Role of autophagy in cancer prevention, development and therapy

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Abstract

Autophagy is a process that takes place in all mammalian cells and ensures homeostasis and quality control. The term autophagy [self (auto)-eating (phagy)] was first introduced in 1963 by Christian de Duve, who discovered the involvement of lysosomes in the autophagy process. Since then, substantial progress has been made in understanding the molecular mechanism and signalling regulation of autophagy and several reviews have been published that comprehensively summarize these findings. The role of autophagy in cancer has received a lot of attention in the last few years and autophagy modulators are now being tested in several clinical trials. In the present chapter we aim to give a brief overview of recent findings regarding the mechanism and key regulators of autophagy and discuss the important physiological role of mammalian autophagy in health and disease. Particular focus is given to the role of autophagy in cancer prevention, development and in response to anticancer therapy. In this regard, we also give an updated list and discuss current clinical trials that aim to modulate autophagy, alone or in combination with radio-, chemo- or targeted therapy, for enhanced anticancer intervention.

Keywords:
anticancer therapy, autophagosome, chloroquine, chronic myeloid leukaemia, haemopoietic stem cell, hydroxychloroquine, tumorigenesis.

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Introduction

Macroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved catabolic process that involves degradation of cellular components in lysosomes. Recycling of these intracellular components can serve as an alternative source of energy during periods of metabolic stress or starvation (e.g. growth factor/nutrient deprivation) to maintain cellular homeostasis and survival. During the autophagy process, double-membraned vesicles, termed autophagosomes, engulf long-lived proteins and organelles and transport these cargoes to lysosomes (Figure 1). Following fusion of the outer membrane of the autophagosome with the lysosomal membrane, the inner membrane, along with the cargo, is degraded by lysosomal hydrolases. Although autophagosomes in mammalian cells have been visualized by electron microscopy since as early as the 1950s, most of the molecular regulators of autophagy have only been characterized in the last two decades, following identification of the ATG

Figure 1. Molecular regulation of autophagy
Under normal nutrient-rich conditions, autophagy can be inhibited following phosphorylation of ULK1 and ATG13 by active mTORC1, or by Bcl-2-mediated inhibition of Beclin1. Induction of autophagy can occur following cellular stress or starvation that leads to mTORC1 dissociation from the ULK1 complex (that involves ULK1/2, ATG13, FIP200 and ATG101). AMPK induces autophagy by phosphorylating ULK1 on active sites. Active ULK1 phosphorylates AMBRA1, freeing Beclin1 to associate with membranes of the phagophore. Beclin1 interacts with several cofactors, such as AMBRA1, leading to activation of the lipid kinase VPS34, a class III PI3K that is critical for the expansion of phagophores. Beclin1 is also associated with UVRAG, ATG14L and Rubicon that are involved in the regulation of autophagy. Autophagosome maturation depends on two ubiquitin-like conjugation systems: (i) the LC3-conjugation system that starts when ATG4 cleaves the C-terminal of LC3 that is then activated by ATG7 (E1-like enzyme), transferred to ATG3 (E2-like enzyme) and eventually conjugated to PE. ATG4 also cleaves the amide bond between LC3 and PE to release the protein from membranes. (ii) The ATG12-conjugation system that starts with conjugation of ATG5 with ATG12 by ATG7 and ATG10 (E2-like enzyme). ATG12–ATG5 interacts then with ATG16 to form an E3-like complex that completes the LC3-conjugation reaction. During the autophagy process, double-membraned autophagosomes engulf proteins and organelles. Completion of the process involves fusion of the outer membrane of autophagosomes with lysosomes to form autolysosomes, where the inner membrane, along with the cargo, is degraded.
(autophagy-related) genes [1]. Most of these genes were initially identified in the budding yeast Saccharomyces cerevisiae and several of them have now been shown to have functional orthologues in mammalian cells. The evolutionarily conserved mechanism of autophagy has been thoroughly investigated and many detailed reviews have been published regarding the protein complexes that contribute to each step [2–4]. In the next section we briefly discuss the main regulators of autophagy and some of the signalling cascades, such as the RAS and PI3K (phosphoinositide 3-kinase)–Akt–mTORC1 [mammalian (also known as mechanistic) target of rapamycin complex 1] pathway, AMPK (AMP-activated protein kinase), p53 and Bcl-2 family proteins, which fine-tune its function and activity in a context-specific manner (also discussed in more detail in other chapters in this volume).

