Targeting Autophagy in Cancer: Therapeutic Implications

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Dipanwita Das Mukherjee, Satabdi Datta Choudhury, and Gopal Chakrabarti

Abstract

Autophagy is an evolutionary conserved self-degradation process that occurs ubiquitously in eukaryotes. It plays an important role in maintenance of cellular homeostasis by balancing the energy resources or through removal of misfolded proteins and damaged organelles. During autophagy, the recycling of the long-lived proteins or organelles is executed through their engulfment into double-membrane autophagosome followed by their lysosomal degradation via formation of autophagolysosome. Interestingly, autophagy is under tight regulation by a group of genes called autophagy-related genes (ATG) in association with various signalling pathways. Literature review suggests that autophagy is implicated in numerous developmental and other physiological processes such as cell differentiation, cell survival, cell death, nutrient starvation response and its dysregulation, often, leads to many pathological conditions including cancer. Generally, under normal physiological conditions, basal autophagy occurs in all cells but it is induced only in response to specific intra- or extra-cellular stimuli. In cancer, depending on the context, autophagy can be paradoxical in nature

D. Das Mukherjee
Department of Biotechnology and Dr. B. C. Guha Centre for Genetic Engineering and Biotechnology, University of Calcutta, Kolkata, West Bengal, India

Division of Molecular Medicine, Bose Institute, Kolkata, West Bengal, India

S. Datta Choudhury
Department of Biotechnology and Dr. B. C. Guha Centre for Genetic Engineering and Biotechnology, University of Calcutta, Kolkata, West Bengal, India

Department of Zoology, Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab, India

G. Chakrabarti (✉)
Department of Biotechnology and Dr. B. C. Guha Centre for Genetic Engineering and Biotechnology, University of Calcutta, Kolkata, West Bengal, India
e-mail: gcbcg@caluniv.ac.in

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(i.e. tumour-suppressive or tumour-promoting) and has also been documented to have the remarkable role in development of chemoresistance, thus, justifying the effectiveness of cancer therapeutic intervention through stimulation or inhibition of autophagy. Henceforth, in this chapter, we have summarised the autophagy in a nutshell, with focus on its mechanism, monitoring methods, regulation and context-dependent role in cancer and explored how the manipulation of autophagy could be beneficial towards improved cancer cure, as evident from the numerous in vitro and in vivo studies as well as clinical trials.

Keywords

Autophagy · Cancer therapy · Chemoresistance · Autophagy regulated genes · Clinical trials

12.1 Introduction

Autophagy is a dynamic process responsible for degradation and turnover of cellular proteins and organelle. It is accomplished through sequestration of target cellular constituents or organelle into double-membrane vesicles called autophagosome, which in turn fuses with lysosome to form autophagolysosome, wherein they are degraded by lysosomal proteases for recycling. It is an evolutionary conserved ubiquitous process occurring in eukaryotes (Klionsky and Emr 2000; Levine and Klionsky 2004). In recent times, autophagy has gained immense attention in clinical research owing to their versatile role in diverse physiological and pathophysiological conditions, amongst which cancer is of particular importance. Autophagy occurs at basal level in most of the cells. However, it can also be induced in response to specific stimuli, wherein autophagy is context-dependent (Mizushima 2007; White 2012; Amaravadi et al. 2016). However, the differences between basal autophagy and stimuli-induced autophagy and their relevance are not yet well-understood.

The Greek term 'autophagy' meaning 'self-eating' was coined by Christian de Duve in 1963 based on the electron microscopic studies displaying single or double-membrane vesicles containing parts of sequestered cytoplasm with variable degree of disintegrated organelles, especially mitochondria and other intracellular structures. Remarkable progress in understanding autophagy has been reported in the last few decades by decoding its molecular mechanism and significance in various physiological processes (Klionsky 2007; Levy et al. 2017). The breakthrough discovery of the detailed mechanism of regulation and execution of autophagy at molecular level in yeast *Saccharomyces cerevisiae* by Yoshinori Ohsumi has been awarded the 2016 Nobel prize in Physiology and Medicine.

