

Review

Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment

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Induction of cell death and inhibition of cell survival are the main principles of cancer therapy. Resistance to chemotherapeutic agents is a major problem in oncology, which limits the effectiveness of anticancer drugs. A variety of factors contribute to drug resistance, including host factors, specific genetic or epigenetic alterations in the cancer cells and so on. Although various mechanisms by which cancer cells become resistant to anticancer drugs in the microenvironment have been well elucidated, how to circumvent this resistance to improve anticancer efficacy remains to be defined. Autophagy, an important homeostatic cellular recycling mechanism, is now emerging as a crucial player in response to metabolic and therapeutic stresses, which attempts to maintain/restore metabolic homeostasis through the catabolic lysis of excessive or unnecessary proteins and injured or aged organelles. Recently, several studies have shown that autophagy constitutes a potential target for cancer therapy and the induction of autophagy in response to therapeutics can be viewed as having a prodeath or a prosurvival role, which contributes to the anticancer efficacy of these drugs as well as drug resistance. Thus, understanding the novel function of autophagy may allow us to develop a promising therapeutic strategy to enhance the effects of chemotherapy and improve clinical outcomes in the treatment of cancer patients.

Cell Death and Disease (2013) 4, e838; doi:10.1038/cddis.2013.350; published online 10 October 2013

Subject Category: Cancer

Facts

- The induction of autophagy in response to metabolic and therapeutic stresses can have a prodeath or a prosurvival role, which contributes to the anticancer efficacy of these drugs as well as drug resistance.
- Anticancer drugs induce different effects of autophagy on cell survival in different cancer types.
- Autophagy as a prosurvival and resistance mechanism against chemotherapy treatment.
- Autophagy-mediated cell death mechanism contributes to efficacy of anticancer drugs.
- Targeting autophagy will hopefully provide a promising therapeutic strategy to circumvent resistance and enhance the effects of anticancer therapies for cancer patients.

Open Questions

- Whether we should try to enhance or inhibit autophagy in cancer treatment?
- Chloroquine and its derivative: just act as autophagy inhibitors?
- Autophagy is shown to precede apoptosis or act in parallel with this cellular process in addition to be an alternative mechanism to cell death when apoptosis is inhibited. Therefore, the autophagy induction may exert other possibilities, which should be considered in the design of new treatments for the malignancies.

Resistance to anticancer drugs is a common clinical issue in the treatment of patients with cancer. Drug resistance,

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Keywords: autophagy; chemotherapy resistance; cancer; therapy

Abbreviations: PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin; AMPK, AMP-activated protein kinase; ER, endoplasmic reticulum; eIF2 α , eukaryotic initiation factor 2 α ; HDAC, histone deacetylase; DDR, DNA damage response; CQ, chloroquine; HCQ, hydroxychloroquine; EPI, epirubicin; 5-FU, 5-fluorouracil; MAPK, mitogen-activated protein kinase; GBM, glioblastoma; TMZ, temozolomide; MTD, maximum tolerated dose; HCC, hepatocellular carcinoma; MCL, mantle cell lymphoma; DAMP, damage-associated molecular pattern; B-CLL, B-chronic lymphocytic leukemia; EGF, epidermal growth factor; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; TPT, topotecan; PDAC, pancreatic adenocarcinoma; HDIL-2, high-dose interleukin-2; NAC1, nucleus accumbens-1; 3-MA, 3-methyladenine; MPNST, malignant peripheral nerve sheath tumor; BITC, benzyl isothiocyanate; LMP, lysosomal membrane permeabilization; MMP, mitochondrial membrane potential; SAHA, suberoylanilide hydroxamic acid; BL, Burkitt's lymphoma

Received 08.5.13; revised 25.8.13; accepted 27.8.13; Edited by GM Fimia

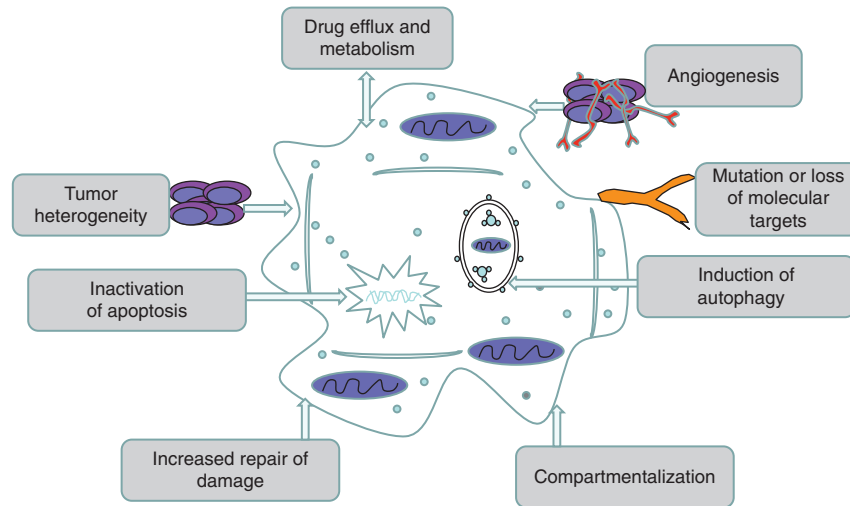


Figure 1 A summary of the approaches by which cancer cells become resistant to chemotherapy and various kinds of genotoxic or metabolic stresses

intrinsic or acquired, can be attributed to a wide variety of mechanisms including tumor cell heterogeneity, drug efflux and metabolism and tumor microenvironment stress-induced genetic or epigenetic alterations as a cellular response to drug exposure (Figure 1).^{1,2} Among these mechanisms, the response or adaptation of cancer cell itself to anticancer drug-induced tumor microenvironment stresses is a vital cause for chemotherapy resistance.

Autophagy is an evolutionarily conserved catabolic process in which portions of cytosol and organelles are sequestered into a double-membrane vesicle and delivered to the lysosome for bulk degradation.^{3–6} In this review, the term ‘autophagy’ refers to macroautophagy. The role of autophagy in regulating cancer cell death or survival remains controversial. Current evidence supports the idea that constitutive autophagy can act as a cellular housekeeper to eliminate damaged organelles and recycle macromolecules, thus protecting against cancer, particularly during malignant transformation and carcinogenesis. In established tumors, autophagy can function as a prosurvival pathway in response to metabolic stresses such as nutrient deprivation, hypoxia, absence of growth factors and the presence of chemotherapy or some targeted therapies that might mediate resistance to anticancer therapies.^{7–9} In this review we will summarize the possible role of autophagy as a novel target for anticancer therapies and discuss the attractive prospect of manipulating this control as a revolutionary strategy for cancer therapy.