Molecular regulators of mammalian autophagy

Basal autophagy levels are usually low under normal conditions, but can be induced following cellular stress. The induction process starts with the regulated assembly of the initiation complex (sometimes referred to as the ULK1 complex) [5], a serine/threonine kinase complex containing ULK1 (uncoordinated-51-like kinase 1) and ULK2 (yeast Atg1), ATG13, FIP200 (focal adhesion kinase family-interacting protein 200 kDa; a mammalian functional homologue of Atg17) and ATG101 (Figure 1). These proteins associate with the phagophore (also known as the isolation membrane) upon autophagy induction.

A central inhibitor of autophagy in mammalian cells is the serine/threonine protein kinase complex mTORC1. mTORC1, which is activated under growing conditions (nutrient-rich), inhibits membrane targeting of the initiation complex by phosphorylating the ULK1 and ATG13 proteins of the complex [6]. Following mTORC1 inhibition, for example induced by starvation or rapamycin treatment, mTORC1 dissociates from the ULK1 complex and AMPK is free to phosphorylate ULK1 directly, allowing the complex to associate with membranes resulting in the initiation of autophagosome formation [7].

The essential autophagy protein Beclin1 (yeast Atg6) is also a key player in autophagosome biogenesis and interacts with several cofactors leading to activation of the lipid kinase VPS34, a class III PI3K, that is critical for the expansion of phagophores to double-membraned autophagosomes [8]. Under normal conditions, Beclin1 is, via its BH3 domain (Bcl-2 homology 3 domain), bound to and inhibited by Bcl-2 or the Bcl-2 homologue Bcl-xL [9]. BH3-only proteins and pharmacological BH3 mimetics can competitively disrupt the interaction between Beclin1 and Bcl-2/Bcl-xL to induce autophagy [10]. Beclin1 can also be found in complex with AMBRA1 (activating molecule in BECN1-regulated autophagy) and phosphorylation of AMBRA1 by ULK1 upon autophagy induction frees Beclin 1 for membrane association. Other Beclin1-binding partners, like UVRAG (UV radiation resistance-associated gene), ATG14L and Rubicon, have been shown to interact with Beclin1 and positively or negatively regulate autophagy [2].

The expansion of phagophores to autophagosomes and maturation of autophagosomes depend on two ubiquitin-like conjugation systems both of which are essential for autophagy. Each system is composed of two ubiquitin-like proteins, ATG8 (also known as microtubule-associated protein 1 light-chain 3, hereafter called LC3) and ATG12, and three enzymes (ATG3, ATG7 and ATG10) that are required for conjugation reactions [11]. The conjugation
The conjugation system of ATG12 starts with conjugation of ATG5 with ATG12 by ATG7, in a similar manner to the conjugation reaction of LC3, except now ATG10 functions as the E2 enzyme instead of ATG3. The ATG12–ATG5 conjugate interacts with ATG16 to form a complex that exerts an E3 enzyme-like function on the LC3 conjugation reaction [12]. Lipidated LC3 is most commonly used to monitor autophagy by various assays although other techniques are also used (researchers in the field have recently updated the comprehensive guidelines for monitoring autophagy [13]).

The completion process of autophagy includes the fusion of the outer membrane of autophagosomes with lysosomes, which contain pH-sensitive degradative enzymes, to form autolysosomes, although the mechanistic details for this step are still not completely clear. Following fusion, the inner single membrane of the autophagosome and its cargo are lysed by lysosomal hydrolases, especially cathepsins, and degraded; this process has been demonstrated to require the lysosomal protein LAMP-2, the small GTPase RAB7 and UVRAG [14–17].

