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Untangling knots between autophagic targets and candidate drugs, in cancer therapy

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Abstract

Autophagy is an evolutionarily conserved lysosomal mechanism implicated in a wide variety of pathological processes, such as cancer. Autophagy can be regulated by a limited number of autophagy-related genes (Atgs) such as oncogenic *Bcl-2*/*Bcl-X*$_L$, *mTORC1*, *Akt* and *PI3KCI*, and tumour suppressive proteins PI3KCIII, Beclin-1, Bif-1, p53, DAPKs, PTEN and UVRAG, which play their crucial roles in regulating autophagy-related cancer. As autophagy has a dual role in cancer cells, with tumour-promoting and tumour-suppressing properties, it has become an attractive target for a series of emerging small molecule drugs. In this review, we reveal new discoveries of related small molecules or chemical compounds that can regulate autophagic pathways and lead to pro-death or pro-survival autophagy, in different types of cancer. We discuss the knots between autophagic targets and candidate drugs, in the hope of shedding new light on exploiting new anti-tumour small molecule drugs for future cancer therapy.

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Introduction

Autophagy is a genetically programmed, multi-step lysosomal process that enables degradation and recycling of long-lived proteins or damaged organelles. It is activated during stress conditions such as amino acid starvation, unfolded protein response or viral infection (1,2). There are three primary forms of autophagy: chaperone-mediated autophagy, microautophagy and macroautophagy, which are different in physiological function and modes of cargo delivery to the lysosome (3). Macroautophagy (hereafter referred to as autophagy) is the most prevalent form, by which a cytosolic double-membrane vesicle termed the autophagosome sequesters portions of cytoplasm and then fuses with a lysosome or vacuole for degradation and recycling of the contents (4).

Autophagy can be dissected into five different steps, induction, vesicle nucleation, vesicle elongation and completion, docking and fusion, and degradation & recycling (5). A limited number of autophagy-related genes (Atg) have been reported to be crucial in autophagosome formation and regulation, and have been known to be associated with cancer initiation and progression (6,7). A variety of oncogenic pathways involved in Bcl-2/Bcl-X_L, mammalian target of rapamycin (mTOR), class I phosphatidylinositol 3-kinase (PI3KCI), Akt and mitogen-activated protein kinases (MAPKs), as well as tumour suppressive pathways of Beclin-1, class III phosphatidylinositol 3-kinase (PI3K-CIII), Bif-1, ultraviolet irradiation resistance-associated gene (*UVRAG*), death-associated protein kinases (DAPKs), PTEN and p53, all have distinct influences on different types of cancer (8,9). Functional autophagy prevents necrosis and inflammation, leading to genetic instability, whereas it might also promote tumour progression by providing energy during unfavourable

metabolic circumstances (10). Thus, autophagy may act as tumour promoter or tumour suppressor, under different circumstances.

The janus-faced role of autophagy in determining destiny of cancer cells makes it an attractive target for a number of small molecular compounds. Some anti-cancer drugs induce autophagy which acts as a survival mechanism and leads to drug resistance. In this case, application of combined treatment with inhibitors of autophagy may be a useful therapeutic strategy. However, there are other drugs that lead to autophagic cell death in cancer cells. Thus, induction of pro-death autophagy has become a new strategy for cancer treatment. Both approaches have significant potential to be translated into ongoing clinical trials that could shed light on exploiting new anti-tumour small molecule drugs for future cancer therapy. In this review, we provide an account of some related small molecule drugs or chemical compounds, which can regulate oncogenic/tumour suppressive autophagic pathways in different types of cancer. Also, we discuss the knot between autophagic targets and these candidate drugs, in the hope of shedding new light on exploiting new anti-tumour small molecule drugs for future cancer therapy.

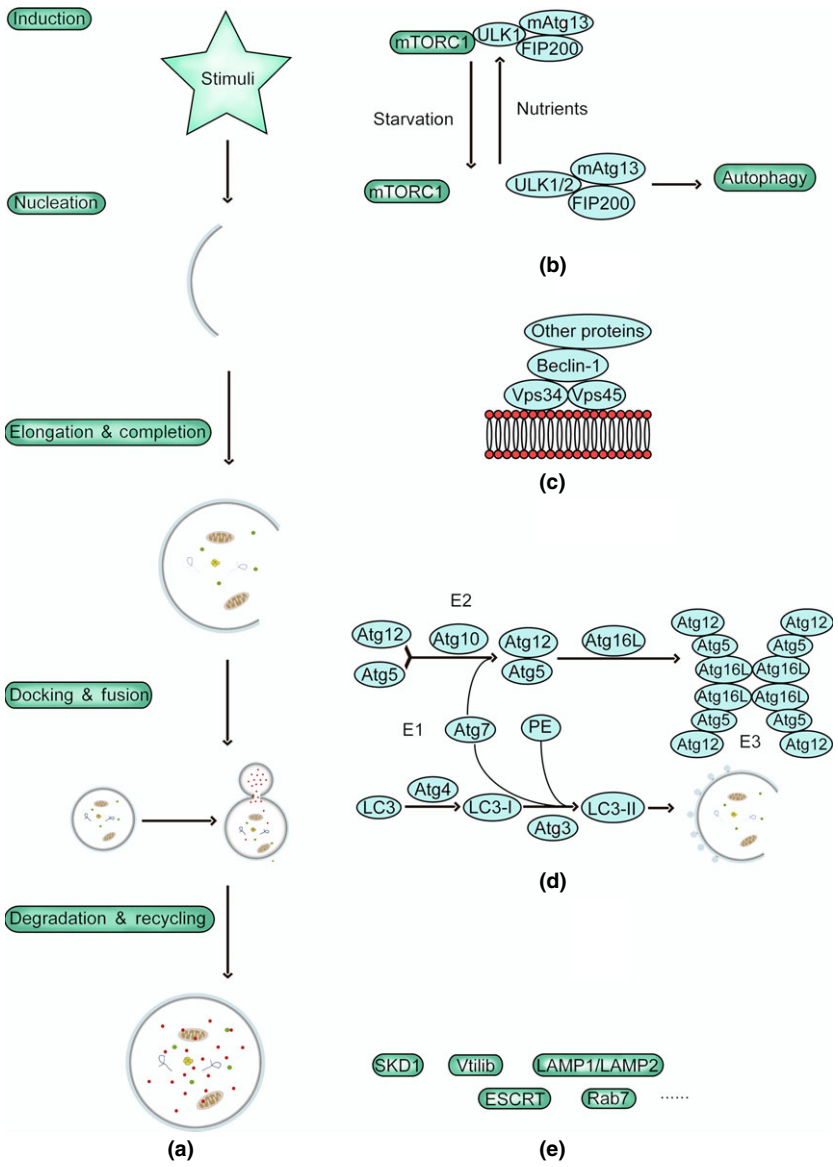


Figure 1. Stages of autophagy and the involved molecular regulators. The complete autophagic flow is a highly regulated process that can generally be divided into the following five stages: (a) induction, (b) vesicle nucleation, (c) vesicle elongation (d) completion, docking and fusion, and (e) degradation and recycling.

Stages of autophagy and the involved molecular regulators

Autophagy has previously been dissected into five different steps, induction, vesicle nucleation, vesicle elongation and completion, docking and fusion, and degradation and recycling (5) (Fig. 1).

Induction is modulated by ULK-Atg13-FIP200 complexes comprised of Unc-51-like kinase 1/2(ULK1/2), Atg13 and focal adhesion kinase family interacting protein of 200 kDa (FIP200) (11). Under nutrient-rich conditions, mTORC1 phosphorylates and subsequently inactivates ULK1/2 and Atg13, whereas under nutrient starvation, mTORC1 phosphorylation of ULK is suppressed, which enhances kinase activity of ULK1/2 and triggers phosphorylation of Atg13 and FIP200, and autophosphorylation of ULK. This results in induction of autophagy (12).