12.2 Types of Autophagy

Autophagy can be categorised into three different types: macro-autophagy, micro-autophagy and chaperon-mediated autophagy. Macroautophagy involves engulfment of cytoplasmic proteins and organelle into double-membrane bound vesicles called autophagosome, which is trafficked to lysosome to form autolysosome for degradation by lysosomal proteases. In contrast, microautophagy is characterised by the internalization of the substrate through invagination of lysosomal or endosomal membrane followed by their lysosomal degradation (Li et al. 2012). However, in chaperon-mediated autophagy (CMA), the cargo protein contains KFERQ-like pentapeptide motif, which is recognised by cytosolic chaperone protein called heat shock cognate 70 (HSP-70) for their translocation to the lysosomal lumen through interaction with lysosomal-associated membrane protein 2A (LAMP 2A) receptor (Kaushik et al. 2011). Although both micro and macro-autophagy are capable of targeting large structures through selective and non-selective mechanism, CMA is constitutively selective in nature and thereby, restricted to turnover of specific protein with well-defined KFERQ motif. It is important to note that non-selective autophagy involves the direct engulfment of the cytoplasm and its components into the autophagosome (in macroautophagy) or through invagination of the lysosomal membrane (in microautophagy). While, in contrast, selective autophagy is mediated by specific targeting of the cargo, either cellular proteins or organelles, hallmarked with degradation signal (most commonly, ubiquitin in mammals) through interaction with autophagy cargo receptor, which serves as molecular bridge, for their degradation by autophagy (Kaur and Debnath 2015; Levy et al. 2017).

12.3 Mechanism of Autophagy

Autophagy is a complex, multi-step process under the intricate control of a set of 30 evolutionary conserved, autophagy-regulated genes (ATG), which were identified in yeast and mostly, have well-recognised mammalian orthologue. It divided into three distinct stages: autophagosome biogenesis, fusion with lysosome and lysosomal degradation of intravesicular constituents (Fig. 12.1). The autophagosome formation is initiated at the phagophore assembly point through the activation of ULK (UNC 51-like kinase) complex comprising of ULK1, ULK2 and ATG13, FIP200 (FAK family kinase interacting protein of 200 kDa) and ATG 101. This is followed by the nucleation stage when the ULK complex targets class III PI3 kinase complex—consisting of Beclin 1 (Atg6 in yeast), VPS34 (vacuolar protein sorting 34; also known as PIK3C3), ATG14, UVRAG (UV radiation resistance-associated gene protein; also known as p63) and AMBRA1 (activating molecule in BECN1-regulated autophagy protein 1)—promotes production of autophagosome-specific phosphatidylinositol-3-phosphate. Finally, the ATG5–ATG12–ATG16 complex along with ATG4B–ATG7 complex facilitates the expansion of the autophagosome membrane through lipidation of the microtubule-associated protein light chain 1 (LC3I), which is the mammalian homologue of yeast Atg8, and GABARAP (γ-aminobutyric type A

