Chapter 3
Small-Molecule Regulators of Autophagy as Potential Anti-cancer Therapy

Qing Li, Mi Zhou, and Renxiao Wang

Abstract Autophagy is an evolutionary conserved lysosomal pathway functioned in the turnover of cellular macromolecules and organelles. It is known that autophagy can have a cytoprotective effect in tumor cells under therapeutic treatment. Autophagy inhibitors thus may be used as auxiliary drugs to augment the anti-tumor activity of cancer therapies. On the other hand, autophagy is a cytotoxic event that can kill tumor cells. Autophagy inducers that increase the level of autophagy thus may be developed as a new class of anti-cancer therapy. This chapter will describe the known pathway of autophagy and its relationship to cancer. The focus of this chapter is to give a summary of the known small-molecule regulators of autophagy, including inhibitors and inducers, discovered as potential therapies for cancer treatment.

Keywords Autophagy • Cell death • Autophagy inhibitor • Autophagy inducer • Anti-cancer treatment

3.1 Introduction

Autophagy plays an essential role in normal physiology. Under normal conditions, autophagy occurs at basal levels to maintain cellular homeostasis by removing long-lived or misfolded proteins and clearing damaged or dysfunctional organelles. Under starved conditions, autophagy can be induced to digest dysfunctional proteins and organelles more rapidly, which will help cell to survive (Green and Levine 2014). It is also well known that autophagy may protect cell by overcoming adversities,

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such as starvation, chemotherapies or radiotherapies. Autophagy can also exhibit cytotoxicity in certain condition, e.g. when apoptosis is blocked (Gewirtz 2014).

In this chapter, we will briefly introduce the molecular mechanism of autophagy and its dual role in anti-cancer therapy development. We will also review the public reported small-molecule regulators of autophagy, including autophagy inhibitors and autophagy inducers, discovered as potential anti-cancer therapies.

3.2 The Process of Autophagy

The known pathway of autophagy includes induction, formation and elongation of isolation membrane, autophagosome completion, fusion of autophagosome and lysosome, and degradation in autolysosome (Fig. 3.1). Autophagy is regulated by a "pre-initiation" ULK complex, which includes ULK1, FIP200 and ATG13. The ULK complex then activates Class III PI3K complex, which requires the disruption of binding of anti-apoptotic Bcl-2 proteins to Beclin 1 and is also regulated by AMPK. The Class III PI3K complex generates Phosphatidylinositol 3-phosphate

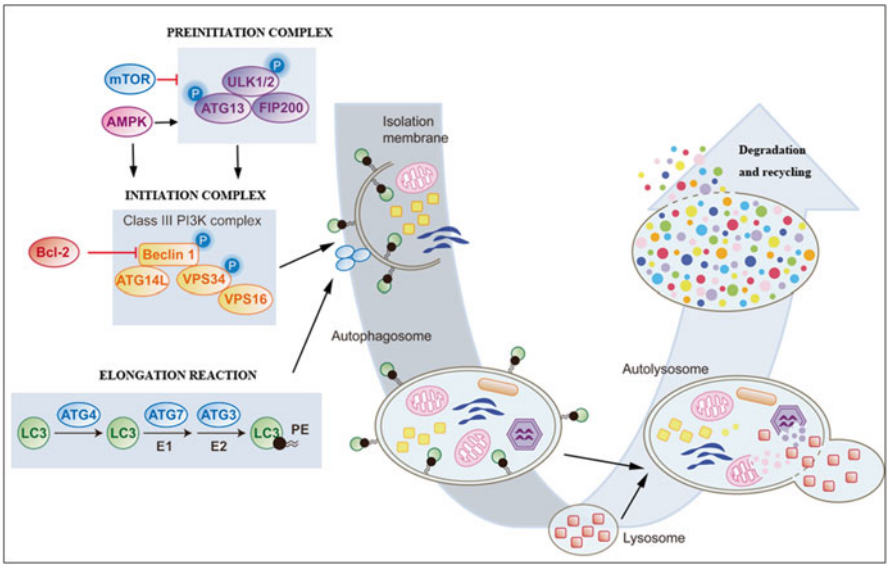


Fig. 3.1 The process of autophagy. The process of autophagy starts with the formation of an isolation membrane, which is regulated by the initiation Class III PI3K complex. The Class III PI3K complex is activated by the pre-initiation ULK complex which is negatively and positively regulated by upstream kinases mTOR and AMPK, respectively. The activated Class III PI3K complex generates PI3P at the site of nucleation of the isolation membrane. This event leads to the binding of proteins involved in the "elongation reaction" to the isolation membrane, resulting in formation of autophagosome. Then, autophagosome fuses with lysosome to form autolysosome, in which its contents undergo degradation and recycling

(PI3P) at the site of nucleation of the isolation membrane (also called the phagophore). The elongation reaction of the isolation membrane is a complicated process to form LC3-II. LC3-II generated by the ATG4-dependent proteolytic cleavage of LC3, and required of ATG7, ATG3, and the ATG12/ATG5/ATG16L complex, which is associated with the mature autophagosome. The autophagosome fuses with a lysosome to form an autolysosome, in which the surrounded contents are degraded and released into the cytoplasm for recycling (Marino et al. 2014).

In the context of nutrient starvation, AMPK is activated and/or mTORC1 is inhibited, which in turn activated the ULK complex to engage autophagy. During starvation-induced autophagy, AMPK is required to release negative regulators of the Beclin 1-VPS34 initiation complex, such as Bcl-2/Bcl-xL (Wirth et al. 2013).

3.3 Role of Autophagy in Cancer

Autophagy has been shown to act as a tumor suppressor, but its role in cancer treatment is still controversial (Maycotte and Thorburn 2011). Almost all of traditional anti-cancer therapies, such as anti-cancer drugs and ionizing radiation, affect autophagy. Most of anti-cancer drugs increase autophagy, which protects treated tumor cells to survive. However, it also has been reported that autophagy is a cell death mechanism when apoptosis is blocked, known as autophagic cell death (Maycotte and Thorburn 2011; Thorburn et al. 2014).

3.3.1 Tumor Promotion

In tumorigenesis, the rapid growth of tumor tissue puts cancer cells under harsh and continuous metabolic stress that results in nutrient deprivation, growth factor limitation, and hypoxia (Zhou and Wang 2013). Nutrient deprivation induces autophagy by mTORC1 inhibition and AMPK activation. Autophagy can also be induced by hypoxia, it has been found to localize to hypoxic tumor regions, supporting cell survival through elimination of autophagic substrate p62, damaged mitochondria and reactive oxygen species (ROS) (Maycotte and Thorburn 2011; Zhou and Wang 2013).

Autophagy may promote tumor cell metastasis by preventing anoikis. When cells are detached from the extracellular matrix (ECM), they may undergo anoikis. However, metastatic tumor cells may escape from anoikis and invade other organs (Frisch and Screaton 2001). Loss of clonogenic capacity is a foundational factor during tumorigenesis, autophagy can be induced to reduce clonogenic capacity after anoikis (Fung et al. 2008).

In tumor treatment, autophagy has been proposed as a protective mechanism to resist chemo- or radio-therapy and to help residual tumor cells to enter dormancy. It is well known that autophagy can function as a survival mechanism which is activated after cancer treatment. In certain instances, tumor can relapse and metas-

tasize after primary tumor treatment in many years later, suggesting residual tumor cells may remain in a dormant state. A recent study showed overexpression of tumor suppressor *aplasia Ras homolog member I* (*ARHI*) promotes the formation of dormant tumors, which was reduced by autophagy inhibitor CQ (Sosa et al. 2013).

3.3.2 Tumor Suppression

Autophagy occurs at basal levels during nutrient rich conditions. The basal autophagy has been shown to be a tumor suppressor mechanism. Cell-cycle check-points are inactivated in tumor cells, but autophagy limits the accumulation of DNA damage and suppresses the mutation rate. It confirms the role for autophagy in protecting the genome in a cellular spontaneous mechanism of tumor suppression (Mathew et al. 2007).