Vesicle nucleation is dependent on Beclin-1-Vps34/PI3KIII-Vps15 core complexes and other Beclin-1 binding proteins such as Atg14L, ultraviolet irradiation resistance-associated gene (*UVRAG*), Bif-1, Ambra1, high-mobility group box 1 (HMGB1), Survivin, PTEN-induced putative kinase 1 (PINK1), Bcl-2, Bcl-X_L and Rubicon, which can mediate PI3KIII activity by interacting with Beclin-1, thus regulating autophagy (13,14).

Vesicle elongation and completion is mediated by two ubiquitin-like systems that together promote assembly of the Atg12–Atg5–Atg16L complex and processing of LC3. The first is an Atg12–Atg5 covalently conjugating system, in which Atg12 is catalysed by E1 protein Atg7 and E2 protein Atg10 fused with Atg5; then the Atg12–Atg5 conjugate interacts with Atg16L to form an Atg12–Atg5–Atg16L complex. In conjunction with Atg12–Atg5, Atg16L directs the complex to elongating autophagic isolation membranes (15,16). The second is an Atg8/LC3 system, in which Atg8(LC3) is converted to LC3-I when carboxy-terminal Arg residue of Atg8 is cleaved away by Atg4. LC3-I is then covalently conjugated to phosphatidylethanolamine (PE). The PE-conjugated form is called LC3-II, an important marker of autophagy (16). In this way, Atg8 proteins recruit lipid molecules to expand autophagosome membranes (17). In addition, Atg12–Atg5 conjugate has E3-like activity for Atg8 lipidation (18). Once autophagosome expansion is complete, Atg8 detaches from PE by Atg4 and then is released to the cytosol (19).

Docking and fusion refer to maturation of autolysosomes and are mediated by LC3, Belclin-1, GTP-binding protein Rab7, lysosomal-associated proteins 1/2(LAMP1/LAMP2), ATPase SKD1, Vtil 1b and the ESCRT complex (20,21). At the final stage, autophagosomal cargoes are digested, and nutrients and energy are recycled.

Autophagic signalling pathways in cancer treatment

mTORC1 serves as the main negative regulator of autophagy in cancer cells. Three major mTORC1-inducing pathways have been identified (Fig. 2). The PI3K–Akt pathway and the extracellular signal-regulated kinase (ERK)–90 kDa ribosomal S6 kinase (RSK)–death-associated protein kinase (DAPK) pathway are the first two and activate mTORC1 and suppress autophagy. The third is the AMP-activated protein kinase (AMPK) pathway, which inhibits mTORC1 and positively regulates autophagy (22). Most signalling pathways regulate TSC2/TSC1 complex, the key point upstream of mTORC1, and suppress mTORC1 by inactivating mTORC1-interacting protein, Ras homologue enriched in brain (Rheb) (22–24). Under conditions of nutrient starvation, mTORC1 phosphorylation of ULK is suppressed, which enhances kinase activity of ULK1/2, triggers phosphorylations of Atg13 and FIP200, and autophosphorylation of ULK, and results in autophagic induction (12). Moreover, mTORC1 regulates autophagy by mediating protein translation by phosphorylating 4E-binding protein 1 (4E-BP1) and $p70^{S6K}$. Phosphorylation of 4E-BP1 leads to its detachment from eukaryotic translation initiation factor 4E (eIF4E), and up-regulates cap-dependent translation. Phosphorylation of $p70^{S6K}$ by mTORC1 allows it to phosphorylate downstream targets, including 40S ribosomal protein S6, a pro-apoptotic BH3-only protein Bad and eukaryotic elongation factor 2 kinase (eEF2K). Phosphorylation of eEF2K inhibits its activity, making it relieve eEF2 from inhibition by eEF2K and promoting autophagy (22,25).

Beclin-1, the mammalian homolog of Atg6, a Bcl-2 interacting coiled-coil protein, is mediated by positive regulators Atg14L, UVRAG, Bif-1, Ambra1, HMGB1, Survivin, and PINK1, as well as negative regulators Bcl-2, Bcl-X_L and Rubicon (13). Atg14L enhances Vps34 lipid kinase activity and up-regulates autophagy (26). Furthermore, Atg14L co-localizes with Atg5 and Atg16L1 on isolation membranes during autophagy (27). UVRAG promotes autophagosome formation by activation of the Beclin-1 complex (28) and Bif-1 interacts with Beclin-1 through UVRAG, and functions as a positive mediator of autophagy (29). AMBRA1 promotes Beclin-1 interaction with its target Vps34/PI3KIII, thus mediating autophagosome nucleation (30) and HMGB1 directly interacts with autophagy protein Beclin-1 displacing Bcl-2 (31). A further positive regulator is Survivin, a member of the inhibitor of apoptosis protein family, which presents a possible mechanism underlying cross-talk between autophagy and apoptosis, by Beclin-1-mediated degradation (32). PINK1, a serine/

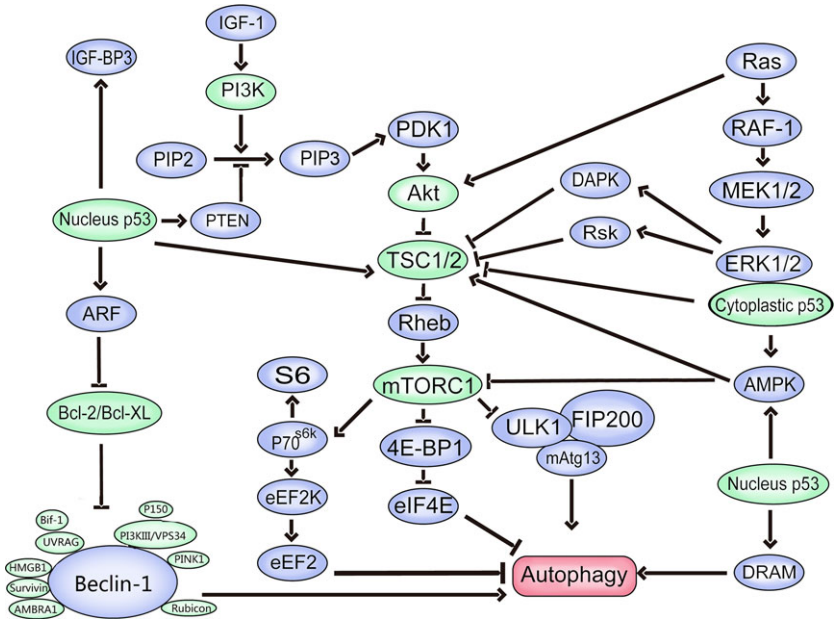


Figure 2. Autophagic signalling pathways in cancer treatment.

threonine protein kinase that localizes to mitochondria, interacts with Beclin-1 and enhances autophagy (33). Bcl-2 and Bcl-X_L, the well-characterized guards of apoptosis, appear to be important factors in autophagy, binding to Beclin-1 to inhibit it (34). Rubicon negatively regulates autophagy, possibly by inhibition of autophagosome maturation (26).

p53, the well-known tumour suppressor protein, becomes activated in response to stresses such as DNA damage and oxidative stress (35). p53 plays a dual regulatory role in autophagy, depending on its subcellular localization, which dictates whether p53 willcontributs to cancer cell survival or to cell death (36). Nuclear p53 activates AMPK to inhibit mTOR and promote autophagy. Also, nuclear p53 promotes autophagy by transactivating autophagy-promoting factors, including AMPK β1/β2 subunits damage-regulated autophagy modulator, pro-apoptotic Bcl-2 proteins (Bad, Bax, BNIP3, PUMA and more), PTEN, Sestrin1/2 and TSC2 (37). In contrast to nuclear p53, cytoplasmic p53 inhibits autophagy by reducing phosphorylation of AMPKα, ACCα and TSC2, as well as by increasing phosphorylation of $p70^{S6K}$ (38).