**Signalling regulation of mammalian autophagy**

The multistep autophagic pathway is tightly controlled by several signalling mechanisms such as growth factor signalling, energy sensing, ER (endoplasmic reticulum) stress, hypoxia, oxidative stress and pathogen infection [2]. Below, we briefly discuss how some of these signalling pathways, such as the RAS–RAF–MEK1/2 [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase 1/2]–ERK1/2, PI3K–Akt–mTORC1 and p53, which are frequently deregulated in cancer and are important for tumorigenesis, can modulate autophagy (Figure 2).

**Growth factor signalling: RAS and the PI3K–Akt–mTORC1 pathway**

A growing body of evidence suggests that increased mTOR activity is important for cancer pathogenesis and may provide cancer cells with the machinery required to sustain high levels of cell growth [18]. mTOR exists in two conserved protein complexes, mTORC1 and mTORC2. mTORC1 has a primary function in regulating autophagy, acts as a nutrient sensor and has been described as the master regulator of autophagy [19]. Upstream of mTORC1 are the class I PI3K and Akt kinases that can lead to inhibition of autophagy when active. PI3K, a factor commonly mutated in human cancers, catalyses the production of PIP<sub>3</sub> (phosphatidylinositol 3,4,5-trisphosphate) at the plasma membrane, which in turn increases membrane recruitment of Akt. Active Akt activates mTORC1 by inhibiting a downstream protein complex, the TSC (tuberous sclerosis complex)1–TSC2. The TSC1–TSC2 complex functions as a GTPase-activating protein for Rheb (Ras homologue enriched in brain), a small GTP-binding protein that binds to and activates mTORC1. The TSC1–TSC2 complex also senses inputs from other kinases such as ERK1/2. It has been shown that phosphorylation of TSC2 by Akt or ERK1/2
leads to the disruption of the complex, resulting in mTORC1 activation [20]. The role of RAS in regulating autophagy is therefore complex as it has been documented to have opposing roles; it can inhibit autophagy by activating ERK1/2 or the PI3K–Akt–mTORC1 pathway [21], or it may activate the RAF–MEK1/2–ERK1/2 pathway leading to phosphorylation of the GIAP (Gα-interacting protein) (protein involved in G-protein signalling regulation) and autophagy induction [22]. This is in line with data suggesting that human cancer cell lines bearing activating mutations in RAS can have high levels of autophagy that is required to maintain oxidative metabolism and tumorigenesis [23].
Energy sensing: positive regulation by AMPK

AMPK is an energy stress sensor that is activated by LKB1 kinase during nutrient and energy depletion (decreased ATP/AMP ratio). In contrast with active Akt and ERK1/2, active AMPK leads to phosphorylation and activation of the TSC1–TSC2 complex, and through Rheb, to inhibition of mTORC1. Thus AMPK is a positive regulator of autophagy. AMPK can also inhibit mTORC1 activity by directly phosphorylating raptor (regulatory associated protein of mTOR), a subunit of mTORC1, and this phosphorylation may be crucial for the inhibition of mTORC1 [24].

Stress response: the dual role of p53

The p53 tumour suppressor is frequently mutated in the majority of human cancers [25], and has been shown to have a dual role in autophagy control. p53 has been shown to induce autophagy in a manner dependent on one of its target genes, DRAM-1 (damage-regulated autophagy modulator 1) [26]. DRAM-1 was also found to be involved in p53-induced death, highlighting the relationship between cell death, p53 and autophagy. p53 has also been shown to induce autophagy through activation of AMPK/inhibition of mTORC1 following glucose starvation [27]. On the other hand, both cytoplasmic wild-type and mutant p53 proteins have been shown to inhibit autophagy [28], in contrast with the pro-autophagic activity of nuclear p53.