A direct link between autophagy and tumor suppression is the discovery that Beclin 1 could function as a tumor suppressor (Liang et al. 1999). The autophagy gene *Beclin 1* is mono-allelic deleted in 40–75 % of cases of human sporadic breast, ovarian, and prostate cancer. Disruption of *Beclin 1* increases the frequency of spontaneous malignancies and accelerates the development of *hepatitis B* virus-induced premalignant lesions in a targeted mutant mouse model (Qu et al. 2003). In addition, animals deficient in autophagy-related *Atg4C* show an increased susceptibility to develop fibrosarcomas induced by chemical carcinogens (Marino et al. 2007).

In apoptosis-deficient cancer cells, autophagy has been induced to maintain cell metabolism and viability during nutrient starvation and protect cells from necrosis. Finally, if the nutrient deprivation persists, continuous autophagy may lead to autophagic cell death, which is type II programmed cell death (PCD) (the others are type I PCD apoptosis and type III PCD necrosis) (Clarke 1990). Autophagic cell death can be suppressed by autophagy inhibitors (e.g., 3-methyladenine and wortmannin) or genetic knockout/knockdown of essential autophagy genes (Shimizu et al. 2014). A recent study indicated that JNK activation is crucial for the autophagic cell death of *Bax*/*Bak* double knockout cells (Shimizu et al. 2010).

3.4 Small-Molecule Regulators of Autophagy

A good number of small-molecule regulators of autophagy have been reported in literature. They have been used either as chemical tools in basic research on autophagy, or developed as drug candidates for cancer treatment (Zhou and Wang 2013; Baek et al. 2012; Fleming et al. 2011; Wu and Yan 2011; Levy and Thorburn 2011; Nagelkerke et al. 2015).

3.4.1 Autophagy Inhibitors

There is extensive and relatively definite evidence showed that the level of autophagy increased in tumor cells. Considering the tumor promotion mechanism of autophagy, many compounds have been developed to treat cancers based on their autophagy inhibition function (Table 3.1).

Multiple clinical trials are currently on-going at every phase by combining autophagy inhibitors with various conventional treatment methods in order to enhance the response to treatment (Gewirtz 2014; Kumar et al. 2015).

3.4.1.1 Class III PI3K Inhibitors

The Class III PI3K, Vps34, shows the positive relationship with autophagy and generates PI3P at the site of nucleation of the isolation membrane by forming a complex with Beclin 1 and other cofactors (Green and Levine 2014). A number of PI3K inhibitors have been developed as autophagy inhibitors, including wortmannin, LY294002, 3-methyladenine (3-MA), and SAR405.

Wortmannin, a steroid metabolite of the fungi *Penicillium funiculosum*, is a nonspecific covalent PI3K inhibitor (Powis et al. 1994). LY294002 is a morpholino derivative of quercetin (Vlahos et al. 1994). Wortmannin derivative PX-866 and LY294002 (Arg-Gly-Asp-Ser)-conjugated SF1126 were shown to be active against various cancer xenografts (Maira et al. 2009). Treatment with wortmannin or LY294002 resulted in a strong inhibition of proteolysis in amino acids-deprivation rat hepatocytes (Blommaart et al. 1997). 3-MA inhibited endogenous protein degradation by about 60 % at 5 mM, and suppressed the formation of autophagosomes (Seglen and Gordon 1982). These three PI3K inhibitors act on PI3K nonselectively, regarding as tools to study PI3K/mTOR pathway and autophagy.

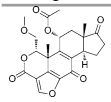
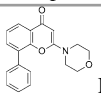
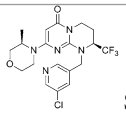
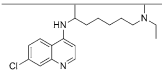
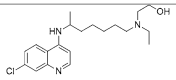
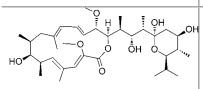
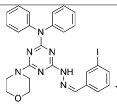
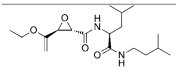
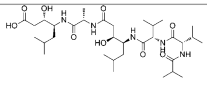
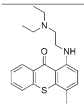
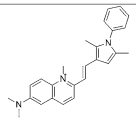
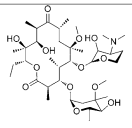
SAR405, a derivative of pyrimidinones, is a first reported selective inhibitor of class III PI3K Vps34. Inhibition of Vps34 by SAR405 affects late endosome-lysosome compartments and prevents autophagy, co-treatment with SAR405 and mTOR inhibitor everolimus results in synergistic anti-proliferative activity in renal tumor cell lines (Ronan et al. 2014).

3.4.1.2 Compounds Disrupting Lysosomal Homeostasis

Lysosome is a membrane-bound cell organelle found in most animal cells. It contains hydrolytic enzymes capable of breaking down virtually all kinds of biomolecules. In the late stage of autophagy, lysosomes fuse with autophagosomes to digest the contents of autophagosomes.

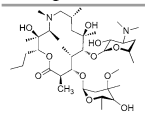
Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) are widely used as the first choice drug for malaria treatment. CQ seems to exert its effects through the weak-base feature by enriching in acidic lysosomes, and thereby destroyed lyso-

Table 3.1 Small-molecule inhibitors of autophagy

Compound	Mechanism and references	Compound	Mechanism and references
 Wortmannin	Pan-PI3K inhibitor (Powis et al. 1994; Maira et al. 2009; Blommaart et al. 1997)	 LY294002	Pan-PI3K inhibitor (Maira et al. 2009; Blommaart et al. 1997; Vlahos et al. 1994)
 3-methyladenine (3-MA)	Pan-PI3K inhibitor (Maira et al. 2009; Seglen and Gordon 1982)	 SAR405	Class III PI3K inhibitor (Ronan et al. 2014)
 Chloroquine (CQ)	Disrupts lysosomal homeostasis (Homewood et al. 1972; Fukuda et al. 2015; Balic et al. 2014; Kimura et al. 2013)	 Hydroxychloroquine (HCQ)	Disrupts lysosomal homeostasis (Homewood et al. 1972)
 Bafilomycin A1	ATPase inhibitor, disrupts lysosomal homeostasis (Harada et al. 1996; Mauvezin and Neufeld 2015)	 Vacuolin-1	Disrupts lysosomal homeostasis (Cerny et al. 2004; Lu et al. 2014)
 Matrine	Disrupts lysosomal homeostasis (Chen et al. 2006; Liu et al. 2010; Wang et al. 2013)	 Thymoquinone	Disrupts lysosomal homeostasis (Racoma et al. 2013)
 E-64d	Cathepsin inhibitor, disrupts lysosomal homeostasis (Tamai et al. 1986; Tanida et al. 2005)	 Pepstatin A	Cathepsin inhibitor, disrupts lysosomal homeostasis (Tanida et al. 2005; Umezawa et al. 1970)
 Lucanthone	Topoisomerase inhibitor, disrupts lysosomal homeostasis (Bases and Mendez 1997; Carew et al. 2011)	 Pyrvinium	Casein kinase activator, inhibits the transcription of autophagy genes (Thorne et al. 2010; Deng et al. 2013)
 NSC185058	ATG4B inhibitor, inhibits formation of LC3-II (Akin et al. 2014)	 Clarithromycin	Macrolide antibiotic (Nakamura et al. 2010)

(continued)

Table 3.1 (continued)

Compound	Mechanism and references	Compound	Mechanism and references
 Azithromycin	Macrolide antibiotic (Renna et al. 2011)		

somal function (Homewood et al. 1972). It shows the antitumor activity in many kind of tumor cells, such as endometrial cancer cells (Fukuda et al. 2015), pancreatic cancer stem cells (Balic et al. 2014). Inhibition of autophagy by CQ could sensitize cisplatin-tolerant cancer cells, as well as injure kidney cells in chemotherapy, leading to acute kidney injury (Kimura et al. 2013).