Autophagy-related drugs/chemical compounds, in different types of cancer

The majority of anti-cancer drugs, as well as ionizing radiation (IR), affect autophagy, mainly by increasing it, in tumour cells. Moreover, autophagy causes cell death after drug treatment, although it may promote or inhibit cell death under different circumstances. Here, we focus on new discoveries in drugs and chemical compounds related to autophagy in some representative types of cancer, in the hope of illuminating exploitation of new anti-tumour small molecule drugs to improve cancer therapy in the future.

Leukaemia

The lymphomas comprise a group of blood cell tumours that develop from lymphocytes; they are the most common form of haematological malignancy. Given the potential function of autophagy in cell survival and death, its manipulation alone may provide a critical means to treat leukaemia or be used together with chemotherapeutic agents to tackle resistance to chemotherapy (Table 1).

A number of drugs has been reported to trigger autophagy in various types of human leukaemia. APO866, a nicotinamide adenine dinucleotide biosynthesis inhibitor, is reported to lead to autophagic cell death in acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (39). Resveratrol inhibits proliferation, and induces apoptosis and protective autophagy in T-cell ALL (T-ALL) cells in a dose and time-dependent manner by inhibiting Akt/mTOR/$p70^{S6K}$/4E-BP1 and activating p38-MAPK signalling pathways (40). Resveratrol also triggers autophagic cell death in CML cells, *via* both AMPK activation and JNK-mediated p62/SQSTM1 expression (41). Arsnic trioxide (As_2O_3) is a potent inducer of autophagy in AML and CML cells by activation of the MEK/ERK

Table 1. Autophagy-related drugs or chemical compounds in leukaemia

Type of cancer	Drugs or chemical compounds	Autophagic pathways	Cell lines and primary cells	Autophagy role pro-death	References
ALL CLL AML	APO866	–	ALL, CLL, AML cell lines and primary cells	Pro-death	(38)
ALL	Resveratrol	Inhibition of Akt/mTOR/$p70^{S6K}$/4E-BP1 and activation of p38-MAPK signalling pathways	Glucocorticoid (GC)-resistant Molt-4/Jurkat/CEM-C1-15/ CEM-C7-14 cells	Pro-survival	(39)
CML		AMPK activation and JNK-mediated p62/SQSTM1 expression	IM-S/IM-R K562 cell line, Ba/f3p210BCR-ABL, p210BCR-ABL T315I	Pro-death	(40)
ALL CML	I3M	–	JM1 cell line K562 cell line	–	(43)
AML CML	As_2O_3	Activation of the MEK/ERK pathway and down-regulation of Bcl-2	U937 cell line KT1 cell line K562 cell line	Pro-death	(41,42)
AML CML	Platonin	BNIP3 overexpression and mTOR/$p70^{S6K}$ inhibition	HL-60/U937 cell line K562 cell line	Pro-death	(44)
ALL	RAD001	mTOR inhibition and Beclin-1 overexpression	Primary cells	Pro-death	(45,46)
ALL	MK-2206	Akt inhibition	Primary cells	–	(48)
ALL	NVP-BEZ235	PI3K/mTOR inhibition	Jurkat/MOLT-4/CEM-S/ CEM-R/RPMI-8402/ BE-13 cell line	–	(47)
ALL	Obatoclax (GX15-070)	Dissociation of beclin-1 from Bcl-2 and inhibition of mTOR	GC-resistant CCM-C7-14/ C1-C15 cells	Pro-death	(51)
ALL	Dexamethasone	Bcl-2 down-regulation and PML/AKT inhibition	RS4; 697, Reh and SUP-B15 cell line	Pro-death	(50)
ALL	Triciribine	Akt1/Akt2/mTORC1 inhibition	T-ALL human cell lines Jurkat, MOLT-4, CEM-S and CEM-R, drug-resistant cells overexpressing P-gp	Pro-survival	(49)
CML	Rapamycin	mTOR/$p70^{S6K}$/4E-BP1 inhibition	K562 cell line	Pro-death	(53)
CML	IM	ER stress and Ca2+ release	CML blast crisis cell lines, CML primary cells, p210BCR/ABL-expressing myeloid precursor cells	Pro-survival	(52)
AML	Vitamin D3	Beclin-1 up-regulation	HL-60 cell line	Pro-death	(54)
AML	Eupalinin A	Inhibition of GSH synthesis	HL-60 cell line	Pro-death	(55)
AML	Vitamin K2	–	HL-60 cell line	Pro-death	(56)

pathway and down-regulation of Bcl-2, but is unrelated to AKT/mTOR or JNK pathways (42,43). Indirubin-3′-monoxime (I3M) is a derivative of indirubin, an active component of a Chinese traditional medicine with known anti-cancer function. I3M can reduce cell viability significantly and dose-dependently, and induce autophagy in ALL and also CML, but the role of autophagy has not been confirmed (44). Platonin is capable of inducing caspase-independent autophagy-associated cell death in AML and CML by BNIP3 over-expression and mTOR/$p70^{S6K}$ inhibition (45).

Autophagy plays a critical role in ALL and Everolimus (RAD001), an mTORC1 inhibitor, reduces cell viability and induces cytotoxic autophagy in ALL and B-precursor ALL (B-pre ALL) (46,47). The novel dual PI3K/mTOR inhibitor NVP-BEZ235, an orally bioavailable imidazoquinoline derivative, induces autophagy and displays strong cytotoxic activity against T-ALL cells. Autophagy plays an unknown role in this case as inhibition of autophagy does not significantly affect cell proliferation. Moreover, combinations of NVPBEZ235 with conventional anti-T-ALL chemotherapeutic agents (cyclophosphamide, cytarabine and dexamethasone) has shown strong synergistic activity, implying that NVP-BEZ235-based combinations could be feasible in the clinic (48). An autophagy and apoptosis inducer, MK-2206, reduces T-ALL cell line viability by Akt inhibition, but the role of autophagy has not been further investigated (49). Akt inhibitor, triciribine, induces autophagy in T-ALL cell lines by Akt1/Akt2/mTORC1

inhibition, which could be a defensive mechanism as the autophagy inhibitor chloroquine (CQ) increases triciribine-induced apoptosis (50). Dexamethasone, a glucocorticoid fundamental drug used in treatment of leukaemia, has fatal consequences for ALL cells by autophagy prior to apoptosis by Bcl-2 down-regulation and PML/AKT inhibition (51). Combination of obatoclax (GX15-070) and dexamethasone triggers cell death by autophagy but not apoptosis in GC-resistant ALL cells. This effect is associated with dissociation of Beclin-1 from Bcl-2 and inhibition of mTOR (52).

Autophagy-related drugs in CML, for example Imatinib mesylate (IM), a potent inhibitor of BCR/ABL tyrosine kinase, has become standard first-line therapy for CML patients and can induce apoptosis and autophagy in CML cells. IM-induced autophagy is associated with endoplasmic reticulum (ER) stress and Ca^{2+} release. Based on suppression of autophagy enhanced cell death induced by IM, autophagy may play a pro-survival role in this case (53). Treatment of CML cell line K56 with rapamycin caused significant reduction in cell viability in a dose-dependent manner by mTOR/4E-BP1/$p70^{S6K}$ inhibition (54).

Autophagic drugs related to AML, have also been demonstrated. In addition to triggering differentiation and inhibiting apoptosis, vitamin D3 up-regulates Beclin-1 to trigger autophagic cell death in HL-60 AML cells (55). Eupalinin A (a sesquiterpene lactone found in Eupatorium chinense L), induces autophagy-mediated cell death in AML cells by inhibition of GSH synthesis (56). Vitamin K2 has also been reported to trigger autophagic cell death in AML but its mechanism of action needs further investigation (57).

