAi (BECN1)	167
CML	Imatinib mesylate (BCR/ABL)	CQ (lysosome), BafA (lysosome), RNAi (ATG5, ATG7)	131
	INNO-406 (BCR/ABL)	CQ (lysosome)	168
	SAHA (histone deacetylation)	CQ (lysosome), 3-MA (PI3K III)	169
	Imatinib mesylate (BCR/ABL)	BafA (lysosome), CQ (lysosome), RNAi (ATG5, ATG7)	131
	Imatinib mesylate (BCR/ABL), TPA (differentiation)	CQ (lysosome)	170
Colorectal cancer	Vorinostat (histone deacetylation)	CQ (lysosome), RNAi (ATG7)	129
	RT (DNA)	RNAi (BECN1, ATG3, ATG4B, ATG5)	128
	Sulindac sulfide (cyclooxygenase)	3-MA (PI3K III)	171
	TRAIL (TNF receptor)	3-MA (PI3K III), RNAi (BECN1, ATG5, ATG7, UVRAG)	172
	5-FU (thymidylate synthase)	3-MA (PI3K III), RNAi (ATG7)	173
	TFT, 5-FU (thymidylate synthase)	3-MA (PI3K III)	174
	5-FU (thymidylate synthase)	3-MA (PI3K III)	132
	Bortezomib (26S proteasome)	RNAi (BECN1)	167
Esophageal cancer	Cisplatin (DNA)	3-MA (PI3K III)	133
Gastrointestinal stromal tumor	Imatinib mesylate (BCR/ABL)	CQ (lysosome), RNAi (ATG5, ATG12)	175
Glioma, malignant	AKTi-1/2 (AKT)	CQ (lysosome)	149
	PI-103 (PI3K, mTOR)	CQ (lysosome)	149
	Imatinib mesylate (BCR/ABL)	BafA (lysosome)	176
	RT (DNA)	3-MA (PI3K III), BafA (lysosome), RNAi (BECN1, ATG5)	177
	RT (DNA)	3-MA (PI3K III), BafA (lysosome)	123
	4-HPR (tyrosine kinase)	3-MA (PI3K III), BafA (lysosome)	178
	Temozolomide, etoposide (DNA)	3-MA (PI3K III)	178
	Temozolomide (DNA)	3-MA (PI3K III), BafA (lysosome)	137
Multiple myeloma	8-Amino-adenosine (AKT/mTOR)	CQ (lysosome)	125
Lung cancer, non-small cell	RT (DNA)	RNAi (BECN1, ATG3, ATG4B, ATG4C, ATG5, ATG12)	128
Pancreatic cancer	Gemcitabine, RT (DNA)	3-MA (PI3K III)	185
Pharyngeal cancer	RT (DNA)	RNAi (BECN1, ATG3, ATG4B, ATG4C, ATG5, ATG12)	128
Prostate cancer	ADI-PEG20 (arginine)	CQ (lysosome), 3-MA (PI3K III), RNAi (BECN1)	130
	TRAIL, FADD (TNF receptor)	3-MA (PI3K III)	179
	Androgen deprivation (androgen receptor)	3-MA (PI3K III), RNAi (BECN1)	180
	Saracatinib (Src kinase)	3-MA (PI3K III), CQ (lysosome), RNAi (ATG7)	181
Rhabdoid tumor, malignant	FK228 (histone deacetylation)	CQ (lysosome)	182
Skin cancer, squamous cell	Cisplatin (DNA)	3-MA (PI3K III), RNAi (ATG5)	183

autophagic cell death.[122] Alkylating agents, such as actinomycin D and arsenic trioxide; hormonal therapies, including tamoxifen and vitamin D analogues; natural compounds, such as resveratrol; cytokines, such as IFNγ; gene therapies, including p53 and $p27^{kip1}$; have all been implicated in the induction of autophagic cell death in various cancer cell lines in vitro, as autophagosomes are commonly observed in dying cells.[55,134-143] However, it is often unclear whether autophagy plays an active role in the cell death