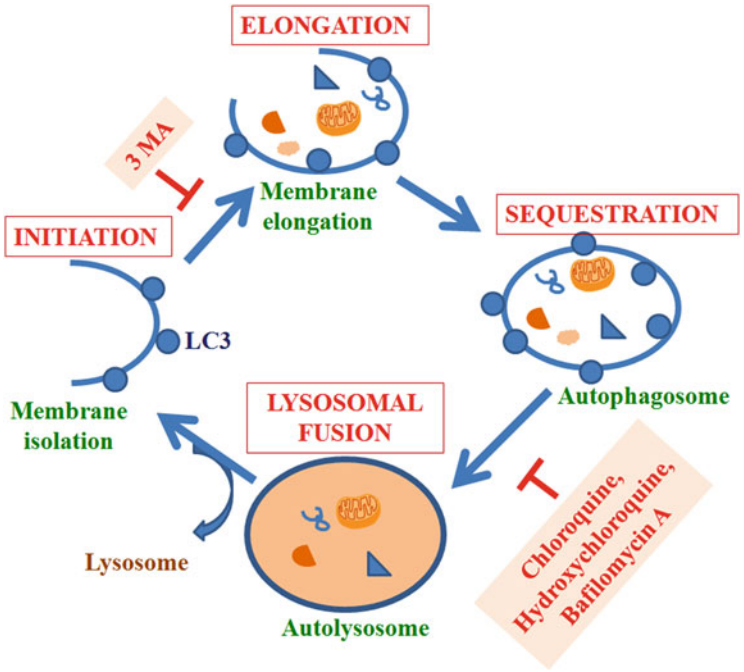

Fig. 12.1 The mechanism of autophagy. Autophagy is a multistep cellular process comprising of autophagosome initiation, elongation of the autophagosomal membrane, sequestration of the cargo and fusion of the autophagosome with the lysosome for degradation of the constituents. It can be inhibited at particular steps by specific inhibitors (such as 3MA—early phase autophagy inhibitor that inhibits autophagosome formation and chloroquine, hydroxychloroquine, bafilomycin A—late phase autophagy inhibitors that prevents the fusion of autophagosome with lysosome)

(GABAA)-receptor associated protein) with phosphatidylethanolamine (PE) to form LC3II and GABARAP-II, which in turn co-localise followed by their recruitment to the membrane. Interestingly, LC3BII, the well-known autophagosome marker found on the autophagosomal membrane, has been reported to facilitate the hemifusion of membranes and cargo selection for degradation, possibly through regulation of variable protein–protein interaction (Mizushima 2007; Levy et al. 2017; Meijer and Codogno 2004; Mizushima et al. 2011; Onorati et al. 2018). However, the significance of LCB-related molecules in autophagy needs further investigation (Fig. 12.1).

12.4 Methods of Monitoring Autophagy

In present-day autophagy research, the detection and quantification of autophagosome along with biochemical validation of the autophagic markers comprises the principal methods of monitoring autophagy. The electron microscopy is the most conventional and oldest method that enables the visualisation of the autophagosome at the ultrastructural level. It is of immense interest to note that in

1950s, the autophagy was first discovered by electron microscopic study of the lysosome (Klionsky 2007). However, advancement of autophagy research called for the formulation of easier and more accessible assays of autophagy detection. The advent of LC3B as the signature of autophagosome has simplified the revelation of autophagy by the light microscopic detection of LC3B or GFP–LC3B puncta. Finally, the conversion of LC3I to LC3 II by immunoblotting with LC3 antibody is the widely employed biochemical assay to confirm autophagy. Furthermore, immunoblot depicting the turnover of p62 is also used to expose autophagy (Mizushima 2004; Mizushima et al. 2010; Yoshii and Mizushima 2017).

Nonetheless, Levine et al. have highlighted the misconception of the direct correlation of the number of autophagosome with the autophagic activity (Mizushima et al. 2010). Owing to the dynamic nature, at any point of time, the number of autophagosome is the function of the balance between their formation rate and fusion rate with the lysosome. Henceforth, the autophagosome accumulation represents either induction of autophagy or suppression of the downstream pathway necessitating the measurement of the autophagic flux, in absence and presence of pharmacological inhibitors and activators, as an essential parameter for uncovering the status of autophagy. The commonly used pharmacological inhibitors include PI3-kinase inhibitors (such as wortmannin, 3-MA and LY294002), microtubule-disrupting agents (e.g. nocodazole), etc. while rapamycin and its analogue, CCI-779, BH3 mimetics (ABT737) and many others are used as autophagy activators. Further, manipulation of the autophagy by knockdown or knockout and over-expression of the ATG genes are also adopted to analyse autophagic flux. The methods used to measure autophagy comprises of LC3 turnover assay, degradation of LC3 and other selective targets, specifically p62 as well as radiolabelled long-lived protein and mRFP-GFP-LC3 assay. The mRFP–GFP–LC3 assay is an interesting test, which exploits the principle of lysosomal stability of RFP versus the quenching of GFP in acidic lysosomal compartment and thus, ascertains the localisation of LC3 depending on their fluorescence properties (Mizushima et al. 2010; Mizushima 2004; Yoshii and Mizushima 2017). Owing to the limitation of each of these assays, combination of the independent experimental methods is usually recommended as the most appropriate technique to estimate autophagy.