Glioblastoma

Glioblastoma (GBM), the most common primary malignant brain tumour in adults, is typically associated with dismal prognosis and poor quality of life. Apoptosis (type I cell death) is reported to be the main mechanism by which most chemotherapeutic drugs and radiation kill GBM cells. Poor prognosis for patients suffering

Table 2. Autophagy-related drugs or chemical compounds in CBM

Drugs or chemical compounds	Autophagic pathways	Cell lines and primary cells	Autophagy role	References
Autophagy inducers				
Quercetin	–	U373MG cell line	Pro-survival	(67)
Bortezomib	–	U251 and U87 cell lines	Pro-survival	(68)
Perifosine	–	U87MG and U251MG cell lines	Pro-survival	(65)
Vodiamine	Calcium-mediated JNK activation	U87MG cell line	Pro-survival	(62)
NVP-BEZ235	PI3K/mTOR inhibition	LN229, SF763, U373 and U87 cell lines and human primary glioma GS2 cells	Pro-survival	(63)
Erlotinib	–	U87MG cell line	Pro-survival	(69)
EMAP-II	PI3K/Akt/FoxO1 axis Inhibition	U87-MG cell line	Pro-death	(70)
TMZ	–	U-87 cell line	Pro-death	(71)
Imipramine	PI3K/Akt/mTOR inhibition	U87-MG cell line	Pro-death	(73)
CM	–	U-87MG and GBM8401 cell lines	–	(74)
Sulfinlsine	–	NCI-H460 and U87 cell lines	–	(75)
Concanavalin-A	Up-regulation of BNIP3 and Atg3/Atg12/Atg16L	U-87 cell line	–	(76)
EDL-291	–	C6, U87 and U251 cell lines	–	(77)
Bromopyruvate	–	GL-15 cell line	–	(78)
Curcumin	–	Glioma-initiating cells from human GBM samples	–	(79)
Autophagy inhibitors				
Ceramide (C6)	–	U87MG and U251MG cell lines	Pro-survival	(65)
ZD6474	PI3K/Akt/mTOR inhibition	U251 and U87MG cell lines	Pro-survival	(66)
TQ	–	T98G and U87MG cell lines	Pro-survival	(58)

from GBMs is strongly related to apoptosis resistance of GBM cells. Inhibition of protective autophagy in chemotherapy and induction of autophagic cell death (type II cell death) become new concepts to trigger glioma cell death and overcome resistance to apoptosis for development of novel glioma therapies (Table 2).

In some cases, autophagy promotes tumour cell survival and adaptation to anti-cancer treatment in GBM. One randomized, double-blind, placebo-controlled trial has revealed that CQ may improve mid-term survival when given in addition to conventional therapy for GBM (58); a further study has shown that GBM cells are sensitive to growth inhibition by CQ (59). These findings suggest that GBM cells may be dependent on the autophagic pathway for survival. Cucurbitacin I, a natural selective inhibitor of JAK2/STAT3, triggers protective autophagy through the AMPK/mTOR/p70S6K pathway and down-regulates HIF-1α (60). However, a further study has shown that quercetin has no effect on autophagic induction in human GBM multiforme T98G cells (61). Piperlongumine (PL), a natural alkaloid present in the fruit of the long pepper, stimulates autophagy by inhibition of Akt/mTORC1. Using CQ, level of PL-induced cell death was significantly increased, indicating a pro-survival role for autophagy (62) and vodiamine, a plant alkaloid, induces calcium/mitochondria-mediated apoptosis and calcium/JNK-mediated autophagy, which is pro-survival, in human GBM cells (63). NVP-BEZ235 promotes cell death and induces autophagy by inhibiting the PI3K/mTOR pathway and autophagy seems to be cytoprotective in this situation (64). Alkylphospholipids, including perifosine, edelfosine, erucylphosphocholine and hexadecylphosphocholine, have been found to induce autophagy in GBM cells (65). A further study has shown that perifosine induces significant protective autophagy, which inhibited cell apoptosis induction. It also showed that inhibition of autophagy by short-chain ceramide (C6) restored perifosine-induced apoptosis and cytotoxicity in GBM cells, without affecting Akt/mTOR signalling (66). In addition to traditional autophagy inhibitors including 3-methyladenine, wortmanin and CQ, other drugs or chemical compounds have recently been reported to be able to inhibit autophagy in GBM. ZD6474 inhibits autophagy by PI3K/Akt/mTOR inhibition and enhances pro-apoptotic effects of glioblastoma (67) and thymoquinone, the most abundant bioactive component of the *Nigella sativa* (black cumin) seed oil, inhibits autophagy and selectively induces cathepsin-mediated, caspase-independent cell death (59); also, quercetin, bortezomib and erlotinib are capable of inducing autophagy and cell death. Autophagy seems to be pro-survival as inhibition of autophagy induces significant increase in erlotinib-induced cell death (68–70).

Although autophagy mainly plays a pro-survival role in GBM cells, there are still some drugs associated with autophagic cell death. Endothelial-monocyte activating polypeptide-II has been shown to be an effective anti-cancer agent for GBM therapy; it can reduce cell viability in GBM stem cells (GSCs) through impaired autophagy mediated by the PI3K/Akt/FoxO1 axis (71). Temozolomide (TMZ) causes autophagy-mediated cell death, markedly reinforced and prolonged by addition of RAD001 (72); however, one further study has revealed that resveratrol potentiates the cytotoxic effect of TMZ on GBM cells, but while resveratrol increases autophagy induced by TMZ, autophagy does not affect acute cell death (73). Imipramine exerts anti-tumour effects on glioma cells by inducing autophagic cell death through inhibiting PI3K/Akt/mTOR signalling (74). Other drugs and chemical compounds, including sulphinosine, *Cordyceps militaris* (CM), concanavalin-A, EDL-291, curcumin and bromopyruvate, have also been reported to be capable of inducing autophagy in GBM (75–80). However, the role of autophagy in these cases requires further study.

Non-small cell lung cancer

Non-small cell lung cancer (NSCLC) represents in the order of 80–90% of lung cancers and is among the leading causes of cancer death. Here, we discuss pro-survival or pro-death role of autophagy of various drugs and chemical compounds in NSCLC, in the hope of illuminating exploration of effective therapeutic strategies for NSCLC treatment (Table 3).

First, drugs which represent a pro-death role of autophagy. Two representative cardiac glycosides, digoxin and ouabain, induce autophagy and autophagy-mediated cell death in A549 and H460 cell lines by activation of AMPK and ERK1/2 (81). Further research has also indicated a mechanism involving JNK-dependent reduction of Bcl-2 in ouabain (82). β-carboline alkaloid harmol, induces cell death *via* autophagy without involving apoptosis in human non-small cell lung cancer A549 cells, the mechanism possibly relating to activation of the ERK1/2 pathway (83). PM02734 (elisidepsin) has been reported to be capable of causing autophagic cell death by a complex mechanism involving inhibition of the Akt/mTOR pathway and activation of DAPK (84). Oleifolioside B (OB) induces apoptosis and triggers autophagy by down-regulation of NF-E2-related factor 2 (Nrf2); OB-induced autophagy functions as a death mechanism in this case (85); SU11274 leads to autophagic cell death in NSCLC A549 cells. It has been demonstrated that p53 can be activated after SU11274 treatment, while interruption of p53 activity reduces

