Autophagy in Cancer Therapy: Progress and Issues

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Abstract: Autophagy is an evolutionarily conserved intracellular self-digestion process, which mediates homeostasis in response to various stresses *via* degradation of damaged organelles or unnecessary proteins. It has been demonstrated that autophagy involves in tumorigenesis and progression. Autophagy serves either as tumor suppressor or promotor in a context-dependent way. It has been revealed in multiple studies that autophagy plays a pro-survival role upon treatment of anticancer drugs. Thus, combination of autophagy inhibitors with anticancer drugs may provide a desirable strategy to improve therapeutic efficacy. In this review, we summarize recent progress in the process and regulation of autophagy with a highlight in advances in the role of autophagy in cancer treatment. We also summarize some recent clinical outcomes of combinatorial use of autophagy inhibitors and anticancer drugs, and introduce latest discovered selective autophagy inhibitors. Some issues which should be paid attention to during the research to improve the clinical outcomes are discussed

Keywords: Autophagy, cancer therapy, chloroquine, selective inhibitor.

INTRODUCTION

Autophagy, which has been proposed as a third mode of cell death, is an evolutionary conserved catabolic process by which redundant, misfolded proteins and damaged organelles or bacterias are sequestered into autophagosomal vesicles then delivered to the lysosome for degradation and recycling. Autophagy is a vital mechanism for proteins, especially for long-lived proteins, degradation and organelles turnover [1, 2]. It is critical to maintain cellular homeostasis and integrity. Under stressed conditions, such as nutrient deprivation, ER stress, reactive oxygen species and hypoxia, autophagy is induced to generate resources for keeping essential cellular functions [3-5]. Recent researches have demonstrated that autophagy has varieties of roles in physiological and pathological (programmed cell death, anti-aging and antigen presentation) circumstances. Defects in this process is associated with numerous human diseases, including cancer, infectious diseases, neurodegenerative disease, pulmonary disease and metabolic disease [6].

Three types of autophagy have been identified according to the ways of how lysosomes receive material for degradation, i.e. macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) [2]. Macroautophagy is a multiple-step process and entails the *de novo* formation of autophagosome, which is a double-membrane organelle and contains a galaxy of cargos. Autophagosome then fuses with late endosome or lysosome to form autolysosome. At the late stage of macroautophagy, the cargo molecules will be degraded by lysosomal hydrolases and the resulting macromolecules are released into the cytosol for recycling [7, 8]. Microautophagy refers the form of autophagy which directly uptakes of cytoplasm at the lysosome surface by invagination of the limiting membrane of the lysosome [8]. Microautophagy is important to maintain organellar size, membrane homeostasis, and cell survival under nitrogen restriction [9]. It has also been reported that microautophagy is associated with early development of mice [10]. Chaperone-mediated autophagy also takes place at the membrane of lysosome, which involves direct translocation of specific proteins containing the KFERQ pentapeptide sequence across the lysosome membrane [11, 12]. Selective autophagy receptors, such as p62/SQSTM1 and NBR1 are required in this process [13]. In this review, we will focus on the most clearly studied autophagy form, i.e. macroautophagy (hereafter referred as autophagy).

AUTOPHAGY MACHINERY

The whole autophagy process can be briefly divided by four phases, including initiation, vesicle elongation, autophagosome maturation and autophagosome-lysosome fusion, and cargo degradation [6]. The executive molecular involved in autophagic pathway are called autophagy-related (Atg) proteins. Up to now, 36 Atg proteins have been identified, and 16 Atg orthologues have been found in human [11, 14]. In mammalian cells, the core autophagy machinery is composed of three major groups (Figure **1**): (1) the ULK1/2 complex, (2) the phosphatidylinositol 3-kinase