The link to cell death: Bcl-2 protein family

Bcl-2 is the prototype of a family of proteins containing at least one BH domain. The Bcl-2 family proteins are subdivided into three groups on the basis of their pro- or anti-apoptotic action and the BH domains they possess: first, anti-apoptotic multidomain Bcl-2-like proteins [such as Bcl-2, Bcl-xL and MCL-1 (myeloid cell leukaemia sequence 1)], which contain four BH domains (BH1–BH4). Secondly, pro-apoptotic multidomain BAX-like proteins (such as BAX and BAK), which also contain four BH domains, and thirdly, the pro-apoptotic BH3-only protein family [such as BID (Bcl-2 homology 3 interacting-domain death agonist), BIM (Bcl-2-interacting mediator of cell death), BAD (Bcl-2/Bcl-xL-antagonist, causing cell death), Noxa and PUMA (p53 up-regulated modulator of apoptosis)] [29]. BH3-only proteins affect cell death by interacting with the anti-apoptotic Bcl-2-like proteins, which possess a hydrophobic cleft (the BH3-binding groove), to inhibit their function and/or by interacting directly with pro-apoptotic multidomain proteins (BAX and BAK) to stimulate their activity. Following activation, BAX and BAK associate with the mitochondrial membrane where they cause outer membrane permeabilization. This induces the release of cytochrome c and other pro-apoptotic factors from the mitochondria, leading to activation of caspases and apoptosis. In addition to cell death regulation, the Bcl-2 protein family also plays a dual role in autophagy regulation. The anti-apoptotic Bcl-2, Bcl-xL and MCL-1 can inhibit autophagy, whereas the pro-apoptotic BH3-only proteins BNIP3, BAD, BIK (Bcl-2-interacting killer), Noxa, PUMA and BIM can induce autophagy [10]. As mentioned earlier, the binding of Bcl-2
to Beclin1 disrupts the association of Beclin1 with VPS34, decreasing VPS34 activity and thereby inhibiting autophagy. The Beclin1–Bcl-2 interaction has been shown to be reduced following starvation or treatment with pharmacological BH3 mimetics, freeing Beclin1 to engage in autophagy activation [30].

The paradoxical role of autophagy in cancer

The first strong link between autophagy and cancer was published in 1999 when it was shown that Beclin1 can inhibit tumorigenesis and that its levels are diminished in human breast carcinoma, suggesting that decreased expression of autophagy proteins may contribute to the development or progression of human cancer [31]. Subsequent studies revealed that Beclin1 is a haploinsufficient tumour suppressor and essential for early embryonic development [32,33]. In line with this notion are studies demonstrating that frameshift mutations of ATG2B, ATG5, ATG9B and ATG12 can be found in human cancers [34] and that deletion of other key autophagy regulators in mice can push cells towards malignant transformation [35,36]. In this regard, recent studies on the role of basal autophagy on the survival and function of HSCs (haemopoietic stem cells) have revealed that autophagy plays a critical role for HSC maintenance [37] and may protect against leukaemia development. The first indication came in 2010 when it was shown that FIP200 is required for the maintenance and function of fetal HSCs and deletion of FIP200 resulted in increased mitochondrial mass and high levels of ROS (reactive oxygen species) followed by severe anaemia and perinatal lethality [38]. In another study, conditional deletion of Atg7 in the haemopoietic system resulted in an accumulation of mitochondria, ROS and DNA damage, followed by loss of HSC function [35]. Moreover, the production of both lymphoid and myeloid progenitors was impaired in the absence of Atg7 and the mice developed a myeloproliferative disorder and died within weeks, indicating that autophagy may protect against leukaemogenesis. Taken together, it is now evident that basal autophagy within normal cells, particularly stem cells, is pivotal since it functions as a ‘guardian’ by promoting adaptation under changing conditions and/or stress, maintains protein/organelle quality control and metabolism, prevents accumulation of p62 [39], regulates removal of damaged mitochondria (that would otherwise produce ROS and damage the DNA), and therefore promotes genetic stability [40].