The Regulation of Autophagy in Cancer During Response to Multiple Stresses

Autophagy is essential for not only cell survival but also organism survival in response to microenvironmental stresses. When cancer cells are subjected to stressful conditions, autophagy is rapidly upregulated to maintain metabolic homeostasis and ensure that cell growth is appropriate to its changing environmental conditions through reduced growth and increased catabolic lysis of excessive or unnecessary proteins and organelles. However, persistent or excessive

autophagy is also shown to promote cell death following treatment with specific chemotherapeutic agents, either by enhancing the induction of apoptosis or mediating ‘autophagic cell death’.

Although the molecular mechanisms whereby autophagy mediates its effects on both normal and cancer cells are far from complete, various signaling pathways have been implicated in the upregulation or downregulation of autophagy.^{10,11} The phosphatidylinositol 3-kinase/mammalian target of rapamycin (PI3K/mTOR) and AMP-activated protein kinase (AMPK) signaling pathways have emerged as the central conduit in the regulation of autophagy (Figure 2). mTOR can be activated by growth factors signal through the class I PI3K/Akt pathway, and inhibited by AMPK and p53.^{12,13} Once activated, mTOR exerts a negative effect on autophagy by phosphorylating a complex of autophagy proteins (ULK1/2), which inhibits the downstream autophagy cascade.^{14,15} In contrast, AMPK can suppress mTORC1 signaling to stimulate autophagy through TSC1/2 phosphorylation.^{16,17} Several of the known tumor-suppressor genes (*p53*, *PTEN*, *TSC1/TSC2*) and tumor-associated genes (*p21*, *AKT*) also respectively stimulate or inhibit autophagy.^{10,15}

Autophagy is also induced by a variety of metabolic stresses such as endoplasmic reticulum (ER) stress, hypoxia, oxidative stress, expression of aggregate-prone proteins, glucose deprivation and so on.¹⁸ ER stress stimulates autophagy through the PERK/eukaryotic initiation factor 2 α (eIF2 α) and IRE1/JNK1 pathways. PERK/eIF2 α phosphorylation has been shown to be essential for the transcription of key autophagy-associated genes during ER stress and may mediate the polyglutamine-induced LC3 conversion.¹⁹ The activation of IRE1/JNK promotes phosphorylation of Bcl-2 and p53, resulting in interfering with Bcl-2 binding to Beclin 1 and autophagic cell death in cancer cells.²⁰ Depletion of nutrients or energy induces autophagy by activating the AMPK pathway or promoting upregulate transcription of certain autophagy genes.^{16,17} The MEK/ERK signaling activation and Rag inactivation contribute to amino acid depletion-induced autophagy.^{7,21} Many anticancer drugs including

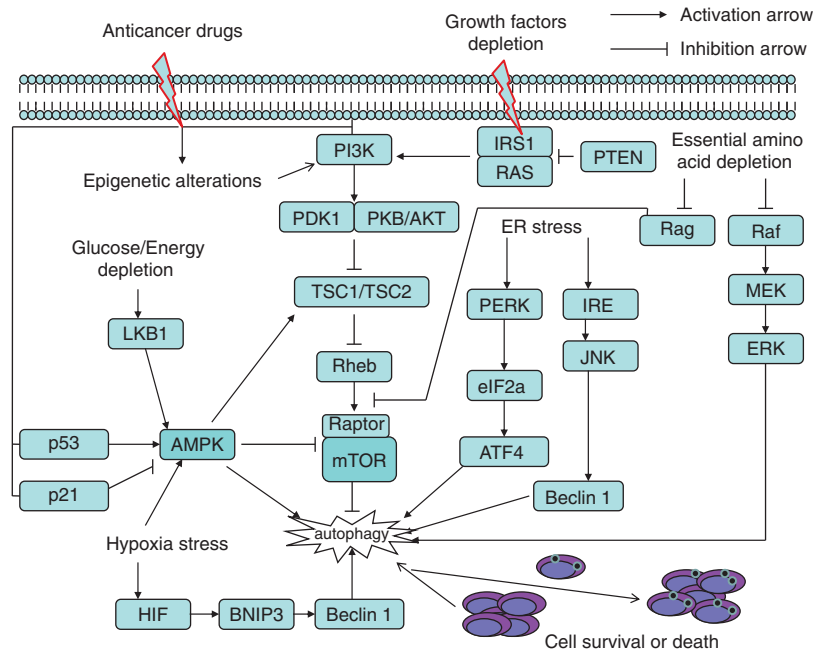


Figure 2 Interrelations between autophagy-related signaling and cell growth control in response to stress. Autophagy can be activated in response to multiple stresses during cancer progression, including nutrient deprivation, endoplasmic reticulum stress, hypoxia, glucose/energy depletion, chemotherapy and other diverse stresses. The AMPK/mTOR pathway functions as a central conduit for autophagic signaling pathways to promote cell survival or death

novel targeted therapies stimulate autophagy by inhibiting the PI3K/Akt/mTOR axis or altering genetic/epigenetic phenotype of cancer cells, which provides a survival advantage for struggling tumor cells.^{22–24} The histone deacetylase (HDAC) inhibitors are recently involved in the control of DNA damage response (DDR) and autophagy. SD118-xanthocillin X (1), a novel marine agent extracted from *Penicillium commune*, induces autophagy through the inhibition of the MEK/ERK pathway.²⁵ Overall, autophagy is a cell biological process that involves diverse signals that have overlapping functions in autophagy and the control of other cellular stress responses.

Autophagy in Response to Chemotherapy

Similar to its potential to either induce cell death or promote cell survival, a growing body of evidence implicates a paradoxical role of autophagy following anticancer treatments, with response increasing or diminishing their anticancer activity. On the one hand, autophagy is activated as a protective mechanism to mediate the acquired resistance phenotype of some cancer cells during chemotherapy. Thus, the inhibition of autophagy can re-sensitize previously resistant cancer cells and augment cytotoxicity of chemotherapeutic agents. On the other hand, autophagy may also play as a death executioner to induce autophagic cell death, a form of physiological cell death which is contradictory to type I programmed cell death (apoptosis) (Figure 3). Based on current genetic and pharmacological studies, it appears that anticancer drugs induce different effects of autophagy on cell survival in different cancer types (Table 1). Here we delineate the possible role of autophagy as a novel target for anticancer therapy.