Table 3. Autophagy-related drugs or chemical compounds in NSCLC

Drugs or chemical compounds	Autophagic pathways	Cell lines and primary cells	Autophagy role	References
Ouabain	JNK-dependent decrease of Bcl-2	A549 and H1975 cell lines	Pro-death	(81)
Digoxin	Activation of AMPK and ERK1/2	A549 and H460 cell lines	Pro-death	(80)
Harmol	Activation of ERK1/2	A549 cell line	Pro-death	(82)
PM02734	Inhibition of the Akt/mTOR pathway, and activation of DAPK	H322 and A549 cell lines	Pro-death	(83)
OB	Down-regulation of Nrf2	A549 cell line	Pro-death	(84)
SU11274	p53 activation	A549 cell line	Pro-death	(85)
TW01001	–	A549 cell line	Pro-death	(86)
Erlotinib	p53 nuclear translocation, AMPK activation and mTOR suppression	HCC827 and HCC4006 cell lines	Pro-survival	(87)
β-Elemene	PI3K/Akt/mTOR/p70S6K1 inhibition	A549 cell line	Pro-survival	(90)
Salinomycin	Akt1-mTOR inhibition	A549 cell line	Pro-survival	(91)
Sodium selenite	ROS	A549 cell line	Pro-survival	(92)
NVP-BEZ235	PI3K/mTOR inhibition	NSCLC cell lines A549, H522, H460 and H1792, *etc.*	Pro-survival	(93)
AT101	–	A549 cell line	Pro-survival	(94)
5-FU	–	A549 cell line	Pro-survival	(95)
MEMC	–	NCI-H460 cell line	Pro-survival	(96)
Plumbagin	Inhibition of PI3K/Akt/mTOR	A549 and H23 cells	–	(97)
Calyxin Y	JNK activation and overexpression of Atg proteins	NCI-H460 cell line	–	(98)
OP-B	Inhibition of PI3K/Akt	NSCLC cell lines H460, NCI-H460 and NCI-H157 *etc.*	–	(99)

SU11274-induced autophagy (86). Moreover, TW01001 has the ability to induce pro-death autophagy, although at the moment its mechanism is uncertain (87).

Concerning cytoprotective mechanisms of autophagy of further drugs, erlotinib induces both apoptosis and autophagy in NSCLC cells *via* p53 nuclear translocation, AMPK activation and mTOR suppression. Autophagy seems to be pro-survival in this case as CQ increases cytotoxicity of erlotinib (88,89). In addition, further research has shown that combination of erlotinib–cisplatin induces synergistic cell death, and a significant reduction in autophagy in erlotinib-resistant lung cancer. However, the pro-survival role of autophagy was not confirmed as being by inhibition of autophagy (90). β-elemene inhibits activity of the PI3K/Akt/mTOR/p70S6K1 signalling pathway in human NSCLC A549 cells, resulting in apoptosis as well as protective autophagy (91) while salinomycin induces pro-survival autophagy in NSCLC cells *via* Akt1-mTOR inhibition (92). Sodium selenite treatment of A549 cells appears to trigger both apoptosis and cytoprotective autophagy, both mediated by reactive oxygen species (ROS) (93) and the novel dual PI3K/mTOR inhibitor NVP-BEZ235 also induces apoptosis and protective autophagy (94). Also, methylene chloride extracts of the *Mori cortex* root, AT-101 and 5-fluorouracil (5-FU), are reported to induce protective autophagy in NSCLC cells, but with unknown mechanisms (95–97).

A variety of drugs and chemical compounds have recently been shown to induce autophagy, but where its role remains unknown. Plumbagin is cytotoxic to NSCLC cells and able to induce apoptosis and autophagy by inhibition of the PI3K/Akt/mTOR pathway, but the role of autophagy in this case is unclear (98). Calyxin Y (isolated from a folk anti-tumour medicine *Alpinia katsumadai*), induces autophagy and apoptosis *via* JNK activation and over-expression of Atg proteins, but whether calyxin Y-induced autophagy is protective or pro-death remains controversial (99). Ophiopogonin B (OP-B), a bioactive component of Chinese traditional medicine Radix *Ophiopogon japonicus*, also has the ability to induce autophagy by inhibition of PI3K/Akt (100).

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC), the most common liver cancer and major cause of cancer death worldwide, is significantly relevant to autophagy. Autophagy-related markers such as Belclin-1 and LC3 have been reported to have prognostic significance in HCC. Regulation of autophagy may be a useful therapeutic strategy for HCC (Table 4).

Considering drugs which have autophagy-mediated anti-tumour activity, Nilotinib has been reported to induce pro-death autophagy, but not apoptosis in HCC

Table 4. Autophagy-related drugs or chemical compounds in HCC

Drugs or chemical compounds	Autophagic pathways	Cell lines and primary cells	Autophagy role	References
Autophagy inducers				
Nilotinib	AMPK activation	PLC5, Huh-7 and Hep3B cell lines	Pro-death	(100)
RAD001	mTORC1 inhibition	SK-Hep1 cells	Pro-death	(101)
Andrographolide	Disruption of MTP and elevation of ROS	Huh-7, QGY-7703 and Bel-7402 cell lines	Pro-death	(102)
Bufalin	Inhibition of Akt/mTOR	SK-HEP-1 cell line	Pro-death	(103)
Oroxylin A	Inhibition of PI3K-PTEN-Akt-mTOR	HepG2 and SMMC-7721 cell lines	Pro-death	(104)
SB203580	Activation of AMPK and DAPK	HepG2, Hep3B, Huh-7, PLC/PRF/5 and SK-Hep-1 cell lines	Pro-death	(105)
Galangin	Increase of p53 expression	HepG2 cell line	Pro-death	(106)
Fangchinoline	Activation of p53/Sestrin2/AMPK signalling	HepG2 and PLC/PRF/5 cell lines	Pro-death	(107)
Baicalin	–	SMMC-7721 cell line	Pro-death	(108)
Cisplatin	Down-regulation of miR-199a-5p	Huh-7 cells, HepG2 cell lines	Pro-survival	(109)
NVP-BEZ235	–	Hep3B and PLC/PRF/5 cell lines	Pro-survival	(110)
C6-ceramide	–	HepG2 cell line	Pro-survival	(111)
Bevacizumab	–	SMMC-7721 and Hep3B cell lines	Pro-survival	(112)
Bortezomib	–	PLC/PRF/5, Huh-7, MHCC-97H cell lines	Pro-survival	(113)
MG-132				
AS-6	–	HepG2 cell line	–	(117)
Panobinostat	–	Huh7, Hep3B and HepG2 cell lines	–	(118)
Neferin	–	Hep3B cell line	–	(114)
Sorafenib	–	HepG2 cell line	–	(115)
Regorafenib	–	Hep3B, HepG2 and PLC/PRF/5 cell line	–	(116)
Autophagy inhibitors				
5-FU	ER stress	Sk-Hep1 cell line	Pro-survival	(119)

by AMPK activation (101). mTOR inhibitor RAD001 augments radiation-induced growth inhibition of HCC cells, and induction of autophagy may account for this effect (102). Andrographolide (a diterpenoid lactone isolated from *Andrographis paniculata*), induces autophagic cell death distinct from apoptosis, by disruption of mitochondrial transmembrane potential (MTP) and elevation of ROS in HCC cells (103). Bufalin, a major component of Chan-Su (a traditional Chinese medicine), is able to trigger autophagic cell death by the AKT/mTOR signalling pathway in SK-HEP-1 HCC cells (104) and oroxylin A exhibits autophagy-mediated anti-tumour activity in a dose- and time-dependent manner *in vivo* and *in vitro*, induction of autophagy being associated with suppression of the PI3K-PTEN-Akt-mTOR signalling pathway (105). SB203580 induces autophagy *via* activating AMPK and DAPK, but is independent of p38-MAPK; induction of autophagy can thus account for the anti-proliferative effect of SB203580 in HCC cells (106). Treatment with polyphenolic compound, galangin, may inhibit cell proliferation and induce autophagy by increasing p53 expression in HepG2 cells (107) and fangchinoline (a highly specific anti-tumour agent) induces autophagic cell death *via* p53/Sestrin2/AMPK signalling in HepG2 and PLC/PRF/5 cells (108). Baicalin, which can significantly inhibit viability of SMMC-7721 cells in a dose- and time-dependent manner, induces apoptosis and pro-death autophagy, however its mechanism of autophagy remains to be revealed (109).

In the case of pro-survival autophagic drugs, cisplatin increases drug resistance by activating autophagy due to down-regulation of miR-199a-5p in HCC cells (110). NVP-BEZ235, nanoliposomal C6-ceramide, bevacizumab, bortezomib and MG-132 act as autophagy inducers and reduce cell viability of this cell type. This autophagy may be a survival mechanism that counteracts their anti-cancer effects as combination with autophagy inhibitors synergistically reduces cell viability (111–114).