12.5 Regulation of Autophagy

Numerous signalling pathways have been involved in up and down-regulation of autophagy. However, the lack of information to understand the detailed molecular mechanism of the autophagy regulation in both cancer and normal cells calls for further investigation (Fig. 12.2).

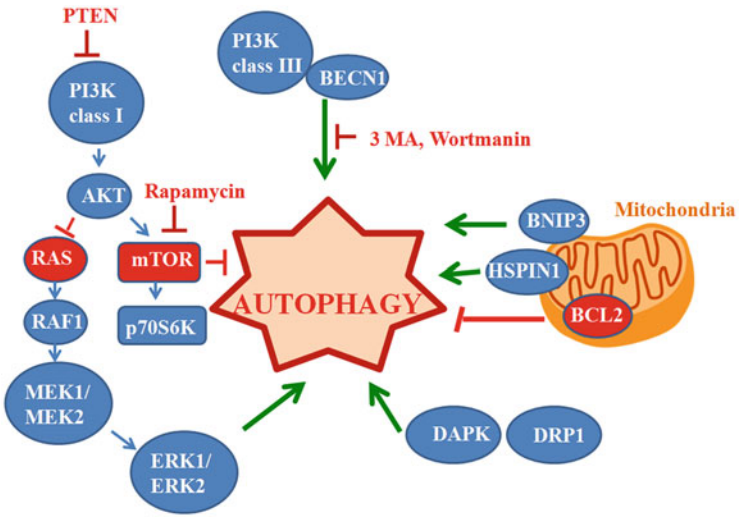

Fig. 12.2 Regulation of autophagy. The different molecular signalling cascade involved in modulation of autophagy. The green arrows indicate activation and the red indicates inhibition of autophagy

12.5.1 The PI3K–AKT–mTOR Signalling Pathway

According to recent reports, phosphatidylinositol 3-kinase (PI3K)–AKT–mTOR pathway is important for negative regulation of autophagy. AKT, a serine-threonine kinase, activates mTOR, a TOR kinase, which leads to suppression of autophagy. Studies in yeast have demonstrated that TOR kinase, which lies upstream of the autophagy-related genes, serves as the guard in autophagy initiation (Schmelzle and Hall 2000). Moreover, in mammalian cells, mTOR integrates with growth factor signalling cascade, thereby, regulating autophagy. It is interesting to note that class I and III PI3K have opposing role in regulation of autophagy: Class I PI3K, which is activated through growth factor receptor, inhibits autophagy while activation of class III PI3K facilitates autophagy by promoting sequestration of cytoplasmic cargo (Petiot et al. 2000). The tumour suppressor genes, like oncogenic RAS and phosphatase and tensin homologue (*PTEN*), also regulate autophagy through PI3K–AKT–mTOR pathway. Oncogenic RAS activates class I PI3K while PTEN deactivates class I PI3K, thereby, suppressing and initiating autophagy, respectively, through modulation of AKT (Arico et al. 2001). In addition, mutation of PTEN, located on chromosome 10q23, in various cancers activates AKT and thus, inhibits autophagy. PI3K–AKT–mTOR signalling pathway is dependent on nutrient availability like nitrogen or amino acids, which leads to transcriptional and translational regulation by p70s6 kinase and 4E binding protein 1 (Wang and Klionsky 2003).

12.5.2 Beclin 1 (BECN1) and Other Pathways

Beclin 1 is a coiled coil protein which is a BCL-2 interacting gene product. It is the first reported molecule to directly link tumourigenesis with autophagy. Previous reports have indicated significant role of class III PI3K in regulation of autophagosome formation and also, promotion of transport of the lysosomal enzymes from trans-golgi network (TGN) to the lysosome. Hence, BECN1 binds to class III PI3K to form BECN1–PI3K complex, which localises in TGN and presumably, facilitates sorting of putative autophagosomal components followed by autophagy induction (Liang et al. 1999).