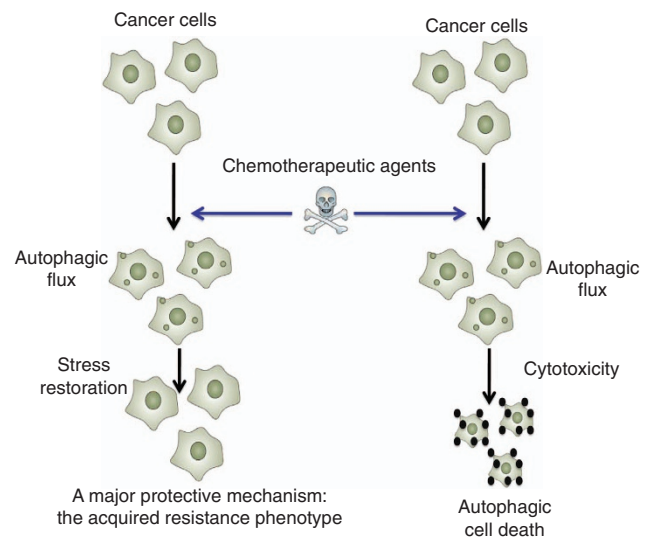


Figure 3 Dual role of autophagy for therapeutic purposes in cancer. On one hand, autophagy is activated as a protective mechanism to mediate the acquired resistance phenotype of some cancer cells during chemotherapy. On the other hand, autophagy may also function as a death executioner to induce autophagic cell death, a form of physiological cell death that is contradictory to apoptosis

Autophagy as a Prosurvival and Resistance Mechanism Against Chemotherapy Treatment

Recent studies have demonstrated that tumor resistance to anticancer therapies including radiation therapy, chemotherapy and targeted therapies can be enhanced through upregulation of autophagy in different tumor cell lines.^{26,27} Moreover, increasing evidence suggests that autophagy inhibition

Table 1 Autophagy in response to chemotherapy in different types of cancers

Class	Target	Type of cancer	Autophagy role	Method used to evaluate autophagy	References
<i>Autophagy inducers</i>					
Aurora kinase A	mTOR	Breast	Prosurvival	siRNA (LC3, Atg5) CQ	27
Suberoylanilide hydroxamic acid (SAHA)	HDAC inhibitor	CML	Prosurvival	Bafilomycin A 3-MA Bafilomycin A	29
Epirubicin (EPI)	Anthracyclines	Breast Breast	Prodeath Prosurvival	3-MA siRNA (Beclin 1, Atg7) Bafilomycin A	97 34
5-Fluorouracil	Thymidylate synthase inhibitor	Colorectal	Prosurvival	siRNA (Atg7) 3-MA	37,40–42
Atorvastatin	AMPK	Digestive malignancies	Prodeath Prosurvival	3-MA siRNA (Atg5) Bafilomycin A	96 38
Irinotecan	MAPK14/p38 α	Colorectal	Prosurvival	siRNA (Atg5, Atg7) Bafilomycin A 3-MA	39
Cisplatin	Genotoxic stress	Esophageal	Prosurvival	3-MA	43
Oxaliplatin	Genotoxic stress	Hepatocellular carcinoma	Prosurvival	siRNA (Atg5) CQ 3-MA	48
Bevacizumab	Angiogenesis inhibitor	Hepatocellular carcinoma	Prosurvival	CQ 3-MA	49
Sorafenib	ER stress	Hepatocellular carcinoma	Prosurvival	CQ	50
High-mobility group box 1 protein (HMGB1)	Genotoxic stress DAMP molecule	CML	Prodeath Prosurvival	siRNA (Beclin 1)	104 52
Gefitinib or Erlotinib	EGFR tyrosine kinase inhibitor	Lung	Prosurvival	siRNA (Atg5, Atg7) CQ 3-MA	55,58
Topotecan	Genotoxic stress	Lung	Prosurvival	CQ	56
RAGE	Genotoxic or metabolic stress	Pancreatic	Prosurvival		59
NVP-BE235	PI3K/AKT/mTOR inhibitor	Renal	Prosurvival		62
Ursolic acid	Genotoxic stress	Urothelial Prostate Cervical	Prodeath Prosurvival Prodeath	CQ siRNA (Atg5, Beclin 1) 3-MA siRNA (Atg5) Wortmannin	98 64 100
Imatinib	Tyrosine kinase inhibitor	Glioma	Prosurvival	Bafilomycin A RTA 203	71
FK-16	Fragment of LL-37	Colon	Prodeath	siRNA (Atg5, Beclin 1) 3-MA	101
Temozolomide	Genotoxic stress	Glioblastoma	Prodeath	siRNA (Bax, Bcl-2)	102
Mono-Pt	Genotoxic stress	Ovarian	Prodeath	mTOR inhibitor RAD001 siRNA (Atg7, Beclin 1) 3-MA CQ	103
Cannabinoids	ER stress AMPK	Glioma Pancreatic Hepatocellular carcinoma	Prodeath Prodeath Prodeath	Bafilomycin A siRNA (Atg1) 3-MA CQ siRNA (Atg5) 3-MA	105 106 108
<i>Autophagy inhibitors</i>					
CQ	Lysosomotropic agent	Breast	Prosurvival		35
HCQ		Esophageal Hepatocellular carcinoma	Prosurvival Prosurvival		43–45 50
		Lung	Prosurvival		56,57
		Pancreatic	Prosurvival		60

augments cytotoxicity in combination with several anticancer drugs in preclinical models.^{28–30} Several pharmacological compounds and strategies have been reported to inhibit autophagy *in vitro* and *in vivo*.

Antimalarial Drugs

The only autophagy inhibitors whose effectiveness *in vivo* and safety in clinical trials have been approved by the FDA are

chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) that suppress autophagy by blocking autophagosome fusion and degradation.^{31,32} Both CQ and HCQ have been investigated in preclinical studies or clinical trials. In comparison with CQ, HCQ can be safely dose escalated in cancer patients.³³ Currently, more than 30 phase I/II cancer clinical trials (<http://clinicaltrials.gov/>) involving CQ or HCQ are open around the world and many of them have evidence of preliminary antitumor activity (Table 2).