On the other hand, increasing evidence suggests that autophagy also acts as a survival mechanism within cancer cells. Leukaemia stem cells living in the bone marrow and other cancer cells, particularly cells in the core of solid tumours, have to overcome adverse conditions such as hypoxia and limited access to the vascular niche and nutrients and may therefore rely more heavily on autophagy than normal cells. Moreover, autophagy appears to be particularly important for cancer cell survival following stress or anticancer therapy. In line with this, we have shown that IM (imatinib) mediated Bcr-Abl inhibition, which has been reported to promote apoptosis by inducing the expression or activity of the BH3-only protein BIM and BAD [41], induces protective autophagy in CML (chronic myeloid leukaemia) cells [42]. Of importance, specific autophagy inhibition, either with ATG7 or ATG5 knockdown, or pharmacological inhibition using CQ (chloroquine), results in enhanced death induced by IM, in cell lines and primary CML stem cells. Furthermore, inhibition of autophagy has been shown to lead to
enhanced apoptosis following irradiation [43], p53 activation [44] and following treatment with other apoptosis activators such as alkylating agents [44] and BH3 mimetics [45,46]. This has provided a rationale for initiation of clinical trials where the use of autophagy inhibitors in combination with chemotherapy or targeted therapy is being tested (Tables 1 and 2).

### Table 1. Drugs used in combination with HCQ-mediated autophagy inhibition in currently active clinical trials

EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; RTK, receptor tyrosine kinase; TK, tyrosine kinase.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Drug</th>
<th>Target</th>
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<tbody>
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<td></td>
<td>Capecitabine</td>
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<td>Paclitaxel</td>
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<td></td>
<td>Temozolomide</td>
<td>DNA (alkylating agent)</td>
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<td>Cyclophosphamide</td>
<td>DNA (alkylating agent)</td>
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<td>Targeted therapy</td>
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<td>Bortezomib</td>
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<td>Akt</td>
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<tr>
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<td>RAD001</td>
<td>mTOR</td>
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<td>Sirolimus/rapamycin</td>
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<td></td>
<td>Sorafenib</td>
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<td></td>
<td>Sunitinib malate</td>
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<td></td>
<td>Temsirolimus</td>
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Table 2. Selective active clinical trials where autophagy inhibition is being tested

BWH, Brigham and Women’s Hospital; CRUK, Cancer Research UK; DFCI, Dana Farber Cancer Institute; DM, Devalingam Mahalingam; LM, Lynn McMahon; MAASTRO, Maastricht Radiation Oncology; MGH, Massachusetts General Hospital; MGH, Massachusetts General Hospital; MP, Millennium Pharmaceuticals; MRC, Medical Research Council; MUMC, Maastricht University Medical Center; NCI, National Cancer Institute; NIH, National Institutes of Health; NYU, New York University; RTK, receptor tyrosine kinase; SKCCC, Sidney Kimmel Comprehensive Cancer Center; TKI, tyrosine kinase inhibitor; UCL, University College, London; UMDNJ, University of Medicine and Dentistry New Jersey; UPenn, University of Pennsylvania; VCU, Virginia Commonwealth University.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
<th>Sponsor, collaborator</th>
<th>Identifier</th>
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<td>Adult solid tumour</td>
<td>HCQ + radiotherapy</td>
<td>I</td>
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<td>HCQ + temsirolimus</td>
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<td>HCQ + MK2206</td>
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<td>NCI</td>
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<td></td>
<td>HCQ + vorinostat</td>
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<td>DM, Merck</td>
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<td></td>
<td>HCQ + sunitinib malate</td>
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<td>NCI</td>
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<td>LM, MRC, CRUK Trials unit Glasgow</td>
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(Continued)
Table 2. Selective active clinical trials where autophagy inhibition is being tested  *(Continued)*

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<th>Phase</th>
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Autophagy therapeutics

Given the paradoxical role of autophagy in cancer, it is not surprising that autophagy modulation has been warned to be a double-edged sword (that is, it could have both favourable and unfavourable consequences) [47]. Establishing how the functional status of autophagy may influence treatment response is therefore critical. Below, we discuss some examples of how autophagy induction could be beneficial and when autophagy inhibition could lead to improved therapy.