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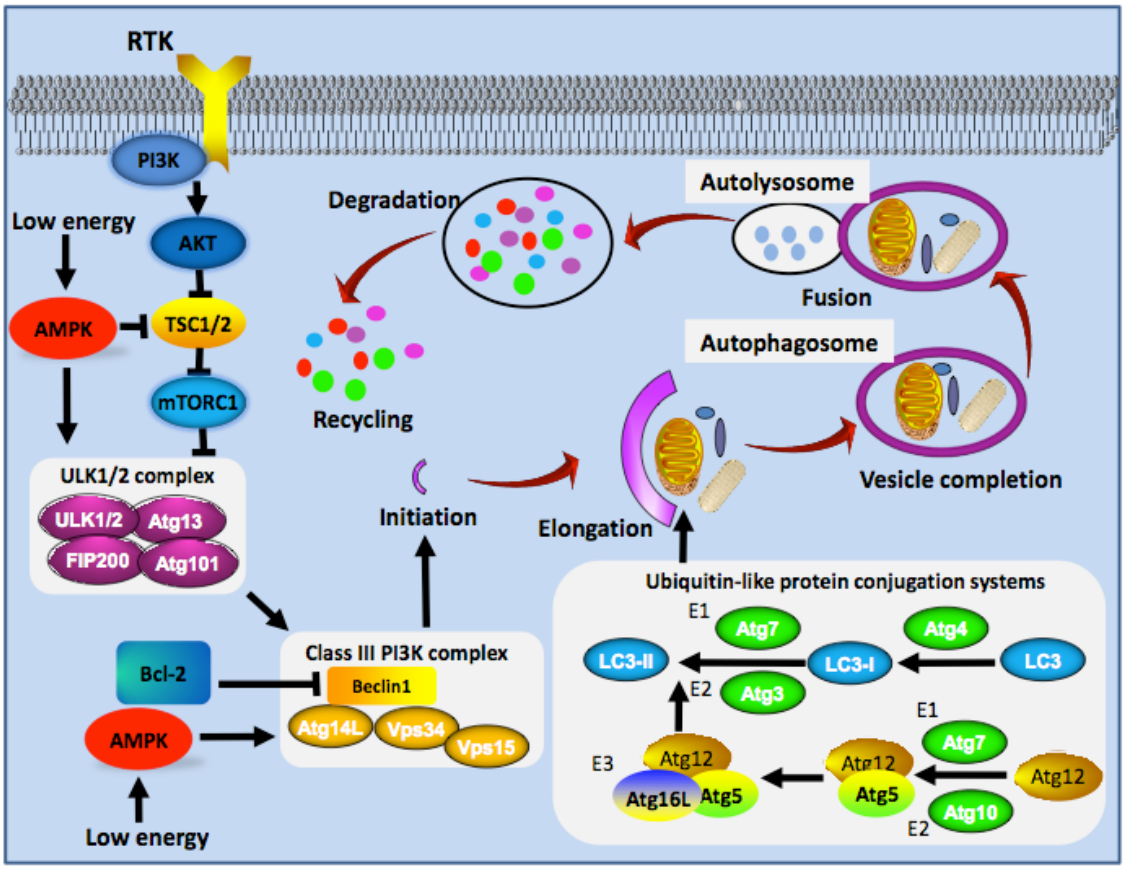

Figure 1: Outline of autophagy pathway. Autophagy is initiated by activation of the ULK1/2 complex, which comprises ULK1/2, Atg13, FIP200 and Atg101. The ULK1/2 complex is negatively or positively regulated by upstream kinases mTORC1 or AMPK respectively. Activation of ULK1/2 complex leads to phosphorylation and activation of class III PI3K complex, which contains four components, Beclin1, Atg14L, Vps34 and Vps15. Class III PI3K complex are positively regulated AMPK and ULK1/2, while Bcl-2 compromises its activity As a result, elongation machinery is activated and promotes formation of autophagosome. Two ubiquitin-like conjugates are involved in elongation process. In the first conjugate, Atg5 binds to Atg12 with the help of E1-like enzyme Atg7 and E2-like enzyme Atg10, and then Atg16L conjugates to the Atg5-Atg12 complex and forms Atg5-Atg12-Atg16L complex. In the second conjugate, microtubule-associated protein 1 light chain 3 (LC3) is cleaved at its C-terminus by Atg4 protease to form LC3-I. With the assistance of Atg7, the E2-like ligase Atg3 and the E3-like Atg5-Atg12-Atg16L complex, the C-terminal of LC3-I conjugates a lipid phosphatidylethanolamine (PE) to form LC3-II (also known as LC3-PE). LC3-II then attaches to the autophagosome membrane and causes to form autophagosome. Autophagosome fuses with a lysosome to generate autolysosome and the contents, which are sequestered into the autolysosome, are degraded and released into the cytoplasm for recycling.