Table 2 Active clinical trials combining the autophagy inhibitor HCQ with anticancer therapies

Identifier	Cancer type	Drugs	Phase	Title
NCT00969306	NSCLC	CQ + cisplatin Etoposide	I/II	Cisplatin, etoposide and escalating CQ in extensive disease SCLC
NCT00809237	NSCLC	HCQ + gefitinib	I/II	Hydroxychloroquine and gefitinib to treat lung cancer
NCT01649947	NSCLC	HCQ + paclitaxel and carboplatin	II	Modulation of autophagy in patients with advanced/recurrent non-small-cell lung cancer – phase II
NCT00977470	Advanced NSCLC and (EGFR) mutations	HCQ + erlotinib	II	Erlotinib with or without hydroxychloroquine in chemo-naïve advanced NSCLC and (EGFR) mutations
NCT00933803	Advanced or recurrent NSCLC	HCQ + carboplatin, paclitaxel, bevacizumab	II	Carboplatin, paclitaxel, bevacizumab and HCQ in advanced or recurrent NSCLC
NCT01292408	Breast cancer	HCQ	II	Autophagy inhibition using hydroxychloroquine in breast cancer patients
NCT00765765	Breast cancer	HCQ + ixabepilone	I/II	Ixabepilone and HCQ in metastatic breast cancer
NCT01023477	DCIS	CQ + tamoxifen	I/II	Neoadjuvant tamoxifen, tamoxifen + CQ, or CQ in DCIS
NCT01510119	Renal cell carcinoma	HCQ and RAD001 (p.o. 10 mg/day)	I/II	Autophagy inhibition to augment mTOR inhibition: a phase I/II trial of RAD001 and hydroxychloroquine in patients with previously treated renal cell carcinoma
NCT01144169	Renal cell carcinoma	HCQ + high dose interleukin-2 and other systemic therapies	I	Study of hydroxychloroquine before surgery in patients with primary renal cell carcinoma
NCT01550367	Renal cell carcinoma	HCQ + IL-2	I/II	Study of hydroxychloroquine and aldesleukin in renal cell carcinoma patients (RCC)
NCT00726596	Prostate cancer	HCQ	II	Hydroxychloroquine in treating patients with rising PSA levels after local therapy for prostate cancer
NCT01128296	Pancreatic cancer	HCQ + gemcitabine	I/II	Study of presurgery gemcitabine + hydroxychloroquine (GcHc) in stage IIb or III adenocarcinoma of the pancreas
NCT01273805	Pancreatic cancer	HCQ	II	Hydroxychloroquine in previously treated patients with metastatic pancreatic cancer
NCT01506973	Pancreatic cancer	HCQ + gemcitabine/abraxane	I/II	A phase I/II/pharmacodynamic study of hydroxychloroquine in combination with gemcitabine/abraxane to inhibit autophagy in pancreatic cancer
NCT01128296	Pancreatic cancer	HCQ + gemcitabine	I/II	Study of Pre-surgery Gemcitabine + hydroxychloroquine (GcHc) in stage IIb or III adenocarcinoma of the pancreas
NCT01494155	Pancreatic cancer	HCQ + capecitabine + proton radiation	II	Short-course radiation therapy with proton beam capecitabine and hydroxychloroquine for resectable pancreatic cancer
NCT01206530	Colorectal cancer	HCQ + FOLFOX/bevacizumab	I/II	FOLFOX/Bevacizumab/Hydroxychloroquine (HCQ) in colorectal cancer
NCT01006369	Metastatic colorectal cancer	HCQ + capecitabine, oxaliplatin, and bevacizumab	II	Hydroxychloroquine, capecitabine, oxaliplatin, and bevacizumab in treating patients with metastatic colorectal cancer
NCT00224978	Glioblastoma	CQ	III	Adjuvant CQ <i>versus</i> placebo in glioblastoma
NCT00486603	Glioblastoma	HCQ + temozolomide	I/II	Adjuvant radiation, temozolomide and HCQ in newly resected GBM
NCT00962845	Melanoma	HCQ	No phase specified	Hydroxychloroquine in patients with stage III or Stage IV melanoma that can be removed by surgery
NCT00568880	Multiple myeloma	HCQ + bortezomib	I/II	Hydroxychloroquine and bortezomib in treating patients with relapsed or refractory multiple myeloma
NCT01480154	Advanced solid tumors or prostate or renal cancer	HCQ + MTD of Akt inhibitor MK2206 (MK-2206)	I	Phase I study of Akt inhibitor MK2206 and hydroxychloroquine in patients with advanced solid tumors or prostate or renal cancer
NCT00909831	Metastatic solid tumors	HCQ + temsirolimus	I	Hydroxychloroquine and temsirolimus in treating patients with metastatic solid tumors that have not responded to treatment
NCT00813423	Advanced solid tumors	HCQ + sunitinib	I	Sunitinib and Hydroxychloroquine in treating patients with advanced solid tumors that have not responded to chemotherapy
NCT01023737	Advanced solid tumors	HCQ + vorinostat	I	Vorinostat and HCQ in advanced solid tumors
NCT01417403	Solid tumors undergoing radiation therapy for bone metastases	HCQ	I	Hydroxychloroquine in treating patients with solid tumors undergoing radiation therapy for bone metastases
NCT01266057	Advanced cancer	HCQ + the highest tolerable dose of sirolimus or vorinostat	I	Sirolimus or vorinostat and hydroxychloroquine in advanced cancer
NCT00714181	Metastatic or unresectable solid tumors	HCQ + temozolomide	I	Hydroxychloroquine and temozolomide in treating patients with metastatic or unresectable solid tumors
NCT01227135	CML	HCQ + imatinib	II	Imatinib mesylate with or without hydroxychloroquine in treating patients with chronic myeloid leukemia
NCT01634893	Ovarian cancer	HCQ + sorafenib	I	Oral hydroxychloroquine plus oral sorafenib to treat epithelial ovarian cancer FIGO stage III or stage IV, or extraovarian peritoneal carcinoma, or fallopian tube carcinoma failing or ineligible for first-line therapy

NSCLC, non-small-cell lung cancer; CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; MTD, maximum tolerated dose; HCQ, hydroxychloroquine

Breast cancer. The role of autophagy in breast cancer is an area of active investigation. There is evidence to suggest that epirubicin (EPI) may induce autophagy in human breast cancer MCF-7 cells, resulting in protecting MCF-7 cells from EPI-induced apoptosis.³⁴ Autophagy is also regarded as a key mechanism of antiestrogen resistance, and blocking autophagosome can significantly reduce the emergence of antiestrogen-resistant breast cancer cells.³⁵ A phase II clinical trial (NCT01292408) is investigating the effects of autophagy inhibition via HCQ on breast cancer patients. The current reports indicate that CQ or HCQ is often used in combination with chemotherapeutic drugs to enhance the efficacy of tumor cell killing; however, its sensitizing effects can also occur independently of autophagy inhibition, which should be considered in the ongoing clinical trials where CQ or HCQ are used in the treatment of breast cancer.³⁶