Autophagy induction in cancer prevention and improved chemotherapy

If autophagy serves to preserve cellular integrity, could boosting autophagy above basal levels cause an even greater protection against tumour development? CR (caloric restriction), i.e. reduced food intake without malnutrition, affects molecular pathways that are also known to be altered in cancer. This can lead to inhibition of mTOR through activation of AMPK or SIRT1, or inhibition of growth factor signalling, and is probably the most physiological inducer of autophagy. Indeed, it has been shown that CR can be effective in cancer prevention in animal models [48–50] and reduce cancer incidence by 50% in monkeys [51]. Epidemiological studies suggest that CR is also beneficial to human health [52]. Whether CR is effective in reducing the risk for developing cancer, such as breast cancer, is now being studied in the clinic (http://clinicaltrials.gov/; search for ‘CR and cancer’). However, whether CR will only prevent cancer from developing in diseases for which being overweight is an accepted risk factor, and if the effect relies partly on autophagy, is still unclear.

Furthermore, is it possible that autophagy induced by lowering caloric intake can prevent tumour progression or even enhance anticancer treatment, either by sensitizing cancer cells to death or by protecting normal cells from highly toxic drug treatment? Interestingly, CR has been shown to decrease tumour progression in p53-deficient mice [53] and reduce the growth of mammary tumours [54]. In addition, CR has been shown to have a similar effect to rapamycin treatment by leading to decreased mTORC1 activity and reduced tumour volume in a murine model of pancreatic cancer [55], suggesting that autophagy may play a role in slowing tumour progression.

Fasting, another way to induce autophagy, has been shown recently to sensitize cancer cells to radiotherapy or chemotherapy and led to extended survival in in vivo GBM (glioblastoma multiforme) models, indicating that fasting could enhance the efficacy of existing cancer treatments in patients [56]. Fasting has also been shown to protect normal, but not cancer, cells against chemotherapy [57], indicating that autophagy modulation may have the potential to maximize the differential toxicity of normal and cancer cells. However, the mechanism for this is still not clear, and whether this effect relies on fasting-induced autophagy needs to be further examined. In fact, initiation of several clinical studies (the ClinicalTrials database; search for ‘fasting and cancer’) suggests that answers regarding whether autophagy-inducing measures may be effective at reducing tumour growth or the toxicity of chemotherapy in humans are on the horizon.
Autophagy inhibition in combination with anticancer therapy

In the clinic, autophagy can be inhibited using the antimalarial drug HCQ (hydroxychloroquine), which has also been approved for treatment of a variety of disorders. HCQ is not a specific autophagy inhibitor, but a lysosomotropic weak base, that raises the pH within lysosomes, impairs lysosomal function and therefore autophagic protein degradation. Despite the controversy regarding the role of autophagy in cancer, since HCQ has been used extensively in the treatment of malaria and rheumatoid arthritis and is quite well tolerated, close to 30 clinical trials are ongoing in cancer patients using HCQ alone or in combination with cytotoxic agents (Table 2). Our promising in vitro data on the effect of IM/CQ combination on survival of CML stem cells [42] have led to a Phase II clinical trial (CHOICES; CHlOroquine and IM Combination to Eliminate Stem cells), the first clinical trial testing autophagy inhibition in CML [58]. The CHOICES trial aims to test the combination of IM with HCQ in IM-sensitive CML patients who continue to show evidence for residual disease caused by the persistence of CML stem cells (Figure 3). Since CML has long been described as a paradigm for targeted therapy with the potential to provide a cure for cancer patients, it is hoped that the CHOICES trial will not only answer the question as to whether autophagy can be effectively inhibited in

Figure 3. IM combined with HCQ for elimination of persistent CML stem cells

CML is a stem cell disorder. The hallmark is the Philadelphia (Ph) chromosome, which forms as a result of translocation between chromosome 9 and 22 in an HSC, leading to expression of the Bcr-Abl fusion protein. This leads to expansion and differentiation of myeloid cells causing the disease. IM, a TKI (tyrosine kinase inhibitor) that inhibits Bcr-Abl activity and used as first-line treatment for CML, is effective in killing differentiated Ph+ CML cells; however, Ph+ CML stem cells are insensitive to IM, leading to disease persistence in the majority of patients. IM has been shown to induce autophagy in CML stem cells and CQ-mediated autophagy inhibition resulted in near complete elimination of CML stem cells in vitro. That has led to CHOICES, a Phase II clinical trial that aims to test a IM/HCQ combination in CML patients who continue to show evidence for residual disease caused by the persistence of CML stem cells following IM treatment.
patients, but also if cancer stem cells/cancer-initiating cells may prove vulnerable to autophagy inhibition, and therefore provide a platform for other stem-cell-driven cancer studies. In line with our data, recent work by Galavotti et al. [59] on the role of autophagy in GBM stem cells showed that DRAM-1 regulates invasive properties of GBM stem cells and its expression is associated with shorter overall survival in GBM patients. Two trials are also currently ongoing to test whether treatment with HCQ in combination with radiation is beneficial in patients with GBM.