Other molecules implicated in regulation of autophagy in cancer cells include BCL2 and its family members (BNIP3 and HSPIN1), death-associated protein kinase (DAPK), death-associated related protein kinase 1 (DRP1) and mitogen-activated kinases. BNIP3 (BCL2–adenovirus E1B 19-kDa-interacting protein 3) and HSPIN1 (a human homologue of the *Drosophila melanogaster* spin gene product) are the member of the BCL2 homology 3 (BH3)-only subfamily of the BCL2 family proteins (Vande Velde et al. 2000). They have been reported to induce caspase-independent autophagic cell death in various cancer cell lines. In addition, literature survey has documented that bone marrow-derived cells from BAX and BAK-deficient mice or murine embryonic fibroblast (MEF) are apoptosis resistant but susceptible to autophagy induction upon withdrawal of growth factor or exposure to the chemotherapeutic agent, etoposide (Lum et al. 2005). These, collectively, strengthens the relevance of BNIP3 and HSPIN1 in regulation of autophagy. The DAPK, DRP1 and mitogen-activated protein kinases belong to the family of serine–threonine kinases that regulate a plethora of cellular responses including autophagy. For example, DAPK and DRP1, which are regulated by Ca^{2+}–calmodulin, induce autophagy in MCF7 and HeLa cell (Inbal et al. 2002). While the stimulation of extracellular signal-regulated kinases ERK1 and ERK2, by the RAS–RAF1–mitogen-activated protein kinase (MEK) signalling cascade, induces autophagy in HT-29 colon cancer cell (Ogier-Denis et al. 2000) and buffers the metabolic stress (Degenhardt et al. 2006). For instance, during nutrient starvation, autophagy serves as the alternative energy reservoir whereas it also expedites the adaptation of cancer cells to cellular damage by removing the damaged proteins and organelles (Mizushima 2007).

12.6 Autophagy: The Double-Edged Sword

Autophagy has versatile role in diverse cellular processes and diseases. Basal autophagy occurs constitutively and performs its homeostatic function in conjugation with proteasome degradation pathway to facilitate protein and organelle quality control (Mathew et al. 2007; Mizushima 2007; Ravikumar et al. 2002). It has also been reported to help in elimination of pathogens and apoptotic bodies (Colombo 2007; Qu et al. 2007).

Mounting evidences suggest that autophagy has a pivotal role in cancer, although, its role in sustaining cell survival or inducing cell death is paradoxical (Baehrecke 2005). Autophagy is a well-conserved survival mechanism in several tumour types, which is rendered by protecting the cancer cells from undergoing programmed cell death. It is the most widely used mechanism of the cancer cells to survive. Therefore, inhibition of the autophagy is often exploited as the most feasible approach to sensitise the tumour cells to apoptosis and forms the basis of numerous cancer clinical trials. Nonetheless, in some situations, autophagy can also induce cell death, which is called programmed cell death type II (PCD II) or lethal autophagy. However, the autophagic cell death and apoptosis can be distinguished based on morphological and biochemical features. For instance, in contrast to apoptosis, autophagic cell death is caspase-independent and characterised by degradation of Golgi apparatus, polyribosome and endoplasmic reticulum prior to nuclear destruction (Bursch et al. 2000). Interestingly, during anti-cancer treatment, protective autophagy is initially triggered at the early stage by sequestering the damaged organelle and protein. But once the cellular damage crosses a certain threshold, lethal autophagy or death-inducing is activated to remove the damaged cells from the tissue (Kondo et al. 2005). Although apoptosis and autophagy are interconnected but little is known about the crosstalk between them. Recently, prothymosin-α, inhibitor of apoptosome formation in neuron, has been identified as plausible candidate for modulating the switch between apoptosis and autophagy (Kondo et al. 2005). Intriguingly, autophagy is dependent on multiple factors such as the nature and duration of stimulus, cell type, etc. For example, arsenic oxide (As_2O_3)-induced autophagy in glioma cells while in leukaemia cells, it triggered apoptotic cell death. Similarly, in contrast to the DNA alkylating agent, cisplatins, temozolomide (TMZ) induced autophagy, instead of apoptosis, in several cancer cell lines (Pelicano et al. 2003; Kanzawa et al. 2004). Moreover, it is interesting to document that while tamoxifen induced apoptosis in some cells, it also induced autophagy in other and both apoptosis and autophagy in the rest of breast cancer cells (Bursch et al. 1996). Henceforth, the modern cancer researchers have focused on investigation of the intricate regulation of autophagy and deciphering the interlink between the apoptosis and autophagy.