(PI3K) complex, (3) the two parallel ubiquitin-like conjugation systems [15, 16]. The ULK1/2 complex is the direct downstream of mammalian target of rapamycin complex 1 (mTORC1), in which the core protein mTOR is a serine/ threonine protein kinase and plays a vital role in protein synthesis, cell growth, proliferation and survival [17-19]. ULK1/2 complex consists of four components - ULK1/2, Atg13, FIP200 and Atg101. Inhibition of mTORC1 by its inhibitors or starvation, led to dephosphorylation of ULK1, ULK2, and Atg13, which results the stabilization and activation of ULK1/2 complex [20-23]. Increased activity of the complex is prerequisite for the phagophore formation. The ULK1/2 complex activates the PI3K complex, which is comprised of Beclin 1, Atg14L, Vps34 and Vps15, through ULK-dependent phosphorylation of key components and, likely, other unknown mechanisms. It should be noted that class III PI3K, rather than class I PI3K, executes functions in this complex. Once the complex is activated, PIP3 will be produced at the surface of phagophore and initiates the recruitment of the Atg machinery to make preparation of elongation reaction [11]. However, the origin of autophagosomal membranes has been controversial for a long time. It has been reported that the membrane originated from endoplasmic reticulum (ER), mitochondria or plasma membrane [24, 25]. Until recently, Maho Hamasaki and colleagues directly observed the autophagosomes formation at the ER–mitochondria contact site [26].

The elongation of membranes and subsequent closure of the autophagosome that is thought to be

critical for autophagosome formation is associated with two ubiquitin-like conjugates [27]. In the first conjugate, Atg12 is conjugated to Atg5 by the sequential activity of Atg7, which is an E1 like ubiquitin-activating enzyme, and Atg10, which is an E2 like ubiquitin-conjugating enzyme. Next Atg16L interacts non-covalently with Atg5-Atg12 conjugates, which resulting the formation of Atg5-Atg12-Atg16L complex[28]. In the second conjugate, microtubule-associated protein 1 light chain 3 (LC3) is cleaved at its C-terminus by Atg4 protease to generate the cytosolic LC3- Ⅰ with a C-terminal glycine residue [11]. With the help of Atg7 (the E1 like enzyme), Atg3 (the E2 like ligase) and Atg5-Atg12-Atg16L complex, the C-terminal glycine residue conjugates a lipid phosphatidylethanolamine (PE) to form LC3-II (also known as LC3-PE) [16, 29]. When the reaction completes, LC3- Ⅱ will attach to the both faces of autophagosome membrane. Once the autophagosome is completed, Atg4 removes LC3-II from the outer autophagosome surface [11].

The composition of the outer and inner autophagosomal membranes is quite different. To date, LC3 is the only known autophagic protein that stably involved in the mature autophagosome [4]. In the next step, autophagosomes fuse with lysosomes or endosomes. Some regulators, including the lysosomal proteins LAMP-1 and LAMP-2, and the small GTP-binding protein RAB7, are involved in this process [30, 31]. The fusion occurs between the outer membrane of autophagosome and lysosome or endosome membrane, and the inner membrane of autophagosome together with the cytoplasm-derived materials contained in the autophagosome are then degraded by lysosomal hydrolases [4]. At last, the small molecules which come from the hydrolysis of macromolecules *via* autophagy will be reused in cellular activity.

REGULATING PATHWAYS INVOLVED IN AUTOPHAGY

In mammalian cells, autophagy is regulated by a complicated network of signaling pathways, most of which are addicted to the PI3K/AKT/mTORC1 axis. mTOR is distinguished into two distinct multi-protein complexes, named mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [32, 33]. mTOR , especially mTORC1, is a nutrient sensor and its activity reflects cellular nutritional states [19]. When glucose is sufficient, representing a high nutrient level, mTORC1 is active and phosphorylates ULK1 at Ser 757 to inactivate ULK1 complex, and results in inhibition of autophagy [34]. Francesca Nazio *et al*. found that mTOR also inhibited autophagy by regulating ULK1 phosphorylation *via* AMBRA1 and TRAF6 [35]. mTOR may also regulate autophagy indirectly *via* S6K and its transcriptional targets or signaling through Akt. Armour and colleagues found that S6K was required for the starvation-induced autophagic response [36].