4-O-carboxymethyl ascochlorin (AS-6), panobinostat, sorafenib, fluoro-sorafenib (Regorafenib) and neferine (a major bisbenzylisoquinoline alkaloid derived from embryos of *Nelumbo nucifera*), exhibit cytotoxicity against HCC cells and are able to induce autophagy. However, neither the role nor mechanisms of autophagy in these examples has been described (115–119).

Except for traditional autophagy inhibitors, 5-FU has been reported to activate apoptotic cell death and induce ER stress to suppress protective autophagy (120).

Carcinoma of the pancreas

Pancreatic cancer is one of the most aggressive human malignancies with very low 5-year survival level. Experimental evidence has pointed out that autophagy acts as a mechanism that pancreatic cancer cells survive under adverse conditions, or as a cytoprotective mechanism, that favours pancreatic cancer cell resistance to treatment. It has been revealed that Beclin-1 over-expression and increase in alteration of autophagy-related proteins, are independently associated with poor prognosis (121). Thus, detection and inhibition of protective autophagy becomes a crucial strategy to fight pancreatic cancer (Table 5).

In most cases, autophagy acts as a survival mechanism. Two p53-reactivating molecules, CP-31398 and RITA, have been reported to reduce cell viability and induce pro-survival autophagy in human pancreatic cancer stem cells (122). Rottlerin (ROT) induces protective autophagy followed by induction of apoptosis *via* inhibition of the PI3K/Akt/mTOR pathway (123). 5-fluorouracil, gemcitabine and eicosapentaenoic acid induce autophagy in pancreatic cancer cells, and induction of autophagy diminishes its ability to induce cell death, but its mechanism here remains unclear (124,125).

Autophagy inhibitors have been discovered to inhibit protective autophagy in pancreatic cells. Treatment with manzamine A (a member of the manzamine alkaloids), results in inhibition of autophagy and cell viability increase (126). TP421 has been shown to have the ability to inhibit autophagy and inhibit cell proliferation in a panel of pancreatic cancer cell lines where its mechanisms seem to depend on accumulation of p62 (127). Dandelion root extract has also been reported to trigger pro-death autophagy in pancreatic cancer cells with the mechanism requiring further investigation (128).

Although autophagy mainly plays a pro-survival role, some drugs have been reported to possibly induce autophagic cell death in pancreatic cancer. Calix[6]arene has been shown to be more potent in reducing Panc-1 cell viability than gemcitabine and 5-fluorouracil, with autophagy being the mode of cell death not apoptosis, by inhibiting the PI3K/mTOR pathway (129). Treated by cannabinoids, pancreatic cancer cells have exhibited strong induction of autophagy and reduction in viability. The mechanism of autophagy lies in AMPK activation, but clear relevance between autophagy and other mechanisms of cell death has not been clarified (130). Moreover, antroquinonol has been reported to be capable of inducing autophagy in pancreatic cancer cells, but with the role of autophagy being unclear (131).

Carcinoma of the breast

Breast cancer is an ancient disease and a leading malignancy for women. Its incidence is high in developed countries, while being low but increasing in developing countries. Research has demonstrated the close relationship between autophagy and breast cancer. Inhibition of protective autophagy and induction of pro-death autophagy has become a new strategy for breast cancer treatment (Table 6).

Many drugs and chemical compounds have recently revealed the ability of inducing autophagic cell death. Treatment with ROT induces dose- and time-dependent cell population growth inhibition, and triggers cell death by apoptosis and pro-death autophagy by activation of

Table 5. Autophagy-related drugs or chemical compounds in pancreatic cancer

Drugs or chemical compounds	Autophagic pathways	Cell lines and primary cells	Autophagy role	References
Autophagy inducers				
CP-31398 RITA	p53 activation	PaCa3 and PANC-1 cell lines	Pro-survival	(121)
ROT	Inhibition of PI3K/Akt/mTOR	Human pancreatic cancer stem cells	Pro-survival	(122)
5-FU Gemcitabine	–	PANC-1 and BxPC-3 cell lines	Pro-survival	(123)
EPA	–	MIA-PaCa-2 and Capan-2 cell lines	Pro-survival	(124)
Calix[6]arene	PI3K/mTOR inhibition	PANC-1 cells	Pro-death	(128)
DRE	–	BxPC-3 and PANC-1 cell lines	Pro-death	(127)
Cannabinoids	AMPK activation	PANC-1 cell line	–	(129)
Autophagy inhibitors				
Manzamine A	The accumulation of p62	PANC-1, AsPC-1, MIA PaCa-2 and BxPC3 cell lines	Pro-survival	(125)
TP421	The accumulation of p62	MIA PaCa-2, PANC-1, BxPC-3 and HPAC pancreatic cancer cell lines	–	(126)

Table 6. Autophagy-related drugs or chemical compounds in breast cancer

Drugs or chemical compounds	Autophagic pathways	Cell lines and primary cells	Autophagy role	References
Rottlerrin	Activation of AMPK	Primary cells	Pro-death	(131)
IR/SAHA combination	–	4T1, MCF-7 and MDA-MB-231 cell lines	Pro-death	(132)
RY10-4	Inhibition of Akt/mTOR/p70^{S6K}	MCF-7 cell line	Pro-death	(133)
Lapatinib	Akt/mTOR/p70^{S6K} inhibition and AMPK activation	BT474 and AU565 cell lines	Pro-death	(134)
SD	Endoplasmic reticulum stress	MCF-7 and MDA-MB-231 cell lines	Pro-death	(135)
DHEA/EPEA	PPARg activation	MCF-7 cell line	Pro-death	(136)
Gefitinib	Down-regulation of AKT and ERK1/2	Cell lines SKBR3, BT474 and JIMT-1 *etc.*	Pro-survival	(137)
CuO NPs	–	MCF-7 cell line	Pro-survival	(138)
Salinomycin	–	MDA-MB-468 cell lines	Pro-survival	(139)
Rapamycin	PI3K/mTOR/Akt inhibition	T-47D cell line	Pro-survival	(141)
HIMOXOL	–	MDA-MB-231 (ER-) cell lines	–	(142)
γ-Tocotrienol	–	MCF-7 and MDA-MD-231 cell lines	–	(143)
Parthenolide	Activation of JNK and PI3K inhibition	MDA-MB231 cell line	–	(144)
Papuamine	Mitochondria damage and JNK activation	MCF-7 cell line	–	(145)
Sulforaphane	Inhibition of Akt and S6K1 kinases	MDA MB 231, MCF-7, SKBR-3 and MDA MB 468 cell lines	–	(146)
Obatoclax	–	MCF-7 cell line	–	(147)
Celastrus paniculatus	–	MCF-7 cell line	–	(148)
Clionamine B	–	MCF-7 cell line	–	(149)
C19	–	MCF-7 cell line	–	(150)

the AMPK pathway (132). Breast cancer cells treated with IR and suberoylanilide hydroxamic acid (SAHA, an inhibitor of histone deacetylase) combination result in significantly enhanced toxicity compared to SAHA or IR treatment; autophagy plays a pro-death role in this case (133). A novel protoapigenone analogue RY10-4, is able to induce breast cancer MCF-7 cell death by autophagy *via* inhibition of the Akt/mTOR/p70^{S6K} pathway (134). Lapatinib, an oral dual tyrosine kinase inhibitor, is a potent inducer of autophagy, thus may be pro-death mechanism, and stimulates apoptosis, with the mechanism of autophagy being Akt/mTOR/p70^{S6K} inhibition and AMPK activation (135). Saxifragifolin D inhibits proliferation of breast cancer cells significantly by interplay between apoptosis and pro-death autophagy, through ROS-mediated ER stress (136). The two ethanolamide derivatives of DHA and eicosapentaenoic acid, docosahexaenoylethanolamine and eicosapentaenoylethanolamine, induce inhibition of cell proliferation triggering autophagy by PPARg activation (137).