Table 2. Ongoing clinical trials exploring autophagy inhibition for cancer treatment

Cancer type	Primary treatment *Autophagy inhibitor*	Study phase	Sponsor	Clinical trial identifier	Title
Advanced solid tumors	Sunitinib *HCQ*	I	CINJ	NCT00813423	Sunitinib and HCQ in advanced solid tumors
	Temozolomide *HCQ*	I	U Penn	NCT00714181	Temozolomde and HCQ in metastatic or unresectable solid tumors
	Temsirolimus *HCQ*	I	U Penn	NCT00909831	Temsirolimus and HCQ in refractory solid tumors
	Vorinostat *HCQ*	I	U Texas HSC S Ant	NCT01023737	Vorinostat and HCQ in advanced solid tumors
Breast cancer	Ixabepilone *HCQ*	I/II	CINJ	NCT00765765	Ixabepilone and HCQ in metastatic breast cancer
CML	Imatinib mesylate *HCQ*	II	U Glasgow	NCT01227135	Imatinib mesylate ± HCQ in CML in major cytogenetic response with residual disease
CLL	*HCQ*	II	NSLIJHS	NCT00771056	HCQ in previously untreated, asymptomatic B-CLL
Colorectal cancer	Capecitabine Oxaliplatin Bevacizumab *HCQ*	II	CINJ	NCT01006369	XELOX, bevacizumab and HCQ in metastatic colorectal cancer
	5-FU/leucovorin Oxaliplatin Bevacizumab *HCQ*	I/II	U Penn	NCT01206530	FOLFOX, bevacizumab and HCQ in colorectal cancer
DCIS	Tamoxifen *CQ*	I/II	Inova Health Care Serv	NCT01023477	Neoadjuvant tamoxifen, tamoxifen + CQ, or CQ in DCIS
Glioblastoma multiforme	Radiation Temozolomide *HCQ*	I/II	NCI	NCT00486603	Adjuvant radiation, temozolomide and HCQ in newly resected GBM
	CQ	III	NINN, Mexico	NCT00224978	Adjuvant CQ versus placebo in glioblastoma
Lung cancer, non-small cell	Carboplatin Paclitaxel Bevacizumab *HCQ*	I/II	CINJ	NCT00933803	Carboplatin, paclitaxel, bevacizumab and HCQ in advanced or recurrent NSCLC
	Erlotinib *HCQ*	II	Mass Gen Hosp	NCT00977470	Erlotinib ± HCQ in previously untreated metastatic NSCLC with EGFR mutations
	Gefitinib *HCQ*	I/II	Nat U Hosp, Singapore	NCT00809237	Gefitinib and HCQ in metastatic NSCLC
Lung cancer, extensive small cell	Cisplatin Etoposide *CQ*	I/II	Maastricht Rad Onc	NCT00969306	Cisplatin, etoposide and escalating CQ in extensive disease SCLC
Lung cancer, limited small cell	RT Cisplatin Etoposide *CQ*	I/II	Maastricht Rad Onc	NCT00969306	RT, cisplatin, etoposide and escalating CQ in limited disease SCLC
Melanoma	*HCQ*	0	CINJ	NCT00962845	Neoadjuvant HCQ in stage III or IV respectable melanoma
Multiple myeloma	Bortezomib *HCQ*	I/II	U Penn	NCT00568880	Bortezomib and HCQ in relapsed or refractory multiple myeloma
Pancreatic cancer	Gemcitabine *HCQ*	I/II	U Pittsburgh	NCT01128296	Neoadjuvant gemcitabine and HCQ in Stage IIb or III pancreatic cancer
Prostate cancer	*HCQ*	II	CINJ	NCT00726596	HCQ in patients with rising PSA after local prostate cancer treatment