AMP-activated protein kinase (AMPK), which contains a catalytic subunit (α) and two regulatory subunits (β and γ), is another pivotal energy sensor. AMPK senses cellular energy status to maintain energy homestasis [37]. AMPK plays critical roles in regulating growth and reprogramming metabolism, and recently has been connected to the cellular process of autophagy [38]. Threes ways have been reported to be implicated in AMPK regulated autophagy. First, when cellular energy is limited, AMPK directly phosphorylates ULK1 on Ser 317 and Ser 777, which stabilize the ULK1 complex and then initiates autophagy [34, 39]. Second, AMPK indirectly leads to induction of autophagy by inhibiting mTORC1 *via* phosphorylation of raptor [40, 41]. Phosphorylated raptor destabilizes mTORC1 which results in decrease in the activity of mTORC1 and in turn autophagy. Third, AMPK also indirectly induces autophagy through phosphorylation of TSC2, a negative upstream regulator of mTORC1 [42].

Bcl-2 is an anti-apoptotic member of Bcl-2 family, which interacts with Beclin 1, and then regulates autophagy. Laurence Lamy *et al*. demonstrated that Bcl-2 and Beclin 1 forms a complex, which limited binding of Beclin 1 to PI3K [43]. In the presence of caspase-10 inhibitors, upregulation of BCLAF1 leads to the dissociation of Beclin1 from Bcl-2, thereby augments autophagy [43]. Bcl-2 inhibitor ABT737 competitively abrogates the inhibitory interaction between Bcl-2 and Beclin 1, then induces autophagy [44, 45].

Non-canonical autophagy, where the entire set of Atg proteins, especially the Beclin 1, is not required to form autophagosome has been reported in recent years [46, 47]. Silencing Beclin 1 or its binding partner Vps34 had no influence on the hallmark of autophagy in some circumstances [48]. Obatoclax, a pan-BCL-2 family inhibitor, has been reported to induce non-canonical autophagy, which is independent on Beclin 1[49]. Nayden G. Naydenov *et al.* reported that loss of N-ethylmaleimide-sensitive factor attachment protein alpha (alphaSNAP) induced Beclin 1-independent autophagy [50]. Besides Beclin 1-independent

autophagy, Yuya Nishida *et al.* discovered Atg5/Atg7-independent non-conaonical autophagy [52]. In this type of autophagy, autophagosomes were generated in a Rab9-dependent manner and regulated by ULK1 and Beclin 1. However, the detailed mechanisms of non-canonical autophagy are still elusive and need to be further investigated.

AUTOPHAGY IN CANCER: SUPPRESSOR OR PROMOTOR?

Accumulating evidence indicates that autophagy is employed by cancer cells as a highly plastic and dynamic mechanism to either suppress tumor initiation or promote established tumor cells survival and growth [53]. The opposite roles of autophagy in cancer cells are seemed to be dependent on tumor type, stage, and genetic context [54-56]. It's not clear at which context autophagy serves as a tumor suppressor and when autophagy acts as a pro-survival role. Therefore, autophagy remains a rigorous investigational field. Elucidation of the action of autophagy in cancer will lead to novel and more efficient anti-cancer treatment strategies.

The Tumor Suppressor Role of Autophagy

The earliest direct evidence of the tumor-suppressing role of autophagy may come from Levine's laboratory [57]. They found overexpression of Beclin 1 in breast carcinoma MCF-7 cells inhibited its proliferation. Furthermore, the authors discovered that Beclin 1 expressed ubiquitously at high levels in normal breast epithelial cells, but the expression levels of Beclin 1 was low in breast carcinoma epithelial cell and tissue [57]. The same group reported that allelic deletions of Beclin 1 were common in breast carcinoma cell lines in the same year [58]. From then on, varieties of proofs sprang up. The importance of Beclin 1 was further validated in mouse models, in which the monoallelic deletion of Beclin 1 developed hepatocellular carcinoma and other types of cancer [59, 60]. Akito Takamura *et al*. established the systemic mosaic deletion of Atg5 and liver-specific Atg7$^{-/-}$ mice and found that these mice developed benign adenomas in liver [61]. Similar results were obtained in Yoshihiro Inami's study, in which conditional deletion of Atg7 in liver caused hepatocellular adenoma [62]. Both studies indicate that tumors caused by deletion of Atg7 or Atg5 are benign tumors rather than cancer, which suggest that deficiency of autophagy contributes to the initiation of tumorigenesis, but not to the progression to advanced cancers [56].