12.7 Role of Autophagy in Chemoresistance

A large number of recent studies suggests autophagy plays pivotal role in development of chemoresistance (Datta et al. 2017; Hu et al. 2012); in addition, various articles provide increasing evidences that inhibition of autophagy, in combination with various anticancer drugs can augment cytotoxicity on cancer cells leading to attenuation of chemoresistance development and metastasis process (Datta et al. 2019; Follo et al. 2018; Levy et al. 2014) (Fig. 12.3).

Epirubicin, one of the leading drugs used for breast cancer treatment, has shown evidences of autophagy induction in MCF7 breast cancer cell lines, which leads to cytoprotection of the cells from the chemotherapeutic stress induced by this drug. Similarly, autophagy inhibition has also shown elevated cytotoxic effect of various

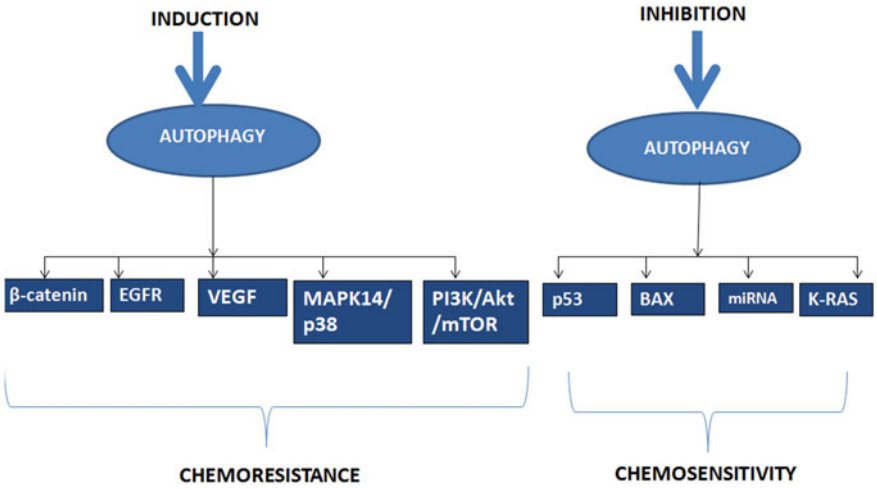

Fig. 12.3 The modulation of chemoresistance and chemosensitivity by autophagy. Schematic diagram depicting the activation of different cellular signalling pathways in cancer cells through autophagy induction or inhibition, leading to chemoresistance or chemosensitivity, respectively

chemotherapeutic drugs like 5-fluorouracil, irinotecan in colorectal cancer, oesophageal cancer, etc. (Chen et al. 2011; Sasaki et al. 2010). Likewise, in human hepatocarcinoma cell lines, autophagy level gets elevated with oxaliplatin treatment, and suppression of autophagy enhances oxaliplatin-induced cell death (Guo et al. 2013). Some of the leading drugs for treatment of lung cancers, like topetocan and paclitaxel, also shows elevated autophagy levels in lung cancer cells, which may ultimately aid in development of chemoresistance against these drugs and inhibition of autophagy has shown promising role in prevention of chemoresistance development against these drugs in lung cancer cells (Datta et al. 2019; Goldberg et al. 2012).