Bafilomycin A1 is an inhibitor of V-ATPase, which is necessary for acidification of the endocytic compartments (Harada et al. 1996). It can also disrupt autophagic flux by inhibiting calcium ATPase-dependent autophagosome-lysosome fusion (Mauvezin and Neufeld 2015).

Vacuolin-1 has been discovered in an image-based phenotypic screen for inhibitors of the secretory pathway, by blocking the Ca^{2+}-dependent exocytosis of lysosomes (Cerny et al. 2004). Treatment with vacuolin-1 alkalinized lysosomal pH and decreased lysosomal Ca^{2+} content in HeLa cells (Lu et al. 2014).

Matrine, derived from traditional Chinese medicine *Sophora flavescens*, has been reported to improve the immune function and life quality of cancer patients by combining standard therapies (Chen et al. 2006). It can also inhibit proliferation and induce apoptosis of pancreatic cancer cells (Liu et al. 2010). Recently matrine has been reported to block autophagic degradation by impairing the activities of lysosomal proteases, and elevating pH values in endosomes/lysosomes (Wang et al. 2013).

Thymoquinone, derived from *Nigella sativa* seed, was reported to inhibit proliferation in glioblastoma cells. It induced lysosomal membrane permeabilization, resulting in a leakage of cathepsin B into the cytosol, which mediates caspase-independent cell death (Racoma et al. 2013).

Cathepsins are proteases distributed in almost all mammalian cells, with functions in tumor progression (Nomura and Katunuma 2005). Most of the members of cathepsins become activated at the low pH level in lysosomes. Their activities are closely linked with the lysosomal function. Cathepsins inhibitors E64d (Tamai et al. 1986) and pepstatin (Umezawa et al. 1970) are frequently used in autophagy-related research as autophagy inhibitors (Tanida et al. 2005).

Lucanthone, an inhibitor of topoisomerase, has been used as an adjuvant in radiation therapy (Bases and Mendez 1997). It induces lysosomal membrane permeabilization to break lysosomal homeostasis, and possesses significantly more potent activity in breast cancer models compared with CQ (Carew et al. 2011).

3.4.1.3 Others Types of Autophagy Inhibitors

An FDA-approved antihelminthic drug pyrvinium shows wide-ranging anti-cancer activity during glucose starvation. It binds casein kinase 1 and increases the kinase activity of casein kinase 1α, which is a negative regulator of Wnt1 pathway (Thorne et al. 2010). Pyrvinium was reported to inhibit autophagy by suppressing the transcription of autophagy genes, such as *Beclin1* and *Vps34*. The inhibition of autophagy by pyrvinium increases the anti-cancer activity of 2-deoxy-D-glucose (Deng et al. 2013).

ATG4B is an essential cysteine proteinase to activate LC3 produce LC3-II. Knockdown of *Atg4b* in Osteosarcoma Saos-2 cells resulted in a failure of forming tumors in mouse models. The antagonist of ATG4B NSC185058 shows a negative impact on the development of Saos-2 osteosarcoma tumors *in vivo* (Akin et al. 2014).

Macrolide antibiotic clarithromycin and aithromycin were reported to block autophagy, whose mechanisms still remain unclear. Clarithromycin increased the anti-tumor activity of thalidomide against multiple myeloma cells (Nakamura et al. 2010). Azithromycin mediated autophagy increases the risk of infection with drug-resistant pathogens (Renna et al. 2011).

3.4.2 Autophagy Inducers

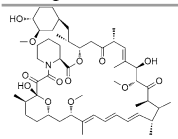
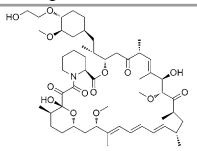
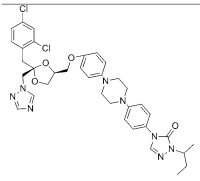
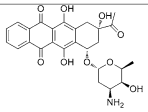
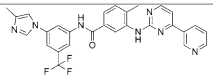
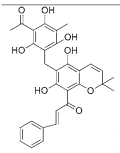
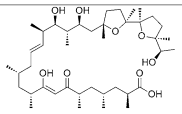
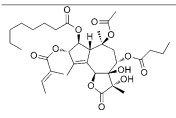
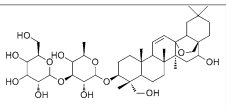
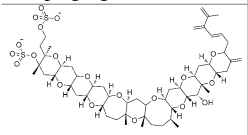
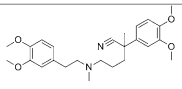
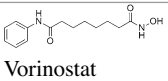
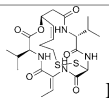
Based on the role of autophagy in tumor suppression, many compounds are used as anti-cancer reagents by inducing autophagy (Fulda and Kogel 2015) (Table 3.2). It should be noted that not all autophagy inducers may be used as anti-cancer reagents. It is because some of them induce protective autophagy, which leads to tumor resistance. These compounds are combined with autophagy inhibitors to treat cancer usually, so they are not discussed in this chapter.

3.4.2.1 mTOR Inhibitors

mTOR (mammalian Target Of Rapamycin) senses cellular nutrient and energy levels, and negatively regulates autophagy. mTOR forms two distinct signaling complexes, mTORC1 and mTORC2. The molecular mechanism of how mTORC2 is regulated by its upstream effectors is largely unknown. mTORC1 (hereafter mTOR) is a master regulator of cellular metabolism and autophagy, regulated by the growth factor/PI3K/AKT signaling pathway. It integrates nutrient and growth factors which signal to promote anabolic metabolism, such as protein synthesis and lipid synthesis, and to inhibit catabolic pathways, such as lysosomal biogenesis and autophagy (Laplante and Sabatini 2012).

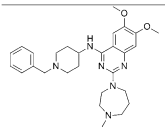
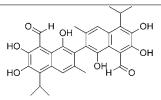
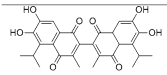
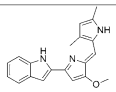
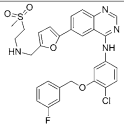
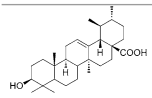
Inhibition of mTOR leads to activation of ULK1, which then phosphorylates other critical subunits of the ULK1 complex, ATG13 and FIP200 (Jung et al.

Table 3.2 small-molecule inducers of autophagy

Compound	Mechanism and references	Compound	Mechanism and References
 Rapamycin	mTOR inhibitor (Kim and Guan 2015; Nam et al. 2013)	 Everolimus	mTOR inhibitor (Kim and Guan 2015; Albert et al. 2006; Cao et al. 2006)
 Itraconazole	Antifungal drug, inhibits mTOR/AKT/PI3K signaling pathway (Liu et al. 2014)	 Idarubicin	Topoisomerase inhibitor, inhibits mTOR activity (Ristic et al. 2014; Plumbridge and Brown 1978)
 Metformin	AMPK activator (Takahashi et al. 2014)	 Nilotinib	Tyrosine kinase inhibitor, activates AMPK (Yu et al. 2013)
 Rottlerin	Protein kinase C inhibitor, activates AMPK (Kumar et al. 2014)	 Ionomycin	Disrupts calcium homeostasis (Hoyer-Hansen et al. 2007; Hoyer-Hansen and Jaattela 2007)
 Thapsigargin	Disrupts calcium homeostasis (Hoyer-Hansen et al. 2007; Hoyer-Hansen and Jaattela 2007)	 Saikosaponin-d	Disrupts calcium homeostasis (Wong et al. 2013)
 Yessotoxin	Disrupts calcium homeostasis (Rubiolo et al. 2014; Azad et al. 2008)	 Verapamil	Disrupts calcium homeostasis (Salabei et al. 2012)
 Valproic acid	HDACi, induces ROS-dependent autophagy (Shao et al. 2004; Fu et al. 2010)	 Vorinostat	HDACi, induces autophagic cell death (Shao et al. 2004; Zhang et al. 2005; Yamamoto et al. 2008; Wei et al. 2010)
 Sodium butyrate	HDACi, induces autophagic cell death (Shao et al. 2004; Hamer et al. 2008)	 FK228	HDACi, induces autophagic cell death (Watanabe et al. 2009)