Colorectal cancer. So far, 5-fluorouracil (5-FU), together with other drugs such as oxaliplatin, remains a widely used chemotherapeutic drug in the treatment of a variety of colorectal carcinomas. Previous researches have demonstrated that inhibition of autophagy augments anticancer effects of chemotherapy or some targeted therapies in colorectal cancer.^{37,38} Recently, it has been shown that mitogen-activated protein kinase 14 (MAPK14)/p38 α is involved in resistance of colon cancer cells to 5-FU and irinotecan, which triggers survival-promoting autophagy to protect tumor cells against the cytotoxic effects of these drugs.^{39,40} Furthermore, autophagy inhibitor CQ significantly enhances the 5-FU-induced inhibition of tumor growth both *in vitro* and *in vivo*.^{41,42} In addition, the combination of FOLFOX/bevacizumab with HCQ is currently being investigated.

Esophageal cancer. The role of autophagy in response to chemotherapy and radiotherapy has been investigated in human esophageal squamous carcinoma cells. Autophagy might play a role as a self-protective mechanism in chemotherapeutic drug-treated esophageal cancer cells, and its inhibition has the potential to improve the efficacy of chemotherapeutic agents such as cisplatin and 5-FU.⁴³⁻⁴⁵ However, the effect of adding HCQ to conventional therapy for esophageal cancer patients remains unclear.

Glioblastoma. Malignant cell clones resistant to chemotherapy is a key reason for treatment failure in patients with (GBM). When treated with bevacizumab alone, human GBM xenografts show increased autophagic flux and hypoxia-associated growth, which indicates that hypoxia-mediated autophagy promotes tumor cell survival and resistance to antiangiogenic therapy. However, in treatment by combining with the autophagy inhibitor CQ, tumor growth is disrupted, which elucidates a novel mechanism of overcoming resistance to antiangiogenic therapy for GBM.⁴⁶ A randomized, double-blind, placebo-controlled trial examines the effect of adding CQ to conventional therapy for GBM. As a result, a median overall survival is 24 months for CQ-treated patients and 11 months for placebo-treated patients.³⁰ Although it is not statistically different because of the small sample size, the rate of death of patients receiving CQ is prominently

lower than controls. Another phase I/II trial concerning dose-limiting toxicities of HCQ with temozolomide (TMZ) and radiation for GBM patients was conducted.⁴⁷ The information about the antitumor activity of this combination is underway.

Hepatocellular carcinoma. The combination of autophagy inhibitor and chemotherapy or molecular-targeted therapies has been regarded as a promising therapeutic strategy in the treatment of hepatocellular carcinoma (HCC). Autophagy is functionally activated in HCC cell lines after oxaliplatin treatment, and suppression of autophagy enhances oxaliplatin-induced cell death.⁴⁸ Autophagy also contributes to HCC cell survival, and the combined treatment of an autophagy inhibitor and bevacizumab markedly inhibits the growth of HCC.⁴⁹ Moreover, the combination of sorafenib with CQ can generate more ER stress-induced cell death in HCC both *in vivo* and *in vitro*.⁵⁰ Therefore, autophagy inhibition may be a promising therapeutic strategy to enhance the effects of chemotherapy and improve clinical outcomes in the treatment of HCC.

Leukemia and MCL. Recently, it is reported that high-mobility group box 1 (HMGB1), a damage-associated molecular pattern (DAMP) molecule, contributes to chemotherapy resistance through the upregulation of autophagy in leukemia.^{51,52} Autophagy inhibitor HCQ is also shown to decrease cell viability of B-chronic lymphocytic leukemia (B-CLL) in a dose- and time-dependent manner.⁵³ The resistance to Akt/mTOR inhibitors such as everolimus (RAD001) is a significant clinical problem in relapsed mantle cell lymphoma (MCL) patients. Fortunately, pretreatment with HCQ can efficiently overcome this resistance, resulting in the activation of the mitochondrial apoptotic pathway.⁵⁴ These data illustrate a strategy of blocking activation of adaptive autophagy pathway to improve treatment outcomes in leukemia and MCL.

Lung cancer. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been widely used in patients with non-small-cell lung cancer (NSCL). Unfortunately, the efficacy of these drugs is limited because of natural or acquired resistance. We report that autophagy can be activated by gefitinib or erlotinib in lung cancer cells, which contributes to the acquired drug resistance of EGFR-TKIs.⁵⁵ Furthermore, the antimalaria drug CQ has been shown to enhance chemotherapy and radiation sensitivity in several preclinical models. CQ can not only potentiate the cytotoxicity of topotecan (TPT), but also substantially increase the effects of PI3K/mTOR inhibitor NVP-BEZ235 on induction of apoptosis, inhibition of colony formation and suppression of xenografts in nude mice.^{56,57} A phase I study in advanced NSCL patients with prior clinical benefit from EGFR-TKIs suggests that HCQ with or without erlotinib is safe, and the recommended phase II dose for HCQ with erlotinib 150 mg is 1000 mg daily.⁵⁸ Although no difference in survival was elaborated, this study explored the safety of adding HCQ to erlotinib. To assess the efficacy of the combination of HCQ with conventional chemotherapeutics, several clinical trials have been launched.

Table 3 The strategies for autophagy inhibition

Strategies	Target	The effect on autophagy
<i>Pharmacological approaches</i>		
Chloroquine	Lysosomal pH	Inhibit autophagosome fusion with lysosomes and autophagosome degradation
Hydroxychloroquine	Lysosomal pH	Inhibit autophagosome fusion with lysosomes and autophagosome degradation
Monensin	Change endocytic and lysosomal pH	Inhibit the initiation/expansion stage of autophagy
Bafilomycin A 1	Class III PI3K inhibitor	Inhibit the initiation/expansion stage of autophagy
3-Methyladenine	Class III PI3K inhibitor	Inhibit the initiation/expansion stage of autophagy
Wortmannin	Class III PI3K inhibitor	Inhibit the initiation/expansion stage of autophagy
LY294002	Class III PI3K inhibitor	Inhibit the initiation/expansion stage of autophagy
Pyrvinium	Class III PI3K inhibitor	Inhibit the initiation/expansion stage of autophagy
Genetic silencing of autophagy regulatory genes		Inhibit the initiation/expansion stage of autophagy

Pancreatic adenocarcinoma. The cytoprotective role of autophagy in response to chemotherapy has been confirmed in the treatment of pancreatic cancer cells.⁵⁹ In agreement with this, Mirzoeva *et al.*⁶⁰ showed that autophagy suppression with CQ promotes antitumor activity of PI3K/mTOR inhibitor for the treatment of pancreatic adenocarcinoma (PDAC) *in vitro* and *in vivo*. In addition to CQ, the efficacy and side effects of adding HCQ to conventional therapy are being investigated through several clinical trials.