As mentioned earlier, since complete inhibition of autophagy using mouse models has significant detrimental effects on survival [32,35,38] and given the tumour suppressive function of autophagy and its role in responses to chemotherapy, is there a potential risk associated with autophagy inhibition in humans? This has been suggested in a previous study by Michaud et al. [60], who showed that autophagy inhibition may limit chemotherapy responses by preventing autophagy-dependent anticancer immune responses, raising the concern that acute autophagy inhibition may actually limit chemotherapy responses in certain cancers for which an immune reaction plays an important role in disease response. However, if treatment regimens involve short-lived and/or impairment as opposed to complete inhibition of autophagy, this may limit toxicity and the propensity to develop secondary malignancies while still achieving therapeutic gain.

**Future directions**

Despite the numerous clinical trials, it is still early days for autophagy inhibition in the clinical setting. Most patients are treated with 200–800 mg of HCQ daily, which in most cases is well tolerated; however, although 1–10 μM concentration HCQ inhibits autophagy in vitro, it is still not clear whether autophagy is blocked in a sustained fashion in all patients receiving up to 800, or even 1200 mg daily. This therefore questions whether any therapeutic effects of HCQ or CQ are even partly related to the inhibition of autophagy. In this regard, recent in vitro studies showed that the ability of CQ to enhance chemotherapeutic responses was the same whether the cells were autophagy competent or deficient [61]. It may therefore be essential to develop more potent/specific autophagy inhibitors for use in future clinical trials. Recently, Lys05, a CQ derivative that is ten times more potent than HCQ [62], spautin-1, a small molecule inhibitor of VPS34 [63] and clarithromycin (a form of macrolide antibiotic) [64] have shown promising results in pre-clinical models or in patients [65].

**Conclusions**

The role of autophagy in cancer has been widely studied in recent years and current knowledge suggests that autophagy can both (i) protect normal cells from accumulation of damaged DNA/proteins and therefore prevent tumour formation, and (ii) help cancer cells to adapt to a hostile environment and protect them from anticancer therapy. Despite this dual role of autophagy, as many studies support the cytoprotective role of autophagy in cancer cells, inhibition of the process is being tested in numerous clinical trials using HCQ, a non-specific autophagy inhibitor and more specific/potent inhibitors are in pre-clinical development. With development of novel autophagy-modulating agents, in addition to greater understanding of
the tissue/cancer-specific role of autophagy, it is hoped that autophagy may provide a therapeutic potential for cancer and other human diseases in the future.

Summary

- Autophagy is an evolutionarily conserved recycling process that takes place in every mammalian cell.
- Many ATG (autophagy-related) genes have been characterized that regulate the process at the molecular level; from initiation/formation of autophagosome (ULK1 complex), to expansion (VPS34–Beclin1 complex), maturation (LC3 and ATG12 conjugation systems) and finally completion/degradation (fusion with lysosomes).
- Under normal conditions autophagy levels are usually low, but can be induced following cellular stress or starvation, often involving inhibition of the PI3K–Akt–mTORC1 pathway.
- Autophagy has dual roles in cancer; it has a tumour suppressive function by preventing DNA damage and genomic instability, yet it promotes tumour development by promoting cancer cell survival under diverse conditions.
- CR has been shown to both prevent tumour progression and enhance anti-cancer treatment, where autophagy has been suggested to play a role.
- Autophagy is induced in cancer cells following radiation or treatment with various apoptosis activators.
- Autophagy inhibition is being tested in the clinic, using HCQ, a non-specific autophagy inhibitor, alone but mostly in combination with chemotherapy and radiotherapy.

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