(continued)

Table 3.2 (continued)

Compound	Mechanism and references	Compound	Mechanism and References
Arsenic trioxide (As_2O_3)	Induces autophagic cell death (Miller et al. 2002; Goussetis et al. 2010)	Sodium arsenite ($NaAsO_2$)	Induces ROS-dependent autophagic cell death (Miller et al. 2002; Zhu et al. 2014; You et al. 2015)
 BIX-01294	EHMT2 inhibitor, induces autophagic cell death (Kim et al. 2013a)	 Gossypol	Bcl-2 inhibitor, induces autophagy by releasing Beclin 1 (Shimizu et al. 2004; Voss et al. 2010)
 Apogossypolone	Bcl-2 inhibitor, induces autophagy by releasing Beclin 1 (Zhang et al. 2010; Arnold et al. 2008; He et al. 2014; Niu et al. 2014)	 Obatoclax	Bcl-2 inhibitor, induces autophagic cell death (Heidari et al. 2010; Bonapace et al. 2010)
 Lapatinib	Tyrosine kinase inhibitor, induces autophagic cell death (Chen et al. 2015; Chen et al. 2014)	 Gemcitabine	Induces autophagic cell death (Wang et al. 2014; Donadelli et al. 2011)
 Ursolic acid	Induces ATG-5-dependent autophagy (Leng et al. 2013)	Salinomycin	Cation ionophore, activates autophagic flux

2009). The growth factor/PI3K/AKT/mTOR pathway is the main pathway regulated by mTOR, its activation is associated with malignant transformation and apoptotic resistance, and hence represents a cell survival mechanism (Polivka and Janku 2014).

In earlier studies, mTOR inhibitors, such as rapamycin and everolimus, were reported to induce autophagy in various model systems. The induction of autophagy by mTOR inhibitors are more inclined to protect cancer cells survival, not death (Kim and Guan 2015). However, recent reports showed rapamycin-induced autophagy may sensitize cancer cells to radiotherapy. Everolimus inhibited radiation-induced AKT/mTOR signaling pathway and enhances the cytotoxic effects of radiation in breast cancer cell models (Albert et al. 2006). It increased the radio-sensitization of PTEN-null prostate cancer cells, and enhances radiation-induced mortality in apoptosis deficient cells, which means everolimus may induce autophagic cell death in certain condition (Cao et al. 2006). In addition, persistent activation of autophagy by mTOR inhibitor rapamycin leads radio-resistant cancer cells into senescence in head and neck cancer cells and a xenograft model (Nam et al. 2013).

Itraconazole, a traditional broad-spectrum antifungal drug, inhibited cell proliferation and induced autophagic progression in glioblastoma cells by repression of AKT/mTOR signaling pathway. Its anti-proliferative activity was inhibited by the blockage of autophagy, suggesting autophagy is responsible for itraconazole-induced inhibition of proliferation (Liu et al. 2014).

Idarubicin is a DNA-binding antileukemic drug (Plumbridge and Brown 1978), has been showed to induce apoptosis and cytotoxic autophagy through mTOR repression. Autophagy inhibitor wortmannin or CQ partially reduced the cytotoxicity of idarubicin in the acute lymphocytic leukemia REH cells (Ristic et al. 2014).

3.4.2.2 AMPK Activators

AMPK are known to induce autophagy. Under starvation condition, AMPK induction and/or mTOR inhibition will lead to autophagy by ULK1 phosphorylation. AMPK can also act directly on the Beclin 1/Vps34 complex (Kim et al. 2013b).

Metformin, a prescribed drug for type 2 diabetes, activated AMPK and reduced cell proliferation, leading to the induction of apoptosis and autophagy. Inhibition of autophagy by knockdown of *Beclin 1* or by 3-MA suppressed the anti-proliferative effects of metformin on endometrial cancer cells, indicating that the anti-proliferative effects of metformin are partially or completely dependent on autophagy (Takahashi et al. 2014).

Nilotinib, a tyrosine kinase inhibitor, significantly reduced cell viability in hepatocellular carcinoma cell lines through autophagy by AMPK activation instead of apoptosis. Knock-down of *Atg5* reduced the effect of nilotinib on autophagy and cell death significantly, co-treatment of nilotinib with a known AMPK activator metformin enhanced the effect of nilotinib on autophagy and cell death (Yu et al. 2013).

Rottlerin, a protein kinase C inhibitor, showed anti-cancer activity in prostate cancer. It induced early stage autophagy via AMPK activation and apoptosis via inhibiting PI3K/AKT/mTOR pathway in human prostate cancer stem cells. Co-treatment with autophagy inhibitors bafilomycin or 3-MA inhibited rottlerin-induced apoptosis. It illustrated that autophagy was required in rottlerin mediated prostate cancer treatment (Kumar et al. 2014).

3.4.2.3 Compounds Disrupting Calcium Homeostasis

Calcium (Ca^{2+}) is one of the most important cellular second messengers. The disorder of Ca^{2+} homeostasis can evoke different types of cell death in cancer cells. Autophagic cell death can be induced by vitamin D3, ATP, ionomycin and thapsigargin, which increased the cytosolic Ca^{2+} in MCF-7 breast cancer cells (Hoyer-Hansen et al. 2007). One of the best investigated mechanisms of calcium-mediated autophagy induction is mTOR-mediated endoplasmic reticulum (ER) stress and unfolded protein response (UPR) activation (Hoyer-Hansen and Jaattela 2007).

Saikosaponin-d is an inhibitor of Ca^{2+} ATPase which induces autophagy by activating the Ca^{2+}/calmodulin-dependent AMPK/mTOR signaling pathway, ER stress and UPR. It leaded to autophagic cell death especially in apoptosis-resistant MEFs cells, which either lack *Caspases 3*, *7* or *8* or have the *Bax/Bak* double knockout (Wong et al. 2013).

Yessotoxin has been shown to modulate Ca^{2+} gating resulted in increasing cytosolic calcium in human lymphocytes. In glioma cells, it induced autophagic cell death mediated by BNIP3 (Rubiolo et al. 2014). BNIP3 inhibits the mTOR pathway through RHEB, which has been shown to induce cell death by autophagy inhibitor 3-MA but not by caspase inhibitor z-VAD-fmk (Azad et al. 2008).

Verapamil is an L-type Ca^{2+} channel antagonist. Treatment with verapamil did not affect cell viability in vascular smooth muscle cells, but inhibited cell proliferation and induced morphological alterations, such as karyokinesis and accumulated perinuclear vacuoles due to enhanced mitochondrial damage and upregulated autophagy (Salabei et al. 2012).

3.4.2.4 Histone Deacetylase Inhibitors

Histone deacetylase (HDAC) is a class of enzymes that remove acetyl groups from an lysine on histone, allowing the histones to wrap the DNA more tightly, leading to chromatin remodeling and transcriptional suppression of key apoptosis and cell cycle regulatory genes (Jazirehi 2010).

It has been reported that HDAC inhibitors preferentially kill transformed cells or cancer cells. According to their chemical structures, HDAC inhibitors can be classified into several groups, including (i) short-chain fatty acids, such as sodium butyrate and valproic acid ; (ii) hydroxamic acids, such as vorinostat; and (iii) cyclic tetrapeptides, such as FK228 (Shao et al. 2004).

Valproic acid, a widely used anti-epilepsy drug, induces autophagy by ROS-dependent pathway in glioma cells. Combination with other autophagy inducers (such as rapamycin, LY294002) increased valproic acid-induced autophagic cell death (Fu et al. 2010). Sodium butyrate exerts potent effects on the inhibition of inflammation and carcinogenesis in colon tissue (Hamer et al. 2008). It induces mitochondria-mediated apoptosis and autophagic cell death in HeLa cells (Shao et al. 2004).