There are varieties of molecular mechanisms via which autophagy induction may lead to chemoresistance development in various cancers; 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 signalling pathways that aids in cancer development and progression, Ras/MAPK, PI3K/Akt and JAK/STATs (Henson and Gibson 2006). In malignant peripheral nerve sheath tumour (MPNST) PD168393, an EGFR-TKI, may induce autophagy as a cytostatic but not a cytotoxic response in malignant peripheral nerve sheath tumour (MPNST) cells that was accompanied by suppression of Akt and mTOR activation. The aberrant expression of PI3K/AKt pathway may also aid in chemoresistance development and PI3K/Akt inhibitors may also lead to increased cytotoxicity of chemotherapeutic drugs against cancer cells by autophagy blockage. In many pre-clinical models autophagy inhibition has shown increased cytotoxic effect, by elevated p53 activity. Vascular

endothelial growth factor-C (VEGF-C) is a secreted growth factor involved in many oncogenic processes, which shows autophagy promoting activities in many cancer cells and VEGF-C inhibitors have been reported to increase cytotoxic effect of anti-cancer drugs by downregulation of cellular autophagy. Activation of MAPK14/p38 also triggers survival-promoting autophagy to protect tumour cells against the cytotoxic effects of chemotherapeutic drugs. In addition, various micro-RNAs may also play key role in chemoresistance development by either inhibition or up-regulation of cellular autophagy, for example inhibition of miR30a (a potent autophagic inhibitor) may lead to chemoresistance development and elevated expression of miR30a may aggravate cytotoxicity of cancer cells by inhibition of autophagy; similarly, miR-199a-5p (an autophagic inducer) may lead to chemoresistance development to cisplatin and vice versa. Moreover, recent reports suggest that some paclitaxel resistant cell lines also show reduced expression of miR16 and 17, which usually exhibits inhibitory effects on beclin-1 expression and elevated expression of these miRNAs may increase sensitivity of these resistant cell lines towards paclitaxel by down-regulation of autophagy (Chatterjee et al. 2015).

However, in spite of its clear prosurvival role, autophagy has also shown to have a prodeath role under certain circumstances, following treatment with a specific set of chemotherapeutic agents, either by enhancing the induction of apoptosis or mediating 'autophagic cell death' by K-RAS, ERK pathways.

12.8 Autophagy Inhibitors

The autophagy inhibitors, whose effectiveness in in vivo and safety in clinical trials have been approved by the FDA, are the antimalarial drugs chloroquine (CQ) and its derivative hydroxychloroquine (HCQ); these are lysomotrophic drugs which raise the lysosomal pH, thereby preventing fusion of lysosomes with autophagosomes and thus, preventing autophagosomal degradation (Fox 1993; Mauthe et al. 2018). Both CQ and HCQ have been investigated in preclinical studies or clinical trials. 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 12.1) also results in sensitisation of cancer cells to a variety of chemotherapeutic drugs. Different autophagy inhibitors block autophagy at different well-defined stages. For example, another antimalarial drug 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 (Liu et al. 2013; Zhao et al. 2012) (Table 12.1).

Although some previous articles have linked autophagy with cell death (Acharya et al. 2011; Lin and Baehrecke 2015; Paul et al. 2020), increasing number of recent research articles have also displayed the promising role of autophagy in cancer cell survival, wherein autophagy inhibition enhanced the chemo-sensitivity of cancer cells towards a wide range of chemotherapeutic drugs (Bhattacharya et al. 2016; Cournoyer et al. 2019; Dyczynski et al. 2018; Ganguli et al. 2014; Pagotto et al.

Table 12.1 Strategies used for inhibition of autophagy

Drug	Target	Effect
I. Pharmacological agents		
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 A1	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
II. Genetic silencing of autophagy regulatory genes		
A. miRNA		
miR-140	ATG12 inhibition	Autophagy inhibition
miR-502	RAB1B	Autophagy inhibition
miR106a/b	ATG16L and ATG12	Autophagy inhibition
miR-183	UVRAG	Autophagy inhibition
miR-22	BTG1	Autophagy inhibition
miR-4093p	Beclin-1	Autophagy inhibition
B. si-RNA		
ATG12-siRNA	ATG12	Autophagy inhibition
ATG5-siRNA	ATG5	Autophagy inhibition
Beclin1-siRNA	Beclin1	Autophagy inhibition
ATG7-SiRNA	ATG7	Autophagy inhibition
ATG6-siRNA	ATG6	Autophagy inhibition

2017). In addition, many reports also suggest that autophagy inhibition may prevent chemo-resistance development in many cancer cell lines (Belounis et al. 2016; Datta et al. 2019). Hence, literature review has established the differential role of autophagy under different conditions. Therefore, finding the exact role of autophagy in a given cancer type, under a given condition is the key factor in determining the clinical approach for apt cancer chemotherapy.