Some research has put an emphasis on pro-survival autophagy in breast cancer. Gefitinibautophagy, a small molecule inhibitor of the epidermal growth factor receptor EGFR tyrosine kinase, has been reported to induce autophagy in breast cancer cells by down-regulation of AKT and ERK1/2 signalling, the role of autophagy being pro-survival (138). Copper oxide nanoparticles (CuO NPs) and salinomycin have been reported to trigger autophagy counteracting cell death, however with unclear mechanisms (139,140). However, a further study indicates that salinomycin may act as an autophagy inhibitor in breast cancer cells. Interplay between apoptosis and autophagy seems to be based on ROS-mediated ER stress (141); traditional autophagy inducer rapamycin is capable of inducing autophagy in T-47D breast carcinoma cells, and its effect seems to be pro-survival (142).

In addition, other drugs and chemical compounds including HIMOXOL (methyl 3-hydroxyimino-11-oxo-olean-12-en-28-oate), γ-tocotrienol, parthenolide, papuamine, sulforaphane, obatoclax,*celastrus paniculatus*, clionamine B and 2-ethyl-3-O-sulphamoyl-estra-1,3,5 (10)16-tetraene(C19), have been reported to be associated with induction of autophagy, but the relationship between autophagy and other types of cell death in breast cancer cells has not been revealed (143–151).

The knot between autophagic targets and candidate drugs in cancer therapy

Autophagy, as we know, is closely associated with both specific autophagic pathways and non-specific ones. Small molecule drugs and chemical compounds can affect autophagy, and induce autophagic cell death or cell survival, through both specific autophagic pathways and non-specific autophagic pathways (Table 7).

The PI3KCI/Akt/mTORC1 pathway, main negative regulator of autophagy in cancer cells, is the major target

Table 7. The knot between autophagic targets and candidate drugs in cancer therapy

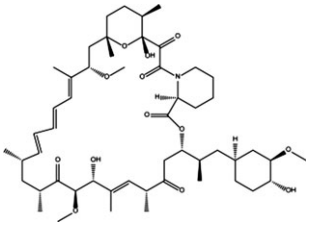
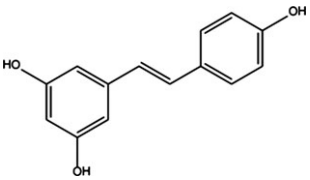
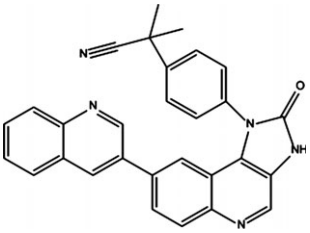
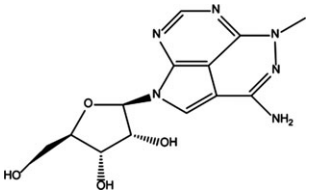
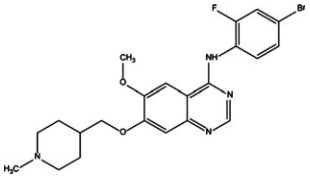
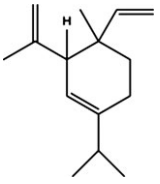
Autophagic pathways	Autophagy role	Drugs or chemical compounds	Chemical structure
PI3KCI/Akt/mTORC1 Inhibition	Dual	Rapamycin	
PI3KCI/Akt/mTORC1 Inhibition	Dual	Resveratrol	
PI3KCI/Akt/mTORC1 Inhibition	Pro-survival	NVP-BEZ235	
PI3KCI/Akt/mTORC1 Inhibition	Pro-survival	Triciribine	
PI3KCI/Akt/mTORC1 Inhibition	Pro-survival	ZD6474	
PI3KCI/Akt/mTORC1 Inhibition	Pro-survival	β-Elemene	

Table 7 (continued)

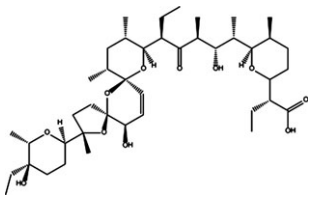
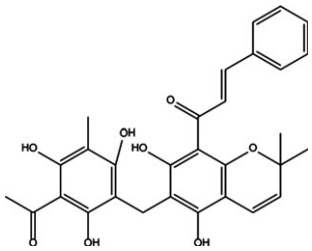
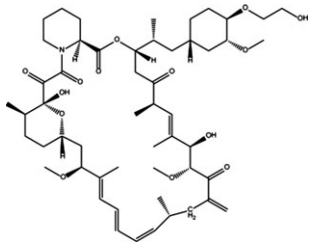
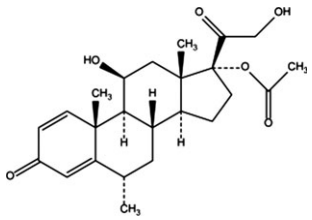
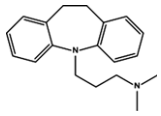
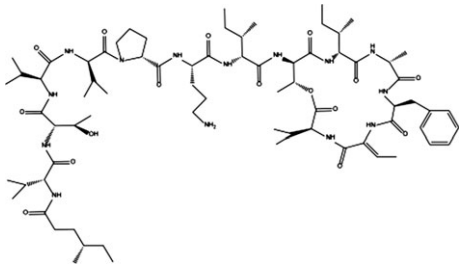
Autophagic pathways	Autophagy role	Drugs or chemical compounds	Chemical structure
PI3KCI/Akt/mTORC1 Inhibition	Pro-survival	Salinomycin	
PI3KCI/Akt/mTORC1 Inhibition	Pro-survival	ROT	
PI3KCI/Akt/mTORC1 Inhibition	Pro-death	RAD001	
PI3KCI/Akt/mTORC1 Inhibition	Pro-death	Dexamethasone	
PI3KCI/Akt/mTORC1 Inhibition	Pro-death	Imipramine	
PI3KCI/Akt/mTORC1 Inhibition	Pro-death	PM02734	

Table 7 (continued)