Table 2. Ongoing clinical trials exploring autophagy inhibition for cancer treatment (continued)

	Docetaxel *HCQ*	II	CINJ	NCT00786682	Docetaxel and HCQ in metastatic prostate cancer
Renal cell cancer	*HCQ*	I	U Pittsburgh	NCT01144169	Neoadjuvant HCQ in renal cell carcinoma

Table 3. Preclinical studies supporting autophagy induction for cancer treatment

Cancer type	Primary treatment (target)	Autophagy induction method (target)	References
*Breast cancer	Doxorubicin (DNA)	RNAi (BCL-2)	184
	Nelfinavir (HIV protease)	Tamoxifen (estrogen receptor)	185
	RT (DNA)	Z-VAD (caspases)	186
AML	MGCD0103, vorinostat (histone deacetylation)	GX15-070 (BCL-2)	187
*Colorectal cancer	RT (DNA)	BCG (immune system)	188
Glioma, malignant	RT (DNA)	Arsenic trioxide (thioredoxin reductase)	189
	Delta-24-RGD (DNA)	RAD001 (mTOR)	190
*	OBP-405	RAD001 (mTOR)	191
Lung cancer, non-small cell	RT (DNA)	ABT-737 (BCL-2) rapamycin (mTOR)	192
	RT (DNA)	Z-VAD (caspases)	186
	RT (DNA)	Berberine (angiogenesis, COX-2, TNF)	193
Lymphoma, mantle cell	Vorinostat (histone deacetylation)	Temsirolimus (mTOR)	194
*Renal cell cancer, VHL-deficient	STF-62247 (ER-Golgi trafficking)	STF-62247 (ER-Golgi trafficking)	145
*Pancreatic cancer	Sorafenib (multiple kinases) + HDACi (histone deacetylation)	GX15-070 (BCL-2)	195
Sarcoma, fibro-	RT (DNA)	Arsenic trioxide (thioredoxin reductase)	196
*Thyroid cancer, papillary	Doxorubicin, RT (DNA)	RAD001 (mTOR)	197

*Studies where genetic deletion or knockdown of autophagy regulator(s) prolonged cancer cell survival.

process or it is a mere bystander representing the stressed cell's futile attempts to preserve viability by upregulating an energy and amino acid recycling program. Thus, the presence of autophagosomes in dying cells is not necessarily synonymous with cell death by autophagy, unless knockdown of essential autophagy regulators prolongs cell survival under the same stress conditions.[144] For example, the autophagy inducer STF-62247 killed VHL-deficient renal cancer cells in association with autophagosome accumulation and reduction of autophagy regulator levels compromised sensitivity to this drug.[145]

The implication of autophagy as a cell death mechanism in tumors with inactivated apoptosis is not surprising, given that cells with functional apoptosis undergo a rapid and 'clean' (not associated with inflammation) apoptotic cell death when severely stressed, making observation, and thus study, of autophagy in an apoptosis-competent background difficult. [99,111] Disabled apoptosis is a frequent occurrence in cancer, thus tumor cells under extreme stress often die by other mechanisms, as already mentioned. However, the conditions-beyond apoptosis inactivation-under which autophagy functions as a primary cell death mechanism remain to be defined; such knowledge will be critical for the rational design and targeted application of therapeutic regimens exploiting autophagy induction for more effective tumor cell killing. **Table 3** lists studies where autophagy inducers potentiated the anticancer effect of other treatment modalities, such as chemotherapy and/or radiation.