Prostate cancer and renal cell carcinoma. Several recent studies have indicated that autophagy functions as a survival mechanism to promote chemoresistance in prostate and renal cancer cells.^{61,62} Autophagy activation protects against ER stress-induced cell death, whereas inhibition of autophagy significantly suppresses PC-3 prostate tumor growth *in vivo*.⁶³ Moreover, inhibition of autophagy by either HCQ or Beclin-1/Atg5 small interfering RNA enhances ABT-737 cytotoxicity and ursolic acid-induced apoptosis in prostate cancer cells.^{64,65} Administration of high-dose interleukin-2 (HDIL-2) has durable complete and partial responses in patients with metastatic renal cell carcinoma. However, HDIL-2 treatment is often limited by side effects, because of a cytokine-induced systemic autophagic syndrome. Liang *et al.*⁶⁶ found that the combination of IL-2 with CQ increases antitumor effects and decreases toxicity when compared with IL-2 treatment alone, which provide a novel clinical strategy to enhance the efficacy of HDIL-2 immunotherapy for patients with renal cell carcinoma. So far, two active trials (NCT 01144169 and NCT 01550367) are investigating the efficacy of adding HCQ to IL-2.

Ovarian cancer. Ovarian cancer has poor prognosis and is frequently resistant to chemotherapy. In this issue, it has been presented that autophagy may be a factor in drug resistance and poor survival in clear cell ovarian cancer patients.⁶⁷ Nucleus accumbens-1 (NAC1) can mediate resistance to cisplatin in ovarian cancer cell lines because of activation of autophagy.⁶⁸ FTY720, a sphingosine analog, may enhance autophagic flux when treated as a new chemotherapeutic agent for ovarian cancer, and blockade of autophagy aggravates necrotic ovarian cancer cell death in response to FTY720.⁶⁹ Now, the effect of combining HCQ with sorafenib is being assessed in FIGO stage III or stage IV ovarian cancer, or extraovarian peritoneal carcinoma, or fallopian tube carcinoma failing or ineligible for first-line therapy.

Other Compounds and Strategies

In addition to antimalarial drugs, inhibition of autophagy by either pharmacological approaches or via genetic silencing of autophagy regulatory genes such as *Beclin 1*, *ATG6*, *ATG5*, *ATG7* or *ATG12* (Table 3) also results in sensitization to a variety of therapeutic agents. Different autophagy inhibitors block the autophagic process at different stages. For example, antimalarial drugs or bafilomycin A1 can inhibit autophagosome fusion with lysosomes and autophagosome degradation in the final stage of autophagy. Class III PI3K inhibitors (3-methyladenine (3-MA), LY294002 and Wortmannin) or knockdown of autophagy regulatory genes are involved in the initiation/expansion stage of autophagy.^{70–73} So far, different autophagy inhibitors or genetic knockout of autophagy regulatory genes have been developed and used in the study of autophagy in cancer chemotherapy.^{74–78}

The Molecular Mechanisms of Protective Autophagy-Mediated Chemoresistance

Although many anticancer therapeutic strategies can induce autophagic cell death, a majority of pertinent studies have been conducted to indicate that autophagy is a protective mechanism associated with increased resistance to chemotherapy. Induction of autophagy has emerged as a drug resistance mechanism that promotes cancer cell survival. There are a number of different mechanisms through which the autophagy-related functions of promoting the survival of tumor cells under the treatment of anticancer drugs (Figure 4).

EGFR signaling. Epidermal growth factor is a key regulatory factor for cell survival. Through its binding to cell surface receptors, EGF can induce the activation of three signaling pathways which are important to the initiation and progression of cancers, Ras/MAPK, PI3K/Akt and JAK/STATs.⁷⁹ In the previous study, we confirmed biochemically and morphologically that autophagy can be activated by gefitinib or erlotinib, which was accompanied by the inhibition of the PI3K/Akt/mTOR signaling pathway. Furthermore, blockage of autophagy by the pharmacological inhibitors or gene silence greatly enhanced cytotoxicity of gefitinib or erlotinib.⁵⁵ PD168393, an EGFR-TKI, may induce autophagy as a cytostatic but not a cytotoxic response in malignant peripheral nerve sheath tumor (MPNST) cells that was accompanied by suppression of Akt and mTOR activation; moreover, suppression of autophagy by CQ increased caspase activation.⁸⁰

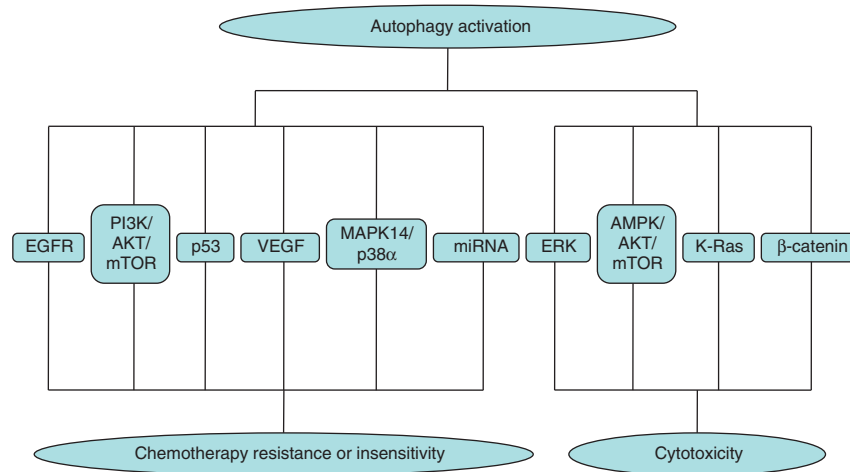


Figure 4 The molecular mechanisms of autophagy activation in response to chemotherapeutic agents. The activation of autophagy either leads to cancer cell chemoresistance via EGFR signaling, PI3K/AKT/mTOR pathways, p53, VEGF, MAPK14/p38 α signaling and microRNA or potentiates autophagic cell death through AMPK/AKT1/mTOR axis, which depends on the tumor types and treatment characteristic

These results indicate that EGFR-TKIs can induce autophagy to promote tumor cell survival in response to targeted chemotherapies, and suppression of autophagy can augment the growth inhibitory effect of these drugs through inhibition of the PI3K/Akt/mTOR signaling pathway.