Many other important autophagy regulators, such as Atg4, Atg12 and UVRAG, have been reported to be mutated or deleted in various cancers [14, 63]. Among these, p62/SQSTM1 is the most studied molecule. More and more evidences indicate that accumulation of p62/SQSTM1 may be a dominant factor in tumorigenesis. In liver tumors caused by Atg7 knockout, tumor size significantly reduced by simultaneous deletion of p62 [61]. During stressed conditions, autophagy-defective tumor cells preferentially accumulated p62/SQSTM1, endoplasmic reticulum (ER) chaperones, damaged mitochondria, reactive oxygen species (ROS), and genome damage [64]. Suppressing ROS or p62 accumulation stopped damage resulting from autophagy defects [64].

Autophagy may also protect against tumorigenesis through limiting apoptosis, necrosis and chronic inflammation, at which HMGB1 takes place of Bcl-2 and displaces Beclin 1 or BCLAF1 interacts with Bcl-2 and releases Beclin 1 [43, 65]. Perhaps senescence is another mechanism that autophagy acts as a tumor suppressor [66]. Inhibition of autophagy resulted in delayed onset of senescence, which is thought to be a barrier to malignant transformation [66].

All these findings likely establish a role for autophagy as a mechanism of tumor suppression. However, some questions still exist and we should take into consideration. Whether autophagy plays a suppression role in human cancer remains to be elucidated. What's more, many autophagy associated proteins, such as Atg5, Atg7 and p62, are not specifically serving in autophagy signaling and may possess other biologic functions. For instance, the TB domain of autophagy adaptor p62 interacts with TRAF6 and activates NF-κB pathway, while its PB1 domain incorporates with ERK, NBR1, MEK5 and αPKC, triggers different downstream signaling [67, 68]. Therefore, further research is needed to elucidate the exact mechanisms of autophagy as a tumor suppressor.

The Tumor Protector Role of Autophagy

In contrast to the suppressing effect in cancer, autophagy has a more prominent role in sustaining cell viability. Knock down of essential genes of autophagy boost the induction of cell death [55]. Inhibition of autophagy in pancreatic cancer cells, which show high level of autophagy, led to tumor regression and extend survival of mice bearing tumor xenografts or simultaneous tumor induced by oncogenes [69].

Autophagy, as an intracellular catabolic process, is to confer stress tolerance, which serves to maintain tumor cell survival *via* degrading toxic molecules [70]. Autophagy may facilitate cancer cell survival during nutrient and oxygen shortage or by preventing apoptosis[71].

Because cancer cells have a high demand for nutrients and oxygen to facilitate their increased metabolic and proliferative rate, cells often suffered metabolic stress and hypoxia, especially in poorly vascularized solid tumors [5]. During this situation, autophagy-proficient cells have more potential viability compared to autophagy-deficient cells [64]. HIF-1α is one of a strong inducer at the region of distal to blood vessels, where is hypoxia. With the help of BNIP3, Atg5 and Beclin 1, autophagy is initiated and stops DNA damage and genomic instability [72]. However, study by Papandreou *et al*. showed that hypoxia launched autophagy in tumor cells *via* AMPK activity, independent of HIF-1, BNIP3, and BNIP3L [73]. The precise action mechanism remains to be elucidated.

Autophagy also promotes cell survival by preventing apoptosis. Degenhardt K *et al*. reported that cells in the interior of solid tumors underwent higher levels of autophagy compared to those in the marginal area, which protected tumor cells from both apoptosis and necrosis [70]. The molecular basis for this phenomenon stays in the physiologic interaction between Bcl-2 and Beclin 1. Under nutrient-sufficient condition, cells are able to initiate apoptosis, but can't induce autophagy because of binding of Bcl-2 to Beclin 1. During starvation or other stressed conditions, Beclin 1 binds to Vps34 and releases free Bcl-2, then Beclin 1 takes part in autophagy initiation and Bcl-2 executes its anti-apoptotic function [74]. However, the mechanism mediating the regulation of apoptosis and autophagy has not yet been fully understood.