Vorinostat (also known as suberoylanilide Hydroxamic Acid) is approved by FDA to treat cutaneous T cell lymphoma (Zhang et al. 2005). It was reported to induce apoptosis and autophagic cell death in chondrosarcoma cell lines and HeLa cells (Shao et al. 2004; Yamamoto et al. 2008). Combination with vorinostat and BH3-mimetic GX15-070 has synergistic effects in acute myeloid leukemia (AML) cell lines and primary AML cells by activating both apoptosis and autophagy (Wei et al. 2010).

Another HDAC inhibitor FK228 can also induce autophagy. Disrupting autophagy with CQ enhanced FK228-induced cell death, which means FK228-induced autophagy is cytoprotective (Watanabe et al. 2009).

3.4.2.5 BH3 Mimetics

Anti-apoptotic Bcl-2 proteins show its anti-apoptotic activity by binding pro-apoptotic protein Bax/Bak, they inhibit autophagy by binding Beclin 1 through their BH3 domain. Consequently, BH3 mimetics are able to activate apoptosis and autophagy (Pattingre and Levine 2006). They have a role in control of autophagic cell death depends on the autophagy genes *Beclin 1* and *Atg5* (Shimizu et al. 2004).

Bcl-2 inhibitor gossypol was reported to induce autophagic cell death by releasing Beclin 1 in malignant glioma, it potentiated the anti-cancer activity of temozolomide, which was used as a first-line treatment of glioblastoma multiforme (Voss et al. 2010). Apogossypolone is a derivative of gossypol, exhibits a higher antitumor activity and lower toxicity than gossypol (Zhang et al. 2010; Arnold et al. 2008). It can also inhibit the binding of Bcl-2 to Beclin 1, it induces autophagy and radiosensitizing in nasopharyngeal carcinoma cells *in vitro* and *in vivo* (He et al. 2014). Moreover, it reduced Bcl-2 expression, and enhanced the expression of Bax and Beclin 1 in MCF-7 cells (Niu et al. 2014).

Obatoclax (also known as GX15-070) has been reported to overcome glucocorticoid resistance in acute lymphoblastic leukemia (ALL) by inducing apoptosis and autophagy, which can be inhibited by downregulation of ATG5 or Beclin 1 (Heidari et al. 2010). In childhood ALL cells, obatoclax plays a role in autophagy-dependent necroptosis, which is required for overcoming glucocorticoid resistance (Bonapace et al. 2010).

3.4.2.6 Other Types of Autophagy Inducers

Inorganic arsenic is a worldwide environmental pollutant. It is widely known as carcinogens that induce cancers in many human tissues (Miller et al. 2002). Arsenic trioxide (As_2O_3) has been used to treat acute promyelocytic leukemia, and also acts as a potent inducer of autophagy in leukemia cells. Treatment with autophagy inhibitors or knockdown of *Beclin 1* or *Atg7* resulted in the decreased inhibition of arsenic trioxide on leukemic cell lines and primary leukemic progenitors from AML patients (Goussetis et al. 2010). Another trivalent arsenicals sodium arsenite ($NaAsO_2$) has showed the positive relationship with the incidence of type 2 diabetes. This phenomenon may be attributed to the ability of sodium arsenite in inducing ROS-dependent autophagic cell death in pancreatic β-cells. Autophagy inhibitor 3-MA protected the cells against sodium arsenite cytotoxicity, and autophagy inducer rapamycin further decreased the cell viability of sodium arsenite-treated INS-1 rat insulinoma cells (Zhu et al. 2014). Sodium arsenite showed an anti-proliferative effect on DU145 prostate cancer cells in xenograft mice, it induced both apoptosis and autophagic cell death via ROS (You et al. 2015).

BIX-01294, a selective inhibitor of euchromatic histone-lysine N-methyltransferase 2 (EHMT2), induced autophagic cell death via EHMT2 dysfunction and intracellular ROS production, and increased autophagy-dependent and caspase-independent cell death in primary human breast and colon cancer cells (Kim et al. 2013a).

Lapatinib, a tyrosine kinase inhibitor, has been widely accepted in the treatment of breast cancer. In breast cancer cells, it induced apoptosis and protective autophagy related with lapatinib resistance (Chen et al. 2015). But in human hepatoma cells, researchers found that lapatinib induced autophagy, a higher percent of dead cells and a lower percent of hypodiploid cells, that suggesting non-apoptotic cell death but autophagic cell death in lapatinib-treated hepatoma cells (Chen et al. 2014).

Gemcitabine (GEM) is currently the first-line treatment for pancreatic cancer. GEM elevated autophagic progress by the MEK/ERK signaling pathway. The autophagic activity was reduced in GEM-resistant human pancreatic cell line KLM1-R compared to GEM-sensitive KLM1 cells, suggesting autophagy was required in GEM-mediated pancreatic cancer treatment (Wang et al. 2014). Combination with GEM and cannabinoid triggers autophagic cell death in pancreatic cancer cells through a ROS-mediated mechanism (Donadelli et al. 2011).

Ursolic acid (UA), a natural pentacyclic triterpenoid carboxylic acid, has been showed potent anti-cancer activity. UA was reported to promote cervical cell lines TC-1 cell death, not through apoptosis but ATG5-dependent autophagy. Treatment with autophagy inhibitor and *Atg5* knockdown increased the survival of TC-1 cells treated with UA (Leng et al. 2013).

Salinomycin, a cation ionophore, has been showed to reduce the viability of breast cancer stem-like/progenitor cells by inhibiting autophagy (Yue et al. 2013). However, it has been reported to induce autophagy in human non-small cell lung cancer cells (Li et al. 2013). In recent study by Li et al. (Jangamreddy et al. 2015), treatment with lower concentration of salinomycin activates autophagic flux in prostate cancer cells while murine embryonic fibroblasts (MEFs) show an inhibition of autophagic flux. But it inhibits autophagic flux in both cell types at a higher concentration, which means salinomycin seems like autophagy inducer instead of inhibitor.

3.5 Summary

Autophagy is a catabolic mechanism mediated by lysosomal degradation, which is required for cellular homeostasis. Disruption of autophagy is associated with various human diseases. Autophagy is regulated by a series of proteins, such as some kinases, some of which in turn may be used as the molecular targets for the development of autophagy inhibitors and inducers. The most notable characteristics of autophagy related to cancer is its dual role, i.e. it can act as a tumor suppressing mechanism as well as a tumor promoting mechanism. Using autophagy inhibitors as an adjuvant therapy in combination with cytotoxic anti-cancer drugs is a promising way to overcome resistance in cancer therapies. On the other hand, a number of compounds achieve their anti-cancer activities by inducing autophagic cell death, presenting another possible strategy for developing novel anti-cancer drugs. Considering the complex context of autophagy, it is important to specify the indication and potential side effects of each type of small-molecule autophagy regulator in anti-cancer drug discovery.

References

Akin, D., Wang, S. K., Habibzadegah-Tari, P., Law, B., Ostrov, D., Li, M., et al. (2014). A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. *Autophagy, 10*(11), 2021–2035.

Albert, J. M., Kim, K. W., Cao, C., & Lu, B. (2006). Targeting the Akt/mammalian target of rapamycin pathway for radiosensitization of breast cancer. *Molecular Cancer Therapeutics, 5*(5), 1183–1189.

Arnold, A. A., Aboukameel, A., Chen, J., Yang, D., Wang, S., Al-Katib, A., et al. (2008). Preclinical studies of Apogossypolone: A new nonpeptidic pan small-molecule inhibitor of Bcl-2, Bcl-XL and Mcl-1 proteins in Follicular Small Cleaved Cell Lymphoma model. *Molecular Cancer, 7*, 20.

Azad, M. B., Chen, Y., Henson, E. S., Cizeau, J., McMillan-Ward, E., Israels, S. J., et al. (2008). Hypoxia induces autophagic cell death in apoptosis-competent cells through a mechanism involving BNIP3. *Autophagy, 4*(2), 195–204.