PI3K/AKT/mTOR pathways. In addition to EGFR, the aberrant expression of PI3K/AKT/mTOR is also known to be a key regulator of autophagy. Genetic and pharmacologic autophagy blockade via PI3K/mTOR inhibition may reverse apoptotic resistance and result in significant cell apoptosis.⁸¹ NVP-BE235 (BEZ235) is a novel, orally bioavailable dual PI3K/mTOR inhibitor that has exerted a positive effect on autophagy. A recent study suggests that NVP-BE235 could induce apoptosis and autophagy; moreover, the combination treatment of NVP with autophagy inhibitors lead to enhanced RCC cell apoptosis.^{62,82} Benzyl isothiocyanate (BITC), a dietary chemopreventive agent, is also found to induce protective autophagy in human prostate cancer cells via inhibition of mTOR signaling pathway, and inhibition of autophagy using 3-MA increased BITC-induced apoptosis.⁸³ Taken together, targeting PI3K/AKT/mTOR-autophagy pathways displays a well-recognized contribution to overcome chemotherapy resistance and sensitize the tumor cells to anticancer therapy.

p53, VEGF and MAPK14/p38 α signaling. As a well-known tumor-suppressor gene, p53 is also involved in autophagic regulation. In a mouse model of c-Myc-driven lymphoma, inhibition of autophagy with either CQ or ATG5 shRNA promotes tumor cell death by p53 activation.⁸⁴ Stanton *et al.*⁸⁵ show that the VEGF-C/NRP-2 axis is involved in the activation of autophagy, and VEGF-C or NRP-2 depletion contributes to cytotoxic drug-mediated cell death. In addition, the recent studies have provided strong evidence that the MAPK14/p38 signaling is involved in cancer cell resistance to chemotherapy treatment. Paillas *et al.*³⁹ investigated the relationship between MAPK14/p38, autophagy and resistance to irinotecan and found that MAPK14/p38

was activated and triggered survival-promoting autophagy to protect tumor cells against the cytotoxic effects of irinotecan. Furthermore, p38MAPK activation is considered a key determinant in the cellular response to 5-FU by controlling the balance between apoptosis and autophagy.⁴⁰

MicroRNAs. MicroRNAs (miRNAs) are a class of small, noncoding, endogenously encoded, single-stranded RNAs that regulate gene expression at the post-transcriptional level. Recently, a number of miRNAs have been reported to be deeply involved in resistance or sensitization to chemotherapy.^{86,87} miR-30a, a member of miR-30 family, is a potent inhibitor of autophagy by selectively downregulating Beclin 1 and Atg5 expression. Targeting miR-30a promotes autophagy in response to imatinib treatment and enhances imatinib resistance against CML including primary stem and progenitor cells.^{88,89} Moreover, the blockade of autophagy by miR-30a expression or 3-MA significantly increased cis-DDP-induced apoptosis in cancer cells.⁹⁰ Downregulation of miR-199a-5p is observed in most HCC tissues of patients. More importantly, downregulation of miR-199a-5p increases cisplatin resistance by activating autophagy in HCC cells.⁹¹ Cisplatin-induced ATM-dependent phosphorylated (p)- Δ Np63 α also plays an important role in chemoresistance. Further research shows that the p- Δ Np63 α -induced miR-885-3p might contribute to regulation of apoptosis and/or autophagy in squamous cell carcinoma cells upon cisplatin exposure.⁹² Although the relationship between miRNA, autophagy and anticancer therapy resistance is quite complicated, and has not been well elucidated, miRNA may underlie key aspects of chemotherapy resistance.

Chloroquine and Its Derivative: Just Act as Autophagy Inhibitors?

CQ and its derivative are currently the only autophagy inhibitors available for clinical treatment of patients. In ongoing cancer treatment clinical trials, HCQ is often used in

combination with chemotherapeutic drugs, radiotherapy, some targeted therapies and immunotherapy. The treatment of CQ or HCQ can inhibit autophagy-related survival function and exert their anticancer action. However, emerging data indicate that the ability of CQ and its derivative to inhibit the final degradative step of autophagy may not be the only mechanism by which they exert anticancer action; CQ and HCQ may also affect other pathways such as lysosomal membrane permeabilization.

Recently, Maycotte *et al.*³⁶ reported that CQ could sensitize breast cancer cells to chemotherapy independent of autophagy inhibition, as sensitization was not mimicked by Atg12, Beclin 1 knockdown or bafilomycin A1 treatment, and occurred even in the absence of Atg12. In further studies, CQ and HCQ are shown to function as lysosomotropic agents to promote lysosomal membrane permeabilization (LMP), resulting in signs of apoptosis. The research from Enzenmüller *et al.*⁹³ shows that PI3K/mTOR inhibitor PI103 enhances the lysosomal compartment by increasing its volume and function, whereas CQ destabilizes lysosomal membranes. Together, CQ overcomes resistance of lung carcinoma cells to PI103-induced apoptosis by cooperating with PI103 to trigger lysosome-mediated apoptosis. An additional report suggests that induction of lysosome-mediated apoptosis rather than inhibition of autophagy is critical for the CQ-mediated sensitization to BEZ235-induced apoptosis, as lysosomal enzyme inhibitors significantly decrease BEZ235- and CQ-induced drop of mitochondrial membrane potential (MMP) and caspase-dependent apoptosis.⁹⁴ Similar to the potential of CQ, HCQ can also induce LMP and Bax/Bak-dependent MMP to trigger caspase activation.⁹⁵

Taken together, these data indicate that the success of clinical trials using CQ or HCQ combined with other anticancer agents might not be due to CQ or HCQ effects on autophagy induced by chemotherapeutic drugs, as the effect may be mediated by mechanisms other than its inhibition of autophagy. Thus, a better knowledge of the molecular mechanisms and cellular targets of CQ or HCQ should be considered in the ongoing clinical trials where CQ or HCQ are used as autophagy inhibitors.

Autophagy-Mediated Cell Death Mechanism Contributes to Efficacy of Anticancer Drugs

Despite its a clear prosurvival role, autophagy has also been viewed as having a prodeath role under certain circumstances and following treatment with a specific set of chemotherapeutic agents, either by enhancing the induction of apoptosis or mediating 'autophagic cell death'.