AUTOPHAGY ARREST CANCER THERAPY

Anticancer Agents Increasing Autophagy

Various anticancer therapies, including conventional cytotoxic drugs and irradiation, have been reported to induce autophagy [75-79]. They may directly affect the core machinery of autophagy or regulate autophagy indirectly [80]. As aforementioned, mTOR is the central coordinator of cell growth and an autophagy negative regulator. mTOR has been recognized as a promising target of cancer therapy. Rapamycin is a naturally occurring allosteric mTOR inhibitor, which binds to FKBP12 to form a complex and inhibits the activity of mTORC1 and then initiate autophagy [81]. Rapamycin analog (rapalogs) temsirolimus (CCI-779), everolimus (RAD-001), and deforolimus (AP-23573) also selectively target mTORC1 and stimulate autophagy [81]. Because of the revert activation of Akt by the S6k-mediated negative feedback loop, rapamycin and rapalogs failed to reach the desired effects in most cancer types [82]. These defects led the development of the second generation ATP-competitive inhibitors (*e.g.*, Torin1, PP242, AZD8055 and WYE132), which inhibit both mTORC1 and mTORC2 and induce autophagy more potently [81]. The dual PI3K-mTOR inhibitor PI-103 also induces autophagy in glioma cells. NVP-BEZ235, another PI3K-mTOR dual inhibitor induces apoptosis in glioma xenografts, when it combined with chloroquine (CQ), which is a widely used autophagy inhibitor [83]. Metformin, an antidiabetic drug which recently has been found displaying anticancer effect, increases autophagy *via* inhibiting mTOR signaling by AMPK [84]. The apoptosis inducer ABT737, which was designed to inhibit Bcl-2 family by acting as BH3 mimetic, interacted with Bcl-2 and released Beclin 1 to form autophagosome [85, 86]. These drugs all directly affect the autophagy core machinery to regulate autophagy.

A lot of anticancer drugs modulate autophagy in tumor cells *via* indirect ways. A good example comes from the anti-estrogen drug-tamoxifen. Tamoxifen and its derivative, 4-hydroxy-tamoxifen (4OHT), induced autophagy in MCF-7 breast cancer cell line [87]. Lately, two groups demonstrated that overexpression of HSBP8, a small heat shock protein 22, protects MCF-7 cells from tamoxifen-induced cell death, by reducing autophagy [88, 89]. Recently, Latika Kohli *et al*. reported a novel mechanism of autophagic death triggered by tamoxifen and 4OHT. In malignant peripheral nerve sheath tumors, 4OHT induces autophagic death through K-Ras degradation [90]. Other classes of drugs, such as HDAC inhibitors, BCR-ABL inhibitors, proteasome inhibitors, are able to induce autophagy [79, 91, 92]. In many cases, the molecular mechanisms which underlie the effects of these drugs on autophagy remain to be elucidated and they seem to act differently at different stages of autophagy. For example, some agents initiated the autophagosomes formation but blocked the fusion with lysosomes [93, 94].

Autophagy Mediates Chemotherapy Resistance

As discussed above, autophagy can be induced during the treatment of anticancer agents. In most of

the studies, autophagy mediates chemotherapy resistance. Combining hydroxychloroquine (HCQ), a derivative of CQ, and everolimus increases the anti-cancer activity compare to monotherapy of everolimus [95]. It has been reported that autophagy limited the cytotoxic effects of the AKT inhibitor AZ7328 in human bladder cancer cells and combination of CQ induced apoptosis in cancer cells [96]. Results from *in vivo* study displayed the same function of autophagy. Combination of AZD5363 (an AKT inhibitor) with CQ significantly reduced tumor volume compared with monotherapy in prostate tumor xenograft models [97].In addition to targeted therapy, autophagy also mediated resistance of conventional chemotherapy. For example, combination of CQ improved the efficacy of 5-FU-based chemotherapy *via* inhibiting autophagy-dependent resistance [98].

It should be noted that autophagy does not always play cyto-protective roles in cancer therapy. An acceptable explanation is that excessive autophagy beyond the bottom line of cancer cell to bear leads to the disruption of cancer cells. In apoptosis-defective cells, autophagy promotes cell death [99]. It has been found that autophagy decreased in tamoxifen-resistant breast cancer cells [88, 89]. The mechanisms of autophagy under antineoplastic drugs treatment is not fully understood, and calls for further research.