Baek, K. H., Park, J., & Shin, I. (2012). Autophagy-regulating small molecules and their therapeutic applications. *Chemical Society Reviews, 41*(8), 3245–3263.

Balic, A., Sorensen, M. D., Trabulo, S. M., Sainz, B., Jr., Cioffi, M., Vieira, C. R., et al. (2014). Chloroquine targets pancreatic cancer stem cells via inhibition of CXCR4 and hedgehog signaling. *Molecular Cancer Therapeutics, 13*(7), 1758–1771.

Bases, R. E., & Mendez, F. (1997). Topoisomerase inhibition by lucanthone, an adjuvant in radiation therapy. *International Journal of Radiation Oncology, Biology, and Physics, 37*(5), 1133–1137.

Blommaart, E. F., Krause, U., Schellens, J. P., Vreeling-Sindelarova, H., & Meijer, A. J. (1997). The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes. *European Journal of Biochemistry, 243*(1–2), 240–246.

Bonapace, L., Bornhauser, B. C., Schmitz, M., Cario, G., Ziegler, U., Niggli, F. K., et al. (2010). Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. *Journal of Clinical Investigation, 120*(4), 1310–1323.

Cao, C., Subhawong, T., Albert, J. M., Kim, K. W., Geng, L., Sekhar, K. R., et al. (2006). Inhibition of mammalian target of rapamycin or apoptotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells. *Cancer Research, 66*(20), 10040–10047.

Carew, J. S., Espitia, C. M., Esquivel, J. A., 2nd, Mahalingam, D., Kelly, K. R., Reddy, G., et al. (2011). Lucanthone is a novel inhibitor of autophagy that induces cathepsin D-mediated apoptosis. *Journal of Biological Chemistry, 286*(8), 6602–6613.

Cerny, J., Feng, Y., Yu, A., Miyake, K., Borgonovo, B., Klumperman, J., et al. (2004). The small chemical vacuolin-1 inhibits Ca(2+)-dependent lysosomal exocytosis but not cell resealing. *EMBO Reports, 5*(9), 883–888.

Chen, Y. J., Chi, C. W., Su, W. C., & Huang, H. L. (2014). Lapatinib induces autophagic cell death and inhibits growth of human hepatocellular carcinoma. *Oncotarget, 5*(13), 4845–4854.

Chen, J., Mei, Q., Xu, Y. C., Du, J., Wei, Y., & Xu, Z. M. (2006). Effects of matrine injection on T-lymphocyte subsets of patients with malignant tumor after gamma knife radiosurgery. *Zhong Xi Yi Jie He Xue Bao, 4*(1), 78–79.

Chen, S., Zhu, X., Qiao, H., Ye, M., Lai, X., Yu, S., et al. (2015). Protective autophagy promotes the resistance of HER2-positive breast cancer cells to lapatinib. Tumour Biology. DIALOG. Retrieved September 14, 2015, from http://link.springer.com/article/10.1007%2Fs13277-015-3800-9

Clarke, P. G. (1990). Developmental cell death: Morphological diversity and multiple mechanisms. *Anatomy and Embryology, 181*(3), 195–213.

Deng, L., Lei, Y., Liu, R., Li, J., Yuan, K., Li, Y., et al. (2013). Pyrvinium targets autophagy addiction to promote cancer cell death. *Cell Death & Disease, 4*, e614.

Donadelli, M., Dando, I., Zaniboni, T., Costanzo, C., Dalla Pozza, E., Scupoli, M. T., et al. (2011). Gemcitabine/cannabinoid combination triggers autophagy in pancreatic cancer cells through a ROS-mediated mechanism. *Cell Death & Disease, 2*, e152.

Fleming, A., Noda, T., Yoshimori, T., & Rubinsztein, D. C. (2011). Chemical modulators of autophagy as biological probes and potential therapeutics. *Nature Chemical Biology, 7*(1), 9–17.

Frisch, S. M., & Screaton, R. A. (2001). Anoikis mechanisms. *Current Opinion in Cell Biology, 13*(5), 555–562.

Fu, J., Shao, C. J., Chen, F. R., Ng, H. K., & Chen, Z. P. (2010). Autophagy induced by valproic acid is associated with oxidative stress in glioma cell lines. *Neuro-Oncology, 12*(4), 328–340.

Fukuda, T., Oda, K., Wada-Hiraike, O., Sone, K., Inaba, K., Ikeda, Y., et al. (2015). The antimalarial chloroquine suppresses proliferation and overcomes cisplatin resistance of endometrial cancer cells via autophagy inhibition. *Gynecologic Oncology, 137*(3), 538–545.

Fulda, S., & Kogel, D. (2015). Cell death by autophagy: Emerging molecular mechanisms and implications for cancer therapy. *Oncogene, 34*(40), 5105–5113.

Fung, C., Lock, R., Gao, S., Salas, E., & Debnath, J. (2008). Induction of autophagy during extracellular matrix detachment promotes cell survival. *Molecular Biology of the Cell, 19*(3), 797–806.

Gewirtz, D. A. (2014). The four faces of autophagy: Implications for cancer therapy. *Cancer Research, 74*(3), 647–651.

Goussetis, D. J., Altman, J. K., Glaser, H., McNeer, J. L., Tallman, M. S., & Platanias, L. C. (2010). Autophagy is a critical mechanism for the induction of the antileukemic effects of arsenic trioxide. *Journal of Biological Chemistry, 285*(39), 29989–29997.

Green, D. R., & Levine, B. (2014). To be or not to be? How selective autophagy and cell death govern cell fate. *Cell, 157*(1), 65–75.

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R. J. (2008). Review article: The role of butyrate on colonic function. *Alimentary Pharmacology and Therapeutics, 27*(2), 104–119.

Harada, M., Sakisaka, S., Yoshitake, M., Kin, M., Ohishi, M., Shakado, S., et al. (1996). Bafilomycin A1, a specific inhibitor of vacuolar-type H(+)-ATPases, inhibits the receptor-mediated endocytosis of asialoglycoproteins in isolated rat hepatocytes. *Journal of Hepatology, 24*(5), 594–603.

He, J. H., Liao, X. L., Wang, W., Li, D. D., Chen, W. D., Deng, R., et al. (2014). Apogossypolone, a small-molecule inhibitor of Bcl-2, induces radiosensitization of nasopharyngeal carcinoma cells by stimulating autophagy. *International Journal of Oncology, 45*(3), 1099–1108.

Heidari, N., Hicks, M. A., & Harada, H. (2010). GX15-070 (obatoclax) overcomes glucocorticoid resistance in acute lymphoblastic leukemia through induction of apoptosis and autophagy. *Cell Death & Disease, 1*, e76.

Homewood, C. A., Warhurst, D. C., Peters, W., & Baggaley, V. C. (1972). Lysosomes, pH and the anti-malarial action of chloroquine. *Nature, 235*(5332), 50–52.

Hoyer-Hansen, M., Bastholm, L., Szyniarowski, P., Campanella, M., Szabadkai, G., Farkas, T., et al. (2007). Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. *Molecular Cell, 25*(2), 193–205.

Hoyer-Hansen, M., & Jaattela, M. (2007). Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death and Differentiation, 14*(9), 1576–1582.

Jangamreddy, J. R., Panigrahi, S., & Los, M. J. (2015). Monitoring of autophagy is complicated—Salinomycin as an example. *Biochimica et Biophysica Acta, 1853*(3), 604–610.

Jazirehi, A. R. (2010). Regulation of apoptosis-associated genes by histone deacetylase inhibitors: Implications in cancer therapy. *Anti-Cancer Drugs, 21*(9), 805–813.

Jung, C. H., Jun, C. B., Ro, S. H., Kim, Y. M., Otto, N. M., Cao, J., et al. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Molecular Biology of the Cell, 20*(7), 1992–2003.