12.9 Clinical Trials

Owing to the opposing, context-dependent role of autophagy in cancer, several studies have proposed that manipulation of autophagy, by stimulation or inhibition, could enhance the efficacy of multiple cancer therapies. However, till date,

Table 12.2 Autophagy inhibitors and their clinical application in different types of cancers

Autophagy inhibitor	Tumour	Additional treatment	Clinical trial phase
Hydroxychloroquine (HCQ)	1. Solid tumours and melanoma	Temsirolimus	I
	2. Malignant solid tumours and colorectal cancer	Vorinostat	I
	3. Non-Hodgkin's lymphoma	Doxorubicin	I
	4. Glioblastoma	Temozolomide and radiation	I/II
	5. Refractory myeloma	Bortezomib	I
	6. Pancreatic adenocarcinoma	Gemcitabine or capecitabine	I/II
	7. Non-small cell lung cancer	Erlotinib	I
	8. Adult solid neoplasm	Sunitinib malate	I
	9. Advanced cancers	MK-2206 (protein kinase B (Akt inhibitor))	I
	10. Small cell lung cancer	Gemcitabine/ carboplatin	I/II
	11. Renal cell carcinoma	IL-2	I/II
	12. Estrogen receptor-positive breast cancer and prostate cancer	None	I and II
Chloroquine	1. Glioblastoma	Temozolomide and radiation	
	2. Brain metastases: Non-small cell lung cancer, small cell lung cancer and ovarian cancer	Radiation	II and Pilot

chloroquine (CQ) and hydroxychloroquine (HCQ) are the only clinically approved and available drugs to inhibit autophagy in clinical models. As tabulated in Table 12.2, mounting preclinical evidences have documented that the inhibition of autophagy with CQ or HCQ alone or in combination with other drugs or radiation caused significant improvement in clinical outcome in cancer patients (Barnard et al. 2014; Briceno et al. 2003; Chude and Amaravadi 2017; Eldredge et al. 2013; Levy et al. 2017; Mahalingam et al. 2014; Rangwala et al. 2014; Rojas-Puentes et al. 2013; Vogl et al. 2014).

12.10 Conclusions

The significance of autophagy in tumourigenesis and cancer treatment makes it an important target for therapeutic intervention. However, till date, autophagy and its role in cancer are poorly understood. Therefore, the attempt to manipulate autophagy should be designed depending on its specific role in that particular scenario of malignancy. The two different and competing approaches of autophagy modulation

are generally adopted towards improvement in cancer therapy. Firstly, cancer cells undergoing lethal autophagy could be exposed to mTOR inhibitors such as rapamycin and its derivatives: CCI-779, RAD001 and AP23573 in order to aggravate autophagic cell death culminating in suppression of a broad range of tumours (Chan 2004). Secondly, in contrast to the above strategy, inhibition of protective autophagy, with autophagy inhibitors such as chloroquine, hydroxychloroquine, bafilomycin A, etc. enhances the therapeutic potential of cancer therapeutics through sensitisation of the cancer cells to apoptotic cell death, as supported by mounting number of clinical trials (Barnard et al. 2014; Chude and Amaravadi 2017; Kanzawa et al. 2003; Levy et al. 2017; Mahalingam et al. 2014; Rangwala et al. 2014). However, both of these attempts in modulation of autophagy yield best outcome when combined with conventional cancer therapies.

Presently, numerous research groups throughout the globe have focused on delineating the detailed mechanism and signalling network of autophagy and understanding its intricate role in various types and stages of cancer. Henceforth, these extensive studies could enlighten new strategies of enhancing the efficacy of the currently available therapeutic options towards successful cancer cure.

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