Increasing evidence supports that autophagy may mediate cell death in cancer cells which are apoptosis defective or hard to induce. Xiong *et al.*⁹⁶ found that autophagic cell death could be induced in PUMA- or Bax-deficient human colon cancer cells after the treatment with 5-FU, consequently followed by decreased cancer proliferation. Suberoylanilide hydroxamic acid (SAHA), a prototype of the newly developed HDAC inhibitor, induces autophagic cell death in tamoxifen-resistant MCF-7 breast cancer cells and significantly reduces the tumor growth *in vitro* and *in vivo*.⁹⁷ NVP-BEZ235 is demonstrated to inhibit cisplatin-resistant urothelial cancer cell proliferation by activating autophagic flux and cell cycle arrest, but not inducing apoptotic cell death.⁹⁸ The antidepressants

maprotiline and fluoxetine induce autophagic cell death in drug-resistant Burkitt's lymphoma (BL), which supports a new mechanistic role for maprotiline and fluoxetine as novel proautophagic agents in the treatment of resistant BL.⁹⁹ These data indicate that autophagic cell death can be induced as an alternative cell death mechanism when cells fail to undergo apoptosis.

In addition, induction of autophagic cell death is also an alternative approach to kill tumor cells without resistance to anticancer drugs. Ursolic acid promotes cancer cell death by inducing Atg5-dependent autophagy.¹⁰⁰ FK-16, derived from the anticancer peptide LL-37, induces caspase-independent apoptosis and autophagic cell death in colon cancer cells.¹⁰¹ The mTOR inhibitor RAD001 potentiates autophagic cell death induced by temozolomide in a GBM cell line.¹⁰² Novel monofunctional platinum (II) complex Mono-Pt induces apoptosis-independent autophagic cell death in human ovarian carcinoma cells, distinct from cisplatin.¹⁰³ Sorafenib and SC-59, which is a novel sorafenib derivative, induce autophagic cell death in hepatocellular carcinoma cells in a dose- and time-dependent manner.¹⁰⁴ Recently, cannabinoids have been shown to exert their anticancer activity in glioma, pancreatic cancer and hepatocellular carcinoma via stimulation of autophagy-mediated cell death.^{105–108} Moreover, the combination of cannabinoids and TMZ strongly activates autophagy-mediated cancer cell death, resulting in a strong antitumoral action in both TMZ-sensitive and TMZ-resistant tumors.¹⁰⁹ These studies present new insights into our understanding of the relationship between autophagy and anticancer efficacy and provide a potential therapeutic strategy for the management of some of these tumors.

Several genes and signal pathways contribute to autophagic cell death in cancer cells (Figure 4). The AMPK/AKT1/mTOR axis is critical for regulation of autophagic cell death. Tanshinone IIA induces autophagic cell death via activation of AMPK/ERK and inhibition of mTOR and p70 S6K in KBM-5 leukemia cells.¹¹⁰ Cannabinoids can trigger autophagic cell death in an ER stress and Akt/mTORC1-dependent manner.^{105–108} 4-Hydroxytamoxifen induces autophagic death through K-Ras degradation.¹¹¹ Diarylquinoline compounds induce a autophagic cell death by inhibiting the Akt pathway and increasing reactive oxygen species in human nasopharyngeal carcinoma cells.¹¹² β -Catenin is also involved in activation of autophagic cell death.¹¹³ These results further strengthen the connection between autophagy and anticancer efficacy.

Recently, autophagy has been shown to precede apoptosis or act in parallel with this cellular process in addition to being an alternative mechanism to cell death when apoptosis is inhibited. It has been acknowledged that autophagy precedes caspase-dependent apoptosis.^{114,115} Several studies demonstrate that autophagy precedes apoptosis and acts as a protective mechanism in cancer cells.^{116–118} Therefore, the autophagy induction may exert other possibilities, which should be considered in the design of new treatments for these malignancies.

Conclusions and Perspectives

Autophagy is a lysosomal degradation process usually activated in response to adverse microenvironmental stresses.

Autophagy itself fulfils a dual role, having tumor-promoting and tumor-suppressing properties. As a response to anticancer treatments, whether autophagy activation leads to cell survival or cell death remains controversial. It is consensus that the outcomes of autophagy activation are highly depended on the tumor types and treatment characteristic.^{119,120}

Resistance to chemotherapy is a major obstacle for the success of cancer therapy. Although the controversy about the pro-survival or anticancer effect of autophagy is still heated, the data *in vitro* and *in vivo* seem more to support the idea that autophagy facilitates the cancer cells' resistance to chemotherapy treatment, and inhibition of autophagy may potentiate the resensitization of therapeutic-resistant cancer cells to the anticancer drugs.^{26,121} Several autophagy inhibitors such as CQ and its derivative HCQ have been studied in preclinical models. Although CQ or HCQ augments cytotoxicity in combination with several anticancer drugs, autophagy researchers should be careful when interpreting experiments in which CQ or HCQ treatment is used, as the effect may be mediated by mechanisms other than its inhibition of autophagy.

Currently, the combination of autophagy inhibitors with cytotoxic drugs is attracting more and more attention in cancer therapy. However, the study of the ability of autophagy inhibitors to overcome resistance to anticancer therapies and how this relates to the regulation of tumor microenvironmental stresses raises many issues. First, the question of whether we should try to enhance or inhibit autophagy in cancer treatment is not straightforward as it might vary according to cell type, the stress signal and other circumstances. What is needed to be better experimentally addressed include elucidating the impact of the tumor microenvironment on autophagy function, determining the role(s) of autophagy in the regulation of therapeutic sensitivity and defining novel mechanism by which autophagy inhibition can overcome chemotherapy resistance and sensitize the tumor cells to anticancer therapy. Second, to maximize the potential to be applied for more stringent clinical study, new and reliable methods for measuring autophagy in clinical samples need to be developed. Third, the ocular toxicities and minimal single-agent anticancer efficacy of CQ or HCQ have restricted its clinical application. New and exciting autophagy inhibitors are worthy of further investigation in the future. Thus, establishment of more effective and safe combinatorial therapeutic strategies using autophagy inhibitors will be necessary in the future. However, our increased understanding of the functional relevance of autophagy within the tumor microenvironment and ongoing dialogue between emerging laboratory and clinical research of targeting autophagy will hopefully provide a promising therapeutic strategy to circumvent resistance and enhance the effects of anticancer therapies for cancer patients.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements. This study is supported by grants from the National Natural Science Foundation of China (grants 81301891, 81272593, 81071651 and 81071963) and the Zhejiang Provincial Natural Science Foundation of China (grant LQ13H160008).

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