Kim, Y. C., & Guan, K. L. (2015). mTOR: A pharmacologic target for autophagy regulation. *Journal of Clinical Investigation, 125*(1), 25–32.

Kim, J., Kim, Y. C., Fang, C., Russell, R. C., Kim, J. H., Fan, W., et al. (2013a). Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell, 152*(1–2), 290–303.

Kim, Y., Kim, Y. S., Kim, D. E., Lee, J. S., Song, J. H., Kim, H. G., et al. (2013b). BIX-01294 induces autophagy-associated cell death via EHMT2/G9a dysfunction and intracellular reactive oxygen species production. *Autophagy, 9*(12), 2126–2139.

Kimura, T., Takabatake, Y., Takahashi, A., & Isaka, Y. (2013). Chloroquine in cancer therapy: A double-edged sword of autophagy. *Cancer Research, 73*(1), 3–7.

Kumar, D., Shankar, S., & Srivastava, R. K. (2014). Rottlerin induces autophagy and apoptosis in prostate cancer stem cells via PI3K/Akt/mTOR signaling pathway. *Cancer Letters, 343*(2), 179–189.

Kumar, A., Singh, U. K., & Chaudhary, A. (2015). Targeting autophagy to overcome drug resistance in cancer therapy. *Future Medicinal Chemistry, 7*(12), 1535–1542.

Laplante, M., & Sabatini, D. M. (2012). mTOR signaling in growth control and disease. *Cell, 149*(2), 274–293.

Leng, S., Hao, Y., Du, D., Xie, S., Hong, L., Gu, H., et al. (2013). Ursolic acid promotes cancer cell death by inducing Atg5-dependent autophagy. *International Journal of Cancer, 133*(12), 2781–2790.

Levy, J. M., & Thorburn, A. (2011). Targeting autophagy during cancer therapy to improve clinical outcomes. *Pharmacology and Therapeutics, 131*(1), 130–141.

Li, T. L., Su, L., Zhong, N., Hao, X. X., Zhong, D. S., Singhal, S., et al. (2013). Salinomycin induces cell death with autophagy through activation of endoplasmic reticulum stress in human cancer cells. *Autophagy, 9*(7), 1057–1068.

Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., et al. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature, 402*(6762), 672–676.

Liu, R., Li, J., Zhang, T., Zou, L., Chen, Y., Wang, K., et al. (2014). Itraconazole suppresses the growth of glioblastoma through induction of autophagy: involvement of abnormal cholesterol trafficking. *Autophagy, 10*(7), 1241–1255.

Liu, T., Song, Y., Chen, H., Pan, S., & Sun, X. (2010). Matrine inhibits proliferation and induces apoptosis of pancreatic cancer cells in vitro and in vivo. *Biological and Pharmaceutical Bulletin, 33*(10), 1740–1745.

Lu, Y., Dong, S., Hao, B., Li, C., Zhu, K., Guo, W., et al. (2014). Vacuolin-1 potently and reversibly inhibits autophagosome-lysosome fusion by activating RAB5A. *Autophagy, 10*(11), 1895–1905.

Maira, S. M., Stauffer, F., Schnell, C., & Garcia-Echeverria, C. (2009). PI3K inhibitors for cancer treatment: Where do we stand? *Biochemical Society Transactions, 37*(Pt 1), 265–272.

Marino, G., Niso-Santano, M., Baehrecke, E. H., & Kroemer, G. (2014). Self-consumption: The interplay of autophagy and apoptosis. *Nature Reviews Molecular Cell Biology, 15*(2), 81–94.

Marino, G., Salvador-Montoliu, N., Fueyo, A., Knecht, E., Mizushima, N., & Lopez-Otin, C. (2007). Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. *Journal of Biological Chemistry, 282*(25), 18573–18583.

Mathew, R., Karantza-Wadsworth, V., & White, E. (2007). Role of autophagy in cancer. *Nature Reviews Cancer, 7*(12), 961–967.

Mauvezin, C., & Neufeld, T. P. (2015). Bafilomycin A1 disrupts autophagic flux by inhibiting both V-ATPase-dependent acidification and Ca-P60A/SERCA-dependent autophagosome-lysosome fusion. *Autophagy, 11*(8), 1437–1438.

Maycotte, P., & Thorburn, A. (2011). Autophagy and cancer therapy. *Cancer Biology and Therapy, 11*(2), 127–137.

Miller, W. H., Jr., Schipper, H. M., Lee, J. S., Singer, J., & Waxman, S. (2002). Mechanisms of action of arsenic trioxide. *Cancer Research, 62*(14), 3893–3903.

Nagelkerke, A., Bussink, J., Geurts-Moespot, A., Sweep, F. C., & Span, P. N. (2015). Therapeutic targeting of autophagy in cancer. Part II: Pharmacological modulation of treatment-induced autophagy. *Seminars in Cancer Biology, 31*, 99–105.

Nakamura, M., Kikukawa, Y., Takeya, M., Mitsuya, H., & Hata, H. (2010). Clarithromycin attenuates autophagy in myeloma cells. *International Journal of Oncology, 37*(4), 815–820.

Nam, H. Y., Han, M. W., Chang, H. W., Lee, Y. S., Lee, M., Lee, H. J., et al. (2013). Radioresistant cancer cells can be conditioned to enter senescence by mTOR inhibition. *Cancer Research, 73*(14), 4267–4277.

Niu, X., Li, S., Wei, F., Huang, J., Wu, G., Xu, L., et al. (2014). Apogossypolone induces autophagy and apoptosis in breast cancer MCF-7 cells in vitro and in vivo. *Breast Cancer, 21*(2), 223–230.

Nomura, T., & Katunuma, N. (2005). Involvement of cathepsins in the invasion, metastasis and proliferation of cancer cells. *The Journal of Medical Investigation, 52*(1–2), 1–9.

Pattingre, S., & Levine, B. (2006). Bcl-2 inhibition of autophagy: A new route to cancer? *Cancer Research, 66*(6), 2885–2888.

Plumbridge, T. W., & Brown, J. R. (1978). Studies on the mode of interaction of 4′-epi-adriamycin and 4-demethoxy-daunomycin with DNA. *Biochemical Pharmacology, 27*(14), 1881–1882.

Polivka, J., Jr., & Janku, F. (2014). Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. *Pharmacology and Therapeutics, 142*(2), 164–175.

Powis, G., Bonjouklian, R., Berggren, M. M., Gallegos, A., Abraham, R., Ashendel, C., et al. (1994). Wortmannin, a potent and selective inhibitor of phosphatidylinositol-3-kinase. *Cancer Research, 54*(9), 2419–2423.

Qu, X., Yu, J., Bhagat, G., Furuya, N., Hibshoosh, H., Troxel, A., et al. (2003). Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *Journal of Clinical Investigation, 112*(12), 1809–1820.

Racoma, I. O., Meisen, W. H., Wang, Q. E., Kaur, B., & Wani, A. A. (2013). Thymoquinone inhibits autophagy and induces cathepsin-mediated, caspase-independent cell death in glioblastoma cells. *PLoS One, 8*(9), e72882.

Renna, M., Schaffner, C., Brown, K., Shang, S., Tamayo, M. H., Hegyi, K., et al. (2011). Azithromycin blocks autophagy and may predispose cystic fibrosis patients to mycobacterial infection. *Journal of Clinical Investigation, 121*(9), 3554–3563.

Ristic, B., Bosnjak, M., Arsikin, K., Mircic, A., Suzin-Zivkovic, V., Bogdanovic, A., et al. (2014). Idarubicin induces mTOR-dependent cytotoxic autophagy in leukemic cells. *Experimental Cell Research, 326*(1), 90–102.

Ronan, B., Flamand, O., Vescovi, L., Dureuil, C., Durand, L., Fassy, F., et al. (2014). A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. *Nature Chemical Biology, 10*(12), 1013–1019.