ISSUES TO BE NOTED IN TARGETING AUTOPHAGY IN CLINICAL TRIALS

Multiple studies over past a few years discovered the connection between autophagy and cancer therapy. As discussed above, most anticancer drugs initiate cyto-protective autophagy and mediate chemotherapeutic resistance. One reasonable drug combination strategy to overcome drug resistance is to combine autophagy inhibitors to sensitize cancer cells to drugs. At present, there are about 30 active clinical trials (Table **1**) undergoing to combine CQ or HCQ with anticancer drugs (ClinicalTrial.gov). Julio Sotelo's group reported that survival time in patients treated with CQ as an adjuvant to the therapy for glioblastoma multiform was 25 months, as compared with that of 11.4 months in control subjects [100]. A phase I trial of HCQ combined with temsirolimus in patients with advanced solid tumors revealed significant antitumor activity and further studies combining mTOR and autophagy inhibitors in cancer patients are warranted [101]. Combination of autophagy inhibitor and HDAC inhibitors also achieved optimal results [102]. However, results from a phase I/II trial of HCQ in combination with radiation therapy and concurrent and adjuvant temozolomide in patients displayed no significant improvement in overall survival [103]. In this trial, dose-limiting toxicity prevented escalation to higher doses of HCQ to inhibit autophagy [103].

Based on the results from clinical trials, some central questions must be considered about autophagy in cancer therapy: whether we should inhibit or stimulate autophagy to improve the efficacy of cancer therapy? Which is the best way to inhibit or stimulate autophagy? Which population could beneficial from the modulation of autophagy?

Inhibiting or Stimulating, that's the Question

Inhibit or stimulate autophagy is really a big issue. Autophagy serves either a cyto-protective role or a pro-death role under different contexts. It should be certain which role of autophagy upon cancer therapy. In addition, other forms of autophagy besides cyto-protective autophagy and cytotoxic autophagy exist, such as cytostatic autophagy and non-protective autophagy [104]. Non-protective autophagy doesn't lead to perceptible alterations in drug or radiation sensitivity, suggesting non-protective autophagy is just a bystander in cancer therapy. Cytostatic autophagy mediates growth inhibition and its inhibition will result in reduced clonogenic survival. The distinct difference between cyto-protective autophagy and cytostatic autophagy is that cytostatic autophagy does not mediate cell killing [104]. Even though most of studies suggest autophagy serves a cyto-protective role, other forms of autophagy should be taken in consideration and modulate autophagy according to its form.

Urgent Need of Selective Modulator of Autophagy

Pharmacological inhibitors and genetic modification are major methods to modulate autophagy. Pharmacological inhibitors are more feasible and convenient than genetic approach in clinical settings. 3-methyladenine (3-MA), bafilomycin A1 (BafA) and CQ are commonly used pharmacological inhibitors. 3-MA is an inhibitor of Vps34 and inhibits formation of autophagosome isolation membrane, which is an early step in autophagy process, by blocking the PI3K complex activity [105, 106]. In contrast, BafA and CQ interfere the late stage of autophagy. BafA is a vacuolar H+-ATPase inhibitor and blocks autophagy by the elevation of pH in lysosomes [107]. CQ and HCQ are the only U.S. Food and Drug Administration approved drugs using in autophagy suppression. They

Table 1: Clinical Trials Undergoing with Chloroquine or Hydroxychloroquine for Cancer Treatment (http://www.cancer.gov/clinicaltrials)