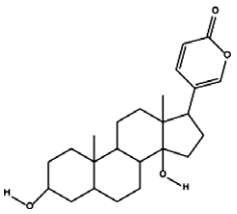
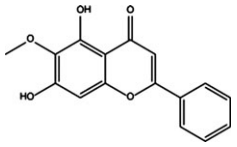
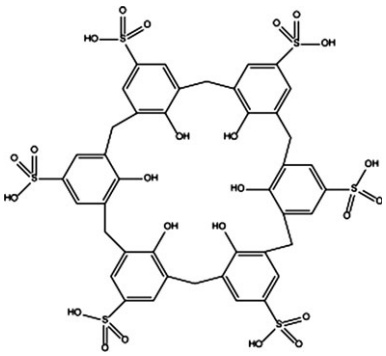
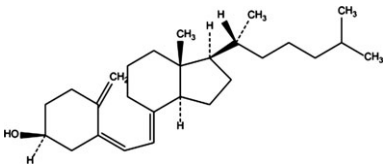
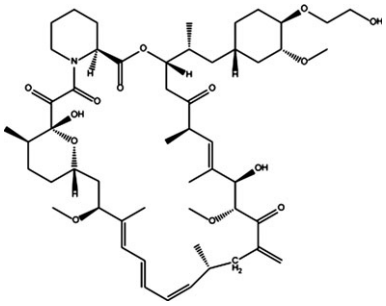
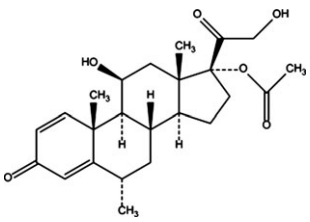
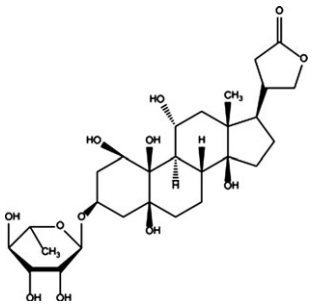
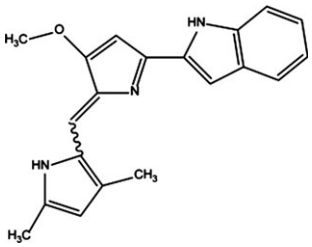
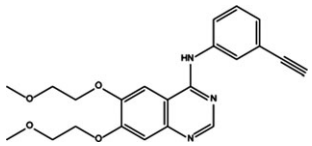
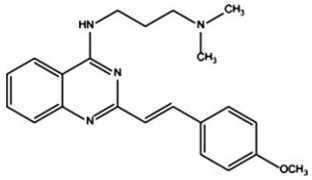
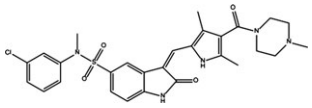
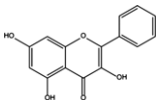
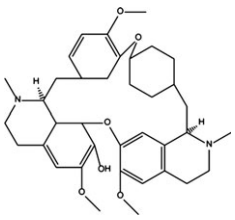
Autophagic pathways	Autophagy role	Drugs or chemical compounds	Chemical structure
PI3KCI/Akt/mTORC1 Inhibition	Pro-death	Bufalin	
PI3KCI/Akt/mTORC1 Inhibition	Pro-death	Oroxylin A	
PI3KCI/Akt/mTORC1 Inhibition	Pro-death	Calix[6]arene	
Beclin-1 up-regulation	Pro-death	Vitamin D3	
Beclin-1 up-regulation	Pro-death	RAD001	
Bcl-2/Bcl-X_L down-regulation	Pro-death	Dexamethasone	

Table 7 (continued)

Autophagic pathways	Autophagy role	Drugs or chemical compounds	Chemical structure
Bcl-2/Bcl-X_L down-regulation	Pro-death	Ouabain	
Beclin-1/Bcl-2 detachment	Pro-death	Obatoclax	
p53 up-regulation	Pro-survival	Erlotinib	
p53 up-regulation	Pro-survival	CP-31398	
p53 up-regulation	Pro-death	SU11274	
p53 up-regulation	Pro-death	Galangin	
p53 up-regulation	Pro-death	Fangchinoline	

for candidate drugs and plays a dual role in cell proliferation, by inducing cell survival or cell death, under different circumstances. Small molecule drugs and chemical compounds such as NVP-BEZ235, triciribine, ZD6474, β-elemene, salinomycin and ROT have been reported to induce autophagic cell survival by using the PI3KCI/Akt/mTORC1 pathway (50,64,67,91,92,94,123,140). Other candidates such as RAD001, dexamethasone, imipramine, PM02734, bufalin, oroxylin A and calix[6]arene have been shown to have the ability to induce pro-death autophagy through the PI3KCI/Akt/mTORC1 pathway (46,47,51,74,84,102,104,105,129). In addition, some candidate drugs such as rapamycin and resveratrol also have a double-faced role, by inducing pro-death or pro-survival autophagy in different cancer cells (40,41,54,142). Exact mechanisms of the different effects that different candidate drugs cause by regulating the PI3KCI/Akt/mTORC1 pathway remains unclear and calls for considerable further investigation.

Beclin-1 can positively regulate pro-death autophagy and is mediated by positive regulators such as Atg14L, UVRAG, Bif-1, Ambra1, HMGB1, Survivin, and PINK1, as well as negative regulators Bcl-2, Bcl-XL and Rubicon. Up-regulating Beclin-1 becomes an attractive target to induce pro-death autophagy. Candidate drugs obatoclax, RAD001 and vitamin D3 have been reported to induce autophagic cell death by up-regulating Beclin-1 in leukaemia cells (46,47,52,55). Bcl-2 and Bcl-X_L binds to Beclin-1 to inhibit autophagy and they have their key roles in cancer cell survival. Down-regulating Bcl-2/Bcl-X_L is also an attractive target for candidate drugs to induce autophagic cell death. Dexamethasone and quabain have been reported to induce autophagic cell death by Bcl-2/Bcl-X_L down-regulation (51,82). Also, some candidate drugs such as obatoclax may also induce pro-death autophagy by inducing dissociation of Beclin-1 from Bcl-2 (52). Although other Beclin-1 cofactors such as Atg14L, UVRAG, Bif-1, Ambra1, HMGB1, Survivin, PINK1 and Rubicon are capable of mediating Beclin-1 activity and have the potential to regulate autophagy, candidate drugs targeting other Beclin-1 cofactors have drawn less attention in recent areas of research.

p53 plays a dual regulatory role in autophagy depending on its subcellular localization, as nuclear p53 boosts autophagy and cytoplasmic p53 reduces it, independent of its transcriptional activity. This may dictate whether p53 contributes to cancer cell survival or death. Recent research has shown that candidate drugs such as erlotinib, CP-31398, SU11274, galangin and fangchinoline induce pro-death or pro-survival autophagy by regulating p53, but the key point to determine cell fate remains unclear (86,107,108,122).

Moreover, autophagy is closely associated with other pathological processes. Some candidate drugs such as IM, eupalinin A, sodium selenite, andrographolide and 5-FU are reported to affect autophagy by regulating pathological processes such as apoptosis, ER stress, oxidative stress and inflammatory responses (53,56,92,103,120). Non-specific autophagic pathways may provide potential targets to regulate autophagy in cancer cells.

Conclusions

Autophagy, an evolutionarily conserved lysosomal degradation process, has a Janus face in cancer: it acts as a survival mechanism or leads to autophagic cell death under different conditions. Autophagy can be modulated by some key oncogenes (*Bcl-2*, *PI3KCI*, *Akt* and *mTORC1*) and tumour suppressors (Beclin-1,PI3KCIII, Bif-1, UVRAG, DAPKs, PTEN and p53), which determine the fate of the cancer cells.

Many anti-cancer drugs affect autophagy. In recent years, many new discoveries of drugs and chemical compounds related to autophagy in different types of cancer have been made. However, for some newly discovered autophagy inducers, neither the role nor the mechanism of autophagy is clear, thus calling for further studies in the future. Moreover, due to the complicated Janus nature of autophagy, establishing the dual role of autophagy in tumour survival versus death may help to improve cancer therapeutic potential. Some anti-cancer drugs have the ability to induce pro-survival autophagy, which diminishes its ability to induce death of tumour cells. Thus, application of combined treatment with autophagy inhibitors may overcome drug resistance and become a new strategy to increase therapeutic effect. However, some drugs are reported to be associated to autophagic cell death, and its induction has become a new concept to trigger tumour cell death. Both strategies have significant potential to be translated into ongoing clinical trials that may shed light on exploiting new anti-tumour small molecule drugs for future cancer therapy.

Small molecule drugs and chemical compounds can affect autophagy, and induce autophagic cell death or cell survival through both specific autophagic pathways and non-specific ones. The PI3KCI/Akt/mTORC1 pathway is a major target for candidate drugs and plays a dual role in cell proliferation, by inducing cell survival or cell death under different circumstances. Exact mechanisms to determine cell fate remain unclear and require further investigation. Up-regulating Beclin-1, down-regulating Bcl-2/Bcl-X_L and dissociating Beclin-1 from Bcl-2 are three major mechanisms for candidate drugs which target the Beclin-1 pathway to induce autophagic cell death in recent research, although, candidate drugs regulating other Beclin-1

cofactors may be of potential research value. Candidate drugs targeting p53 have been reported to cause autophagic cell death and survival under different circumstances, but mechanisms of the different effects remain unclear. In addition, non-specific autophagic pathways may provide potential targets to regulate autophagy in cancer cells.

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Conflict of interest

We declare that none of the authors have a financial interest related to this work.

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