Rubiolo, J. A., Lopez-Alonso, H., Martinez, P., Millan, A., Cagide, E., Vieytes, M. R., et al. (2014). Yessotoxin induces ER-stress followed by autophagic cell death in glioma cells mediated by mTOR and BNIP3. *Cellular Signalling, 26*(2), 419–432.

Salabei, J. K., Balakumaran, A., Frey, J. C., Boor, P. J., Treinen-Moslen, M., & Conklin, D. J. (2012). Verapamil stereoisomers induce antiproliferative effects in vascular smooth muscle cells via autophagy. *Toxicology and Applied Pharmacology, 262*(3), 265–272.

Seglen, P. O., & Gordon, P. B. (1982). 3-Methyladenine: Specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. *Proceedings of the National Academy of Sciences of the United States of America, 79*(6), 1889–1892.

Shao, Y., Gao, Z., Marks, P. A., & Jiang, X. (2004). Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. *Proceedings of the National Academy of Sciences of the United States of America, 101*(52), 18030–18035.

Shimizu, S., Kanaseki, T., Mizushima, N., Mizuta, T., Arakawa-Kobayashi, S., Thompson, C. B., et al. (2004). Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nature Cell Biology, 6*(12), 1221–1228.

Shimizu, S., Konishi, A., Nishida, Y., Mizuta, T., Nishina, H., Yamamoto, A., et al. (2010). Involvement of JNK in the regulation of autophagic cell death. *Oncogene, 29*(14), 2070–2082.

Shimizu, S., Yoshida, T., Tsujioka, M., & Arakawa, S. (2014). Autophagic cell death and cancer. *International Journal of Molecular Sciences, 15*(2), 3145–3153.

Sosa, M. S., Bragado, P., Debnath, J., & Aguirre-Ghiso, J. A. (2013). Regulation of tumor cell dormancy by tissue microenvironments and autophagy. *Advances in Experimental Medicine & Biology, 734*, 73–89.

Takahashi, A., Kimura, F., Yamanaka, A., Takebayashi, A., Kita, N., Takahashi, K., et al. (2014). Metformin impairs growth of endometrial cancer cells via cell cycle arrest and concomitant autophagy and apoptosis. *Cancer Cell International, 14*, 53.

Tamai, M., Matsumoto, K., Omura, S., Koyama, I., Ozawa, Y., & Hanada, K. (1986). In vitro and in vivo inhibition of cysteine proteinases by EST, a new analog of E-64. *Journal of Pharmacobio-Dynamics, 9*(8), 672–677.

Tanida, I., Minematsu-Ikeguchi, N., Ueno, T., & Kominami, E. (2005). Lysosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. *Autophagy, 1*(2), 84–91.

Thorburn, A., Thamm, D. H., & Gustafson, D. L. (2014). Autophagy and cancer therapy. *Molecular Pharmacology, 85*(6), 830–838.

Thorne, C. A., Hanson, A. J., Schneider, J., Tahinci, E., Orton, D., Cselenyi, C. S., et al. (2010). Small-molecule inhibition of Wnt signaling through activation of casein kinase 1alpha. *Nature Chemical Biology, 6*(11), 829–836.

Umezawa, H., Aoyagi, T., Morishima, H., Matsuzaki, M., & Hamada, M. (1970). Pepstatin, a new pepsin inhibitor produced by Actinomycetes. *Journal of Antibiotics, 23*(5), 259–262.

Vlahos, C. J., Matter, W. F., Hui, K. Y., & Brown, R. F. (1994). A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *Journal of Biological Chemistry, 269*(7), 5241–5248.

Voss, V., Senft, C., Lang, V., Ronellenfitsch, M. W., Steinbach, J. P., Seifert, V., et al. (2010). The pan-Bcl-2 inhibitor (-)-gossypol triggers autophagic cell death in malignant glioma. *Molecular Cancer Research, 8*(7), 1002–1016.

Wang, Y., Kuramitsu, Y., Tokuda, K., Baron, B., Kitagawa, T., Akada, J., et al. (2014). Gemcitabine induces poly (ADP-ribose) polymerase-1 (PARP-1) degradation through autophagy in pancreatic cancer. *PLoS One, 9*(10), e109076.

Wang, Z., Zhang, J., Wang, Y., Xing, R., Yi, C., Zhu, H., et al. (2013). Matrine, a novel autophagy inhibitor, blocks trafficking and the proteolytic activation of lysosomal proteases. *Carcinogenesis, 34*(1), 128–138.

Watanabe, M., Adachi, S., Matsubara, H., Imai, T., Yui, Y., Mizushima, Y., et al. (2009). Induction of autophagy in malignant rhabdoid tumor cells by the histone deacetylase inhibitor FK228 through AIF translocation. *International Journal of Cancer, 124*(1), 55–67.

Wei, Y., Kadia, T., Tong, W., Zhang, M., Jia, Y., Yang, H., et al. (2010). The combination of a histone deacetylase inhibitor with the BH3-mimetic GX15-070 has synergistic antileukemia activity by activating both apoptosis and autophagy. *Autophagy, 6*(7), 976–978.

Wirth, M., Joachim, J., & Tooze, S. A. (2013). Autophagosome formation—The role of ULK1 and Beclin1-PI3KC3 complexes in setting the stage. *Seminars in Cancer Biology, 23*(5), 301–309.

Wong, V. K., Li, T., Law, B. Y., Ma, E. D., Yip, N. C., Michelangeli, F., et al. (2013). Saikosaponin-d, a novel SERCA inhibitor, induces autophagic cell death in apoptosis-defective cells. *Cell Death & Disease, 4*, e720.

Wu, L., & Yan, B. (2011). Discovery of small molecules that target autophagy for cancer treatment. *Current Medicinal Chemistry, 18*(12), 1866–1873.

Yamamoto, S., Tanaka, K., Sakimura, R., Okada, T., Nakamura, T., Li, Y., et al. (2008). Suberoylanilide hydroxamic acid (SAHA) induces apoptosis or autophagy-associated cell death in chondrosarcoma cell lines. *Anticancer Research, 28*(3A), 1585–1591.

You, D., Kim, Y., Jang, M. J., Lee, C., Jeong, I. G., Cho, Y. M., et al. (2015). KML001 induces apoptosis and autophagic cell death in prostate cancer cells via oxidative stress pathway. *PLoS One, 10*(9), e0137589.

Yu, H. C., Lin, C. S., Tai, W. T., Liu, C. Y., Shiau, C. W., & Chen, K. F. (2013). Nilotinib induces autophagy in hepatocellular carcinoma through AMPK activation. *Journal of Biological Chemistry, 288*(25), 18249–18259.

Yue, W., Hamai, A., Tonelli, G., Bauvy, C., Nicolas, V., Tharinger, H., et al. (2013). Inhibition of the autophagic flux by salinomycin in breast cancer stem-like/progenitor cells interferes with their maintenance. *Autophagy, 9*(5), 714–729.

Zhang, X. Q., Huang, X. F., Hu, X. B., Zhan, Y. H., An, Q. X., Yang, S. M., et al. (2010). Apogossypolone, a novel inhibitor of antiapoptotic Bcl-2 family proteins, induces autophagy of PC-3 and LNCaP prostate cancer cells in vitro. *Asian Journal of Andrology, 12*(5), 697–708.

Zhang, C., Richon, V., Ni, X., Talpur, R., & Duvic, M. (2005). Selective induction of apoptosis by histone deacetylase inhibitor SAHA in cutaneous T-cell lymphoma cells: Relevance to mechanism of therapeutic action. *Journal of Investigative Dermatology, 125*(5), 1045–1052.

Zhou, M., & Wang, R. (2013). Small-molecule regulators of autophagy and their potential therapeutic applications. *ChemMedChem, 8*(5), 694–707.

Zhu, X. X., Yao, X. F., Jiang, L. P., Geng, C. Y., Zhong, L. F., Yang, G., et al. (2014). Sodium arsenite induces ROS-dependent autophagic cell death in pancreatic beta-cells. *Food and Chemical Toxicology, 70*, 144–150.