Cancer type	Therapy	Phase	Protocol ID
Stage IV Small Cell Lung Cancer	CQ	I	NCT00969306
Stage I-III Small Cell Lung Cancer	CQ	I	NCT01575782
Breast cancer	CQ	II	NCT02333890
Primary renal cell carcinoma	HCQ	I	NCT01144169
Advanced BRAF mutant melanoma	HCQ+Vemurafenib	I	NCT01897116
Resectable pancreatic cancer	HCQ+Radiation	II	NCT01494155
Advanced solid tumors	HCQ+Vorinostat	I	NCT01023737
High grade gliomas	HCQ+Radiation	II	NCT01602588
Colorectal cancer	HCQ+Vorinostat	II	NCT02316340
Advanced solid tumors	CQ+Carboplatin/Gemcitabine	I	NCT02071537
Advanced solid tumors, melanoma, prostate or kidney cancer	HCQ+MK2206	I	NCT01480154
Pancreatic cancer	CQ+Gemcitabine	I	NCT01777477
Progressive metastatic castrate refractory prostste cancer	CQ+Navitoclax/Abiraterone	II	NCT01828476
Renal cell carcinoma	CQ+RAD001	I/II	NCT01510119
Lung cancer	HCQ+Gefitinib	I/II	NCT00809237
Metastatic or unresectable solid tumors	HCQ+Temozolomide	I	NCT00714181
Ductal carcinoma in situ	CQ	I/II	NCT01023477
Soft tissue sarcoma	CQ+Sirolimus	II	NCT01842594
Relapsed or refractory multiple myeloma	CQ+Bortezomib	I/II	NCT00568880
Metastatic solid tumors	HCQ+Temsirolimus	I	NCT00909831
Lymphangioleiomyomatosis	HCQ+Sirolimus	I	NCT01687179
Chronic Myeloid Leukemia	HCQ+Imatinib mesylate	II	NCT01227135
Renal cell carcinoma	HCQ+Aldesleukin	I/II	NCT01550367
Advanced cancer	HCQ+Sirolimus/Vorinostat	I	NCT01266057
Resectable pancreatic adenocarcinoma	HCQ+Gemcitabine/Nab Paclitacel	II	NCT01978184
Relapsed or refractory solid tumors	HCQ+Sorafinib	I	NCT01634893
Breast cancer	HCQ	II	NCT01292408
Pancreatic cancer	HCQ+Gemcitabine/Abraxane	I/II	NCT01506973
Resectable solid tumors	HCQ	I	NCT02232243
Unresectable HCC	HCQ+TACE	I/II	NCT02013778
Advanced or metastatic breast cancer	CQ+Taxane	II	NCT01446016

are widely used in clinical trials and preclinical research due to their safety. CQ is a weak base, which can be trapped in acidic vesicles and interfere autophagy by preventing lysosome acidification [108]. All of these inhibitors can efficiently block autophagy, while none of them are specific inhibitors of autophagy. They have significant off-target effects and modulate other cellular activities [5]. Take CQ as an example, CQ also sensitizes breast cancer cells to chemotherapy via a way independent of autophagy [109]. It has been reported that CQ functioned in tumor vessel normalization, which is dependent on Notch1 signaling [110].

Owing to the defects of existed autophagy inhibitor, developing new inhibitors is urgent. In 2014, at least three groups discovered different types of Vps34 selective inhibitors [111-113]. The selectivity of the small molecule compound SAR405 against class III PI3K is more than 1000 times than that against other

classes of PI3K. SAR405 strongly inhibits autophagy without effect on PI3K/mTOR signaling [113]. ATG4B could be another potential target against autophagy, which is necessary for LC3 processing. Robert Young's group has identified some potent compounds [114]. Sharon Gorski's team discovered a lead ATG4B inhibitor and has identified four potential binding sites on ATG4B [114].

Biomarker Monitoring Autophagy in Clinic Needs to be Developed

At present, overall survival is measured to evaluate whether patients are benefited from modulation of autophagy. Efficient and reliable biomarkers are needed to monitor the efficacy at earlier time. In laboratory, detections of morphology by electron microscope, fluorescence of LC3-GFP-mCherry fusion protein by confocal microscopy and the transition of LC3-Ⅰ to LC3-Ⅱ by western blot are routine methods to detect autophagy [115], but they are difficult to be performed in clinic settings. Moreover, high LC3 has been reported to correlate with poor survival in oral squamous cell carcinoma patients [116]. However, LC3 expression level is not the indicator of autophagy, as inhibition of autophagy at late stage will accumulate LC3 level. Therefore, discovery of reliable biomarkers is urgent.

CONCLUDING REMARKS

Over the past decades, there has been a tremendous amount of progress in our understanding of the mechanism of autophagy and the role of autophagy in cancer. Despite the recent advancements, it is still a challenge to have a comprehensive understanding of autophagy in cancer because of the complexity both of autophagy and cancer. Currently, it is well recognized that autophagy serves as tumor suppressor at the early stages of tumor initiation and as pro-survival role in established tumors in response to stresses imposed during cancer progression and due to chemotherapy. Inhibition of autophagy during chemotherapy may be an ideal combination strategy. CQ and 3-MA are widely used as autophagy inhibitors in pre-clinical and clinical studies. Owing to the multiple targets and high operation concentration, they are not perfect autophagy inhibitors. Developing high selective autophagy inhibitors are extremely urgent and some potential compounds are reported. In clinical trials, most of them achieved positive results, but failure cases also existed. The failure maybe caused by different reasons, which should be paid high attention to and be resolved to achieve optimal clinical outcome.

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