Autophagy Modulation for Cancer Prevention

In the long run, the most effective (and also the least expensive) way to treat cancer is to prevent it from arising in the first place. Cancer prevention strategies must be accessible, easily implemented, well tolerated and safe over time. Accumulation of protein aggregates due to defective autophagy is a well-recognized culprit in neurodegeneration, including Alzheimer's, Parkinson's and polyglutamine diseases, and in hepatic dysfunction, but its contribution to cancer development and progression was not so clear.[146-148] Recent studies indicated that protein aggregation in autophagy-defective tumor cells is a likely source of genotoxic stress, mostly due to the resultant ER and oxidative stress, in turn contributing to the genomic damage and instability, and thus increased tumorigenicity, associated with autophagy defects.[100-102,149] Since basal autophagy plays a critical role in cellular homeostasis by degrading aged or malfunctioning organelles and damaged or misfolded proteins, thus maintaining genome integrity, and as such exhibiting a tumor suppressive effect, it is reasonable to target autophagy for cancer prevention. Autophagy stimulators are currently being explored as preventative agents in neurodegeneration;[150] their predicted role in cancer prevention is also worthy of investigation and has potentially significant clinical implications, as supported by accumulating scientific evidence.

For example, cancer risk highly correlates with age.[151] Caloric restriction (CR) involving limited food intake and physical exercise

induces autophagy and delays the aging process, thus extending lifespan and possibly inhibiting tumorigenesis. The longevity resulting from CR was further validated in *Tp53*$^{-/-}$ mice, rhesus monkeys, yeast, *Caenorthabditis elegans* and Drosophila.[151-157] Thus, CR may be one way to modulate autophagy for cancer prevention. However, this hypothesis is far from clinical application, as it still needs to be extensively investigated and ultimately validated in humans. Diet-associated autophagy upregulation may be another approach to cancer prevention. Autophagy induction by carotenoids, lycopene, lutein, polyphenols, resveratrol, curcumin and epigallocatechin-3-gallate has been implicated in the ability of these dietary compounds to inhibit ROS and reactive nitrogen species accumulation.[158,159] Pharmacological activation of autophagy by drugs, such as rapamycin analogs, class I PI3K inhibitors, chloroquine and metformin, has also been reported to inhibit malignant transformation, and thus likely prevent cancer, by limiting genomic damage.[160-162] The possibility of preventing cancer by autophagy modulation is intriguing, but clearly in need of further investigation, as the most effective and safest way to manipulate autophagy for cancer prevention remains to be defined.

Concluding Remarks

The role of autophagy in cancer and treatment responsiveness is undoubtedly complicated. The double-edged sword function of autophagy, as both a tumor suppressor and a protector of cancer cell survival, likely impacts anticancer treatment efficacy in opposing ways. Exploitation of the functional autophagy status in tumors and pharmacologic autophagy modulation for cancer treatment and prevention presents novel opportunities in cancer management. Autophagy defects are associated with susceptibility to metabolic stress, DNA damage accumulation, genomic instability and accelerated tumorigenicity. It is, thus, reasonable to hypothesize that autophagy upregulation may preserve cellular fitness and genome integrity to prevent cancer development and progression; chronically autophagy-deficient tumors may also be particularly sensitive to genotoxic and/or metabolic stress-inducing anticancer agents, such as DNA damaging and anti-angiogenic drugs. On the other hand, autophagy-competent tumors (even if their autophagy potential is partially compromised due to cancer-associated cellular events, such as constitutive PI3K/AKT/mTOR axis activation) likely utilize, and potentially rely on, this pathway for survival under metabolic stress conditions, such as during rapid tumor growth, metastasis and treatment. In the latter case, concurrent (i.e., acute) autophagy inhibition is expected to increase the efficacy of any anticancer modality, including radiation, chemotherapy, biological agents and combinatorial regimens.

In conclusion, the multifaceted nature of autophagy and its diverse crosstalk with other biological processes, including cell death pathways, must be carefully considered when the autophagic system is targeted for anticancer benefit. Areas of great interest in cancer research and with potentially significant therapeutic implications include autophagy regulation in tumor cells, impact of autophagy functional status in tumors on cancer progression and response to treatment, and elucidation of how to best modulate autophagy for therapeutic benefit and cancer prevention, so as to achieve the ultimate goal of cancer